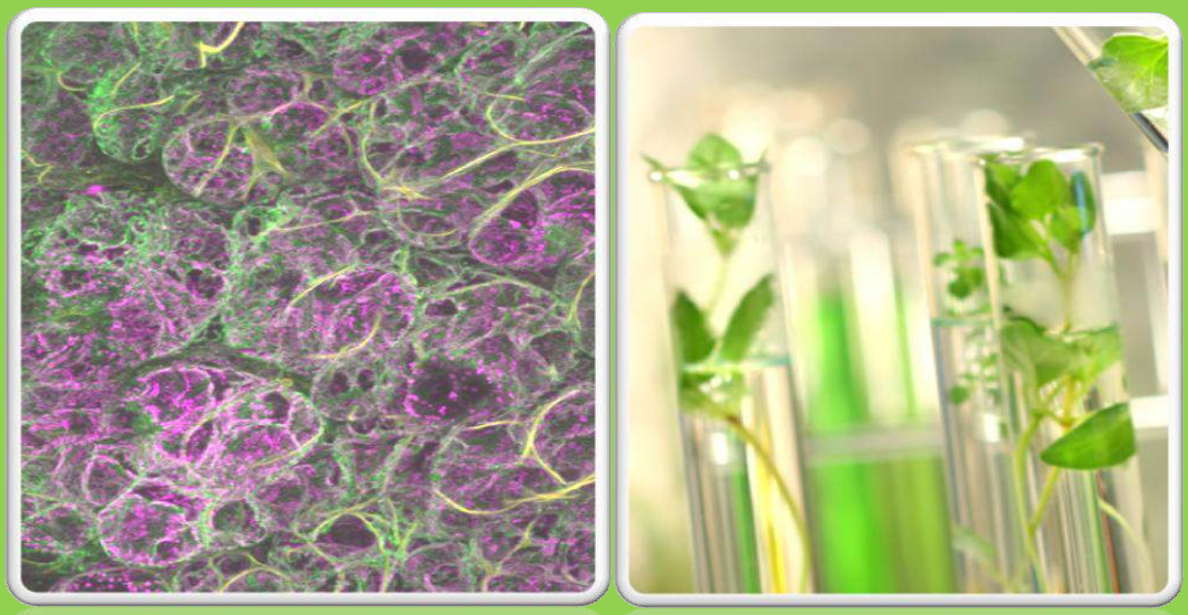
BSCBO- 301

B.Sc. III YEAR

Cell Biology, Molecular Biology And Biotechnology



DEPARTMENT OF BOTANY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

BSCBO-301

CELL BIOLOGY, MOLECULAR BIOLOGY AND BIOTECHNOLOGY

SCHOOL OF SCIENCES
DEPARTMENT OF BOTANY
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BLOCK-1 CELL BIOLOGY

UNIT-1THE CELL

1.1 OBJECTIVES

After reading this unit student will be able to understand about-

- the advancements in cell biology
- brief idea about the great diversity shown by cells in their shapes and sizes
- Also, to give an outline information about structures and purposes of basic components of prokaryotic and eukaryotic cells.
- to know how the cellular components are arranged in both types of cell.

1.2 INTRODUCTION

The basic structural and functional unit of cellular organization is the *cell*. Within a selective and relative semi permeable membrane, it contains a complete set of different kinds of units necessary to permit its own growth and reproduction from simple nutrients. All organisms, more complex than viruses, consist of cells, yet they consist of a strand of nucleic acid, either DNA or RNA, surrounded by a protective protein coat (the capsid). The word cell is derived from the Latin word *cellula*, which means small compartment. Hooke published his findings in his famous work, *Micrographia*. Actually, he only observed cell walls because cork cells are dead and without cytoplasmic contents. A.G. Loewy and P. Siekevitz have defined cell as "*A unit of biological activity delimited by a semi permeable membrane and capable of self reproduction in a medium free of other living organisms*". John Paul has defined the cell as "The simplest integrated organization in living systems, capable of independent survival".

On the basis of internal organization and architecture, all cells can be subdivided into two major classes, prokaryotic cells and eukaryotic cells. Cells which have the unit membrane bound nuclei are called eukaryotic, whereas cells that lack a membrane bound nucleus are prokaryotic. Besides the nucleus, the eukaryotic cells have other membrane bound organelles (small organs) like the Endoplasmic reticulum, Golgi complex, Lysosomes, Mitochondria, Microbodies and Vacuoles. The prokaryotic cells lack such unit membrane bound organelles.

1.3 HISTORICAL BACKGROUND

Ancient Greek philosophers such as Aristotle 384-322 B.C and Paracelsus concluded that "*All animals and plants, however, complicated, are constituted of a few elements which are repeated in each of them*". They were referring to macroscopic structures of an organism such as roots, leaves and flowers common to different plants, or segments and organs that are repeated in the animal kingdom. Many centuries later, owing to the invention of magnifying lenses, the world of microscopic dimensions was discovered. Da Vinci (1485) recommended the uses of lenses in viewing small objects. In 1558, Swiss biologist, Conard Gesner (1516-1565) published results on

his studies on the structure of a group of protists called foraminifera. His sketches of these protozoa included so many details that they could only have been made if he had used form of magnifying lenses. Perhaps, this is earliest recorded use of a magnifying instrument in a biological study.

Further growth and development of cell biology are intimately associated with the development of optical lenses and to the combination of these lenses in the construction of the compound microscope. Thus, the invention of the microscope and its gradual improvement went hand-in-hand with the development of cell biology.

Growth of Cell Biology during 16th and 18th Centuries

The first useful compound microscope was invented in 1590 by Francis Janssen and Zacharias Janssen. Their microscope had two lenses and total magnifying power between 10X and 30X. Such types of microscopes were called “flea glasses", since they were primarily used to examine small whole organisms such as fleas and other insects. In 1610, an Italian Galileo Galilei (1564-1642) invented a simple microscope having only one magnifying lens. This microscope was used to study the arrangement of the facets in compound eye of insects.

The Italian microanatomist Marcello Malpighi (1628-1694) was among the first to use a microscope to examine and describe thin slices of animal tissues from such organs as the brain, liver, kidney, spleen, lungs and tongue. He also studied plant tissues and suggested that they were composed of structural units that he called “utricles”. An English microscopist Robert Hooke (1635-1703) is credited with coining the term cell in 1665. He examined a thin slice cut from a piece of dried cork under the compound microscopes which were built by him.

Fig. 1.1: Hooke's compound microscope

1.4 CELL THEORY

In biology, cell theory is a scientific theory which describes the properties of cells. These cells are the basic unit of structure in all organisms and also the basic unit of reproduction. With continual improvements made to microscopes over time, magnification technology advanced enough to discover cells in the 17th century. This discovery is largely attributed to Robert Hooke, and began the scientific study of cells, also known as cell biology. Over a century later, many debates about cells began amongst scientists. Most of these debates involved the nature of cellular regeneration, and the idea of cells as a fundamental unit of life.

Fig.1.2 Mathias Jakob Schleiden (1804–1881)

Fig.1.3 Theodor Schwann (1810–1882)

Matthias Jakob Schleiden (5 April 1804 – 23 June 1881) was a German botanist and co-founder of cell theory, along with Theodor Schwann (7 December 1810 – 11 January 1882) a German physiologist and Rudolf Ludwig Carl Virchow (13 October 1821 – 5 September 1902) was a German physician, anthropologist, pathologist, prehistorian, biologist, writer, editor, and politician, known for his advancement of public health. Credit for developing cell theory is usually given to these scientists- Schleiden and Schwann. While Rudolf Virchow contributed to the theory, he is not as credited for his attributions toward it. In 1838, Schleiden suggested that every structural part of a plant was made up of cells or the result of cells. He also suggested that cells were made by a crystallization process either within other cells or from the outside. However, this was not an original idea of Schlieden. He claimed this theory as his own, though Barthelemy Dumortier had stated it years before him. This crystallization process is no longer accepted with modern cell theory. In 1839, Theodor Schwann states that along with plants, animals are composed of cells or the product of cells in their structures. This was a major advancement in the field of biology since little was known about animal structure up to this point

compared to plants. From these conclusions about plants and animals, two of the three tenets of cell theory were postulated.

1. All living organisms are composed of one or more cells.
2. The cell is the most basic unit of life.

Schleiden's theory of free cell formation through crystallization was refuted in the 1850s by Robert Remak, Rudolf Virchow, and Albert Kolliker. Robert Remak (26 July 1815 – 29 August 1865) was a Jewish Polish-German embryologist, physiologist, and neurologist, born in Posen, Prussia, who discovered that the origin of cells was by the division of pre-existing cells. In 1855, Rudolf Virchow added the third tenet to cell theory. In Latin, this tenet states "*Omnis cellula e cellula*". This translated to-

3. All cells arise only from pre-existing cells.

However, the idea that all cells come from pre-existing cells had in fact already been proposed by Robert Remak; it has been suggested that Virchow plagiarized Remak and did not give him credit. Remak published observations in 1852 on cell division, claiming Schleiden and Schawnn were incorrect about generation schemes. He instead said that binary fission, which was first introduced by Dumortier, was how reproduction of new animal cells was made. Once this tenet was added, the classical cell theory was complete. Barthélemy Charles Joseph Dumortier (3 April 1797 in Tournai – 9 June 1878) was a Belgian who conducted a parallel career of botanist and Member of Parliament.

Modern Interpretation

The generally accepted parts of modern cell theory include:

1- All known living things are made up of one or more cells.
2- All living cells arise from pre-existing cells by division.
3- The cell is the fundamental unit of structure and function in all living organisms.
4- The activity of an organism depends on the total activity of independent cells.
5- Energy flow (metabolism and biochemistry) occurs within cells.
6- Cells contain DNA which is found specifically in the chromosome and RNA found in the cell nucleus and cytoplasm.
7-All cells are basically the same in chemical composition in organisms of similar species.

The modern version of the cell theory:

The modern version of the cell theory includes the ideas that:

1- Energy flow occurs within cells.
2- Heredity information (DNA) is passed on from cell to cell.
3- All cells have the same basic chemical composition.

1.5 SIZE AND STRUCTURE OF CELL

There are many cells in an individual, which performs several functions throughout the life. The different types of cell include- prokaryotic cell, plant and animal cell. The size and the shape of the cell range from millimeter to microns, which are generally based on the type of function that it performs. A cell generally varies in their shapes. A few cells are in spherical, rod, flat, concave, curved, rectangular, oval and etc. These cells can only be seen under microscope.

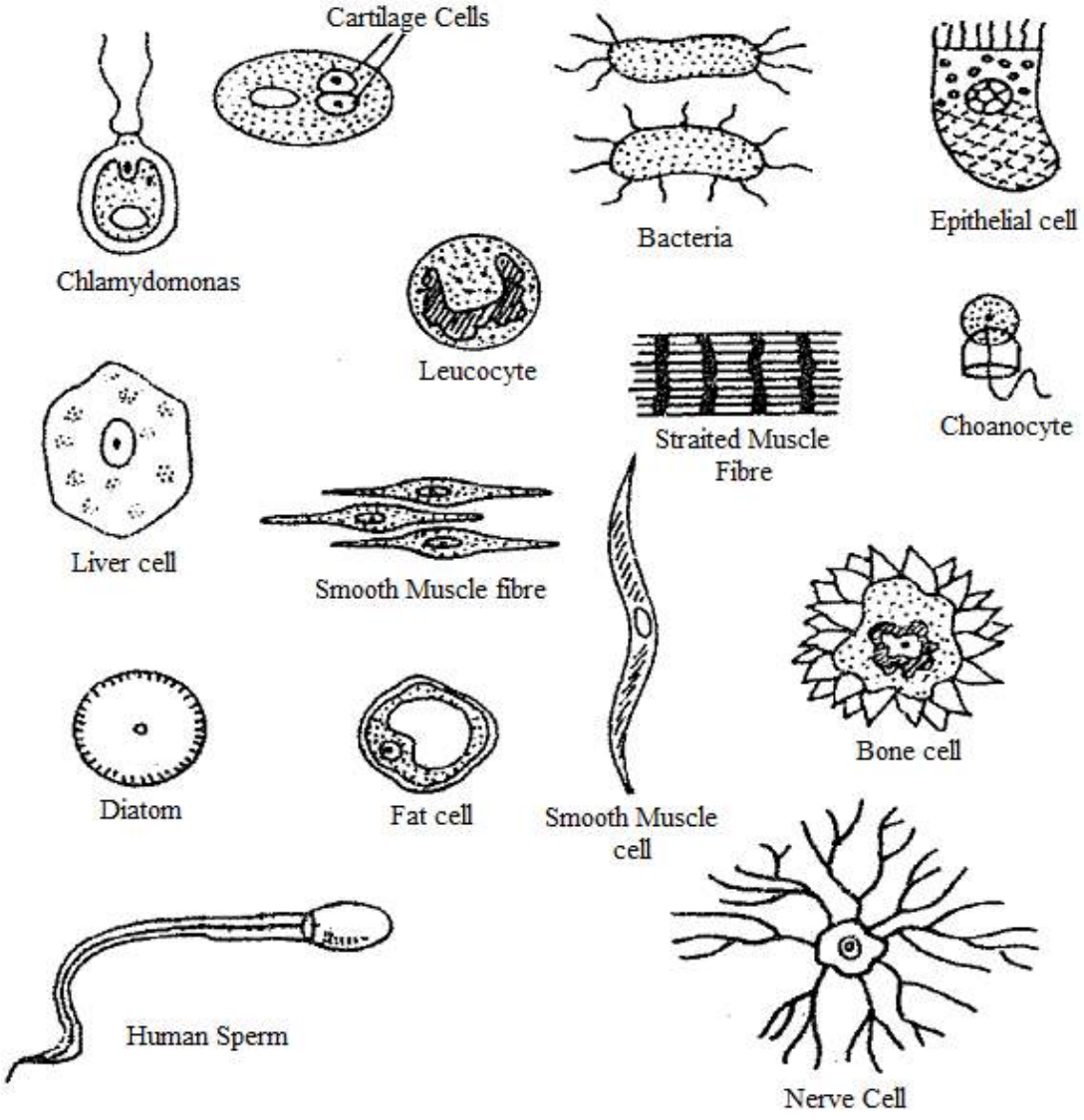


Fig. 1.4 Different types of cells

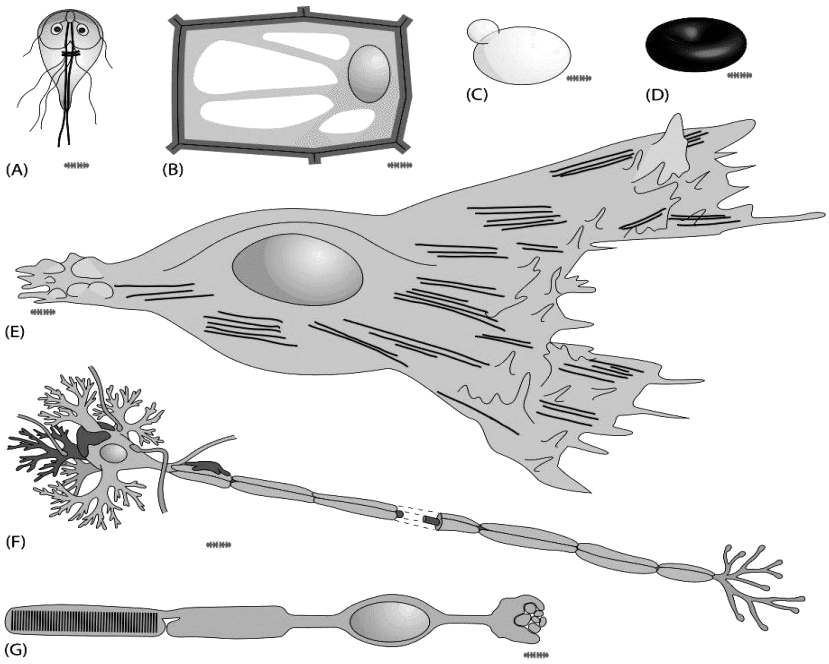


Fig.1.5 : Several different types of cells all referenced to a standard E. coli ruler of 1 micron (A) The protist *Giardia lamblia*, (B) a plant cell, (C) a budding yeast cell, (D) a red blood cell, (E) a fibroblast cell, (F) a eukaryotic nerve cell, and (G) a rod cell from the retina.

Cell Size

One may wonder why all cells are so small. If being able to store nutrients, is beneficial to the cell, how come there are no animals existing in nature with huge cells? Physical limitations prevent this from occurring. A cell must be able to diffuse gases and nutrients in and out of the cell. A cell's surface area does not increase as quickly as its volume, and as a result a large cell may require more input of a substance or output of a substance than it is reasonably able to perform. Worse, the distance between two points within the cell can be large enough that regions of the cell would have trouble communicating, and it takes a relatively long time for substances to travel across the cell.

That is not to say large cells don't exist. They are, once again, less efficient at exchanging materials within themselves and with their environment, but they are still functional. These cells typically have more than one copy of their genetic information, so they can manufacture proteins locally within different parts of the cell. Features of such large cells are following:

1. Is limited by need for regions of cell to communicate
2. Diffuse oxygen and other gases
3. Transport of mRNA and proteins
4. Surface area to volume ratio limited

Larger cells typically:

a) Have extra copies of genetic information
b) Have slower communication between parts of cell

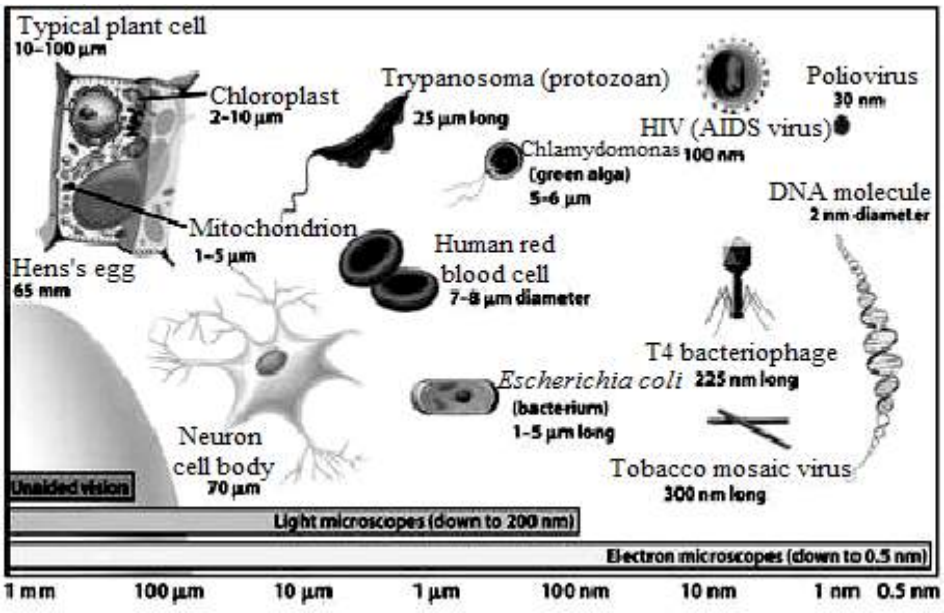


Fig. 1.6- Various types of cells ranging in different sizes

The shapes of cells are quite varied with some, such as neurons, being longer than they are wide and others, such as parenchyma (a common type of plant cell) and erythrocytes (red blood cells) being equidimensional. Some cells are encased in a rigid wall, which constrains their shape, while others have a flexible cell membrane (and no rigid cell wall).

The size of cells is also related to their functions. Eggs (or to use the Latin word, ova) are very large, often being the largest cells an organism produces. The large size of many eggs is related to the process of development that occurs after the egg is fertilized, when the contents of the egg (now termed a zygote) are used in a rapid series of cellular divisions, each requiring tremendous amounts of energy that is available in the zygote cells. Later in life the energy must be acquired, but at first a sort of inheritance/trust fund of energy is used. Cells range in size from small bacteria to large, unfertilized eggs laid by birds and dinosaurs. Here are some measurements and conversions that will aid your understanding of biology.

1 meter = 100 cm = 1,000 mm = 1,000,000 µm = 1,000,000,000 nm
1 centimeter (cm) = 1/100 meter = 10 mm
1 millimeter (mm) = 1/1000 meter = 1/10 cm
1 micrometer (µm) = 1/1,000,000 meter = 1/10,000 cm
1 nanometer (nm) = 1/1,000,000,000 meter = 1/10,000,000 cm

1.6 PROKARYOTIC AND EUKARYOTIC CELL

Body of all living organisms except virus has cellular organization and may contain one or many cells. The organisms with only one cell in their body are known as unicellular (bacteria, protozoa etc.) and organisms with many cells in their body are known as multicellular organisms (most plants and animals). Any cellular organization may contain only one type of cell from the following types:

A- Prokaryotic cell

B- Eukaryotic cell

These terms were suggested by Hans Ris in the 1960's.

A: PROKARYOTIC CELL

The prokaryotic (Gr., *pro*= primitive or before and *karyon* = nucleus) are small, simple, and most primitive. They are probably first to come into existence perhaps 3.5 billion years ago. These cells occur in bacteria (i.e., Mycoplasma, Cyanobacteria etc). Prokaryotic cell is a one envelope system organized in depth. It consists of central nuclear components surrounded by cytoplasmic ground substance, with whole enveloped by a plasma membrane. The cytoplasm of prokaryotic cell lacks nuclear envelope and any other cytoplasmic membrane and well defined cytoplasmic organelles.

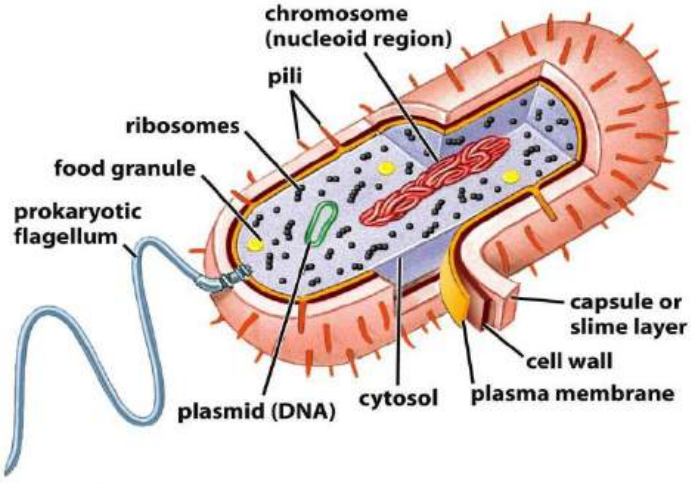


Fig.1.7- Structure of a prokaryotic cell

Bacteria

The bacteria are amongst smallest organisms, most primitive, prokaryotic and microscopic organisms. They occur almost everywhere; in air, water, soil and inside other organisms. They lead either autotrophic or heterotrophic mode of existence.

1. Size of Bacteria: It ranges between 1-3µm and are barely visible under light microscope.

2. Shape of Bacteria: The three basic bacterial shapes are:

Cocci – (spherical shaped). e.g., *Diplococcus pneumonia, Streptococcus pyogenes* etc.

Bacilli – (rod-shaped) e.g. *Mycobacterium, Clostridium botylinum* etc.

Spirilla (spiral or twisted) e.g. *Treponema pallidum* etc

However pleomorphic bacteria can assume several shapes.

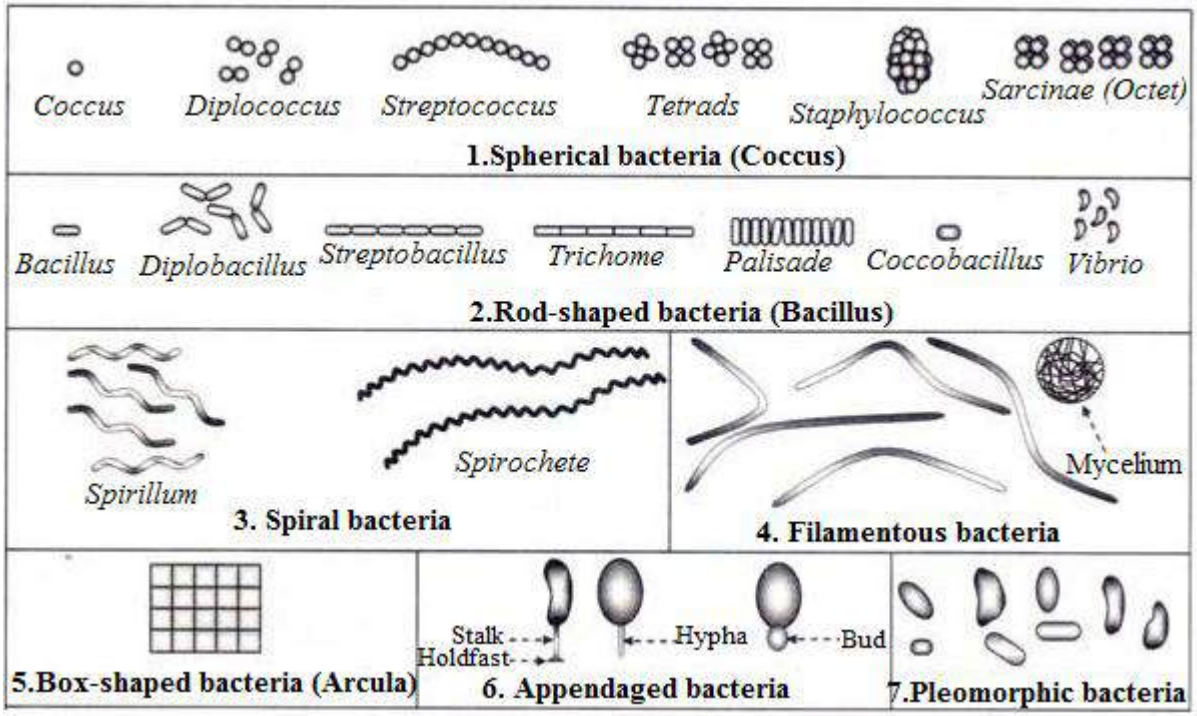


Fig. 1.8 Different shapes of bacteria

3. Structure of Bacteria:

(i) Plasma membrane- It is an ultra thin membrane 6-8 nm thick, chemically comprised of molecules of lipids and proteins, arranged in a fluid mosaic pattern. Infoldings in it gives rise to two main types of structures:

(a) Mesosomes- (Also known as Chondriods); are extensions involving complex whorls of convoluted membranes. They increase surface area of plasma membrane and enzymatic contents.

(b) Chromatophores- These are photosynthetic pigment- bearing membranous structures of photosynthetic bacteria and are present as vesicles, thylakoids, tubes etc.

(ii) Cell Wall- It is strong and rigid and covers plasma membrane to provide chemical protection and characteristic shape of bacteria. It is made up of peptidoglycan and contains muramic acid.

(iii) Capsule- In some bacteria, cell wall is surrounded by an additional slime or gel layer called capsule that acts as protective layer against viruses and phagocytes.

(iv) Cytoplasm- It is the ground substance surrounded by plasma membrane and is site of all metabolic activities of bacteria. It consists of water, proteins, enzymes, different types of RNA molecules and reserve materials like glucogen, volutin and sulphur. The dense nuclear areas of cytoplasm contain 70S ribosomes granules, composed of RNA and protein and are the site of protein synthesis.

(v) Nucleoids- The nuclear membrane includes a single, circular and double stranded DNA molecule often called as bacterial chromosome. It is not separated by nuclear membrane and is usually concentrated in a specific clear region of the cytoplasm called nucleoid. It has no ribosomes, nucleolus and histone proteins.

(vi) Plasmids – Many species of bacteria may also carry extrachromosomal genetic elements in the form of small, circular, and closed DNA molecules called plasmids. They produce antibiotically active protein or colicins which inhibit the growth of other bacterial strain in their vicinity. They may also act as sex or fertility factors (F factor) which stimulate bacterial conjugation. R factors are also plasmid carrying genes for resistance to drugs.

(vii) Flagella- Many bacteria are motile and contain one or more flagella for cellular locomotion. They are 15-20nm in diameter and up to 20µm long. e.g., *E.coli* etc

4. Nutrition: They show diversity in their nutrition from being chemosynthetic, to photosynthetic; but most of them are heterotrophic. Heterotrophic bacteria are mostly either saprophytic or parasitic. Parasitic lives on the bodies of other organisms. Most bacteria are pathogenic.

5. Mode of Respiration: It is of both types; aerobic (which respire in the presence of oxygen.eg *Lactobacillus*) and anaerobic (which respire in the absence of oxygen. e.g. *Pseudomonas*).

6. Reproduction: Bacteria reproduce through asexually by binary fission and endospore formation and sexually by conjugation. In conjugation, genetic exchange and recombination occurs through sex pili, but this is a form of horizontal gene transfer and is not a replicative process, simply involving the transference of DNA between two cells.

B: Eukaryotic Cell

The Eukaryotic cells are essentially two envelope systems and they are very much larger than prokaryotic cells. Secondary membranes envelop the nucleolus and other internal organelles and to a great extent they pervade the Cytoplasm as the Endoplasmic reticulum. The Eukaryotic cells are true cells which occur in the plants (from algae to angiosperms) and the animal (from Protozoa to mammals). Though the Eukaryotic cells have different shape, size, and physiology; all the cells are typically composed of plasma membrane, cytoplasm and its organelles, viz. Mitochondria, Endoplasmic reticulum, ribosomes, Golgi apparatus etc; and a true nucleus. Here

the nuclear contents, such as DNA, RNA, Nucleoproteins and Nucleolus remain separated from the Cytoplasm by the thin perforated nuclear membranes. Before going into the detail of cells and its various components; it will be advisable to consider the general features of different types of eukaryotic cells which are as follow:

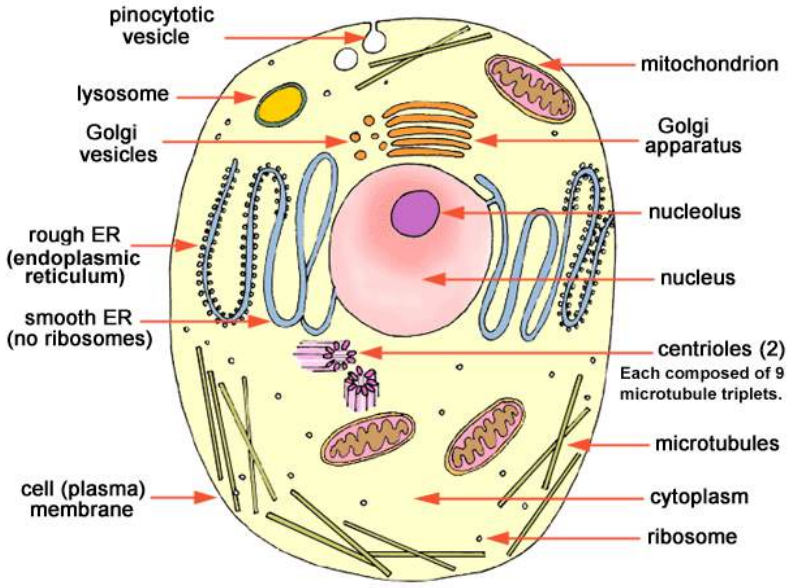


Fig.1.9- Structure of a Eukaryotic cell

1. Cell Shape: The basic shape of Eukaryotic Cells is **Spherical**; however the shape is ultimately determined by the specific function of the cell. Thus, the shape of the cell may be **Variable** or **Fixed.** Variable or irregular shape occurs in Amoeba and white blood cells or Leucocytes. Fixed shape cells occur in almost all protests', plant, animals. In unicellular organisms the cells shape is maintained by tough plasma membrane and exoskeleton. The shape of cells may vary from animal to animal and from organ to organ. Even cells of the same organ may display variations in the shape. Thus cells may have diverse shape such as Polyhedral, Flattened, Cuboidal, Columnar, Discoidal, Spherical, Spindle Shaped, Elongated or Branched.

2. Cell Size: The eukaryotic cells are typically larger (mostly ranging between 10 to 100 µm) than the prokaryotic cells (mostly ranging between 1 to 10 µm). Size of the cell of the unicellular organisms is larger than typical multicellular organisms' cells. For example, *Amoeba proteus* is biggest among the unicellular organisms. One species of *Euglena* is found up to 500 µm in length. Diatoms have length of 200µm or more.

The size of multicellular organism ranges between 20 to 30µm. Among animal's the smallest cells have a diameter 4µm. (e.g. Polocytes); Human erythrocytes being 7 to 8 µm in diameter. Largest animal cell is the egg of Ostrich, having a diameter of 18cm and the longest cell is human nerve cell, a meter long.

3. Cell Volume: The volume of cell is fairly constant for a particular cell type and is independent of the size of the organism (**Law of Constant Volume**). For example, kidney or liver cells are about the same size in the bull horse and mouse. The difference in the total mass of organ depend upon the number not on the volume of the cells. If a cell is to be efficient, the ratio of volume to surface should be within a limited range. An increase in the cell volume is accompanied by much smaller expansion in surface area of the cells. In other words, a large cell has a proportionately smaller surface area and a higher volume: Surface ratio than a small a cell.

4. Cell Number: The number of cell present in organism is varies from a single cell in unicellular organism to many cell in multi cellular organism. The number of cell in multicellular organism usually remains correlated with the size of organism and, therefore, a small sized organism has a less number of cells in comparison to large sized organism. Further, the number of cells in most of multicellular organism is indefinite, but the number of cells may be fixed in some multicellular organism. For example, in rotifers, numbers of nuclei in the various organs are found to be constant in any given species. The phenomenon of cells or nuclear constancy is called Eutely. In one species of rotifers, Martini (1912) always found 183 nuclei in the brain, 39 in stomach and so on.

5. Structure:

(i) Cell Wall: The outermost structure of most plant cells is a dead and rigid layer called cell wall. It is mainly composed of carbohydrates such as cellulose, pectin hemicelluloses and lignin and certain fatty substances like waxes. There is pectin- rich cementing substance between the walls of adjacent cells which is called middle lamella. The cell wall which is formed immediately after the division of cell, constitute the primary cell wall. In certain types of cells such as phloem and xylem, an additional layer is added to the inner surface of primary cell wall called, secondary cell wall and it consist mainly of cellulose, hemicelluloses and lignin. In many plant cells, there are tunnels running through the cell wall called **Plasmodesmata** which allow communication with the other cells in a tissue.

(ii) Plasma Membrane: Every kind of animal cell is bounded by a living, extremely thin and delicate membrane called Plasma lemma, cell membrane or plasma membrane. In plant calls plasma membrane occurs just inner to cell wall, bounding the cytoplasm. The plasma membrane exhibits a tri- laminar structure with a translucent layer sandwiched between two dark layers. The plasma membrane is selectively permeable membrane; its main function is to control selectively the entrance and exit of materials. This allows then cell to maintain a constant internal environment (Homeostasis). Molecules of water, oxygen, carbon-dioxide, glucose etc., are transported across the plasma membrane takes place by various means such as osmosis, diffusion, and active transportation.

(iii) Cytosol: The plasma membrane is followed by the colloidal organic fluid called matrix or cytosol. The cytosol is aqueous part of cytoplasm and nucleoplasm. Cytosol is particularly rich in differentiation cells and many fundamental properties of cells are because of this part of cytoplasm. The cytosol serves to dissolve or suspend the great verity of small molecules concerned with cellular metabolism, e.g., glucose amino acids, nucleotides, vitamins, minerals, oxygen. In all type of cells, cytosol contains the soluble proteins and enzymes which form 20-25 % of the total protein content of the cells. In many types of cells, the cytosol is differentiated into two parts:

(a) Ectoplasm or cell cortex is the peripheral layer of cytosol which is relatively non granular, viscous, clear and rigid.

(b) Endoplasm is the inner portion of cytosol which is granular and less viscous.

Cytoskeleton and Microtrabecular Lattice

The cytosol of cells also contains fibers that help to maintain cell shape and mobility and that probably provide anchoring points for the other cellular structures. These fiber's are called cytoskeleton. At least three general classes of such fibers have been identified.

1-The thickest are the microtubules (20 nm in diameter) which consists primarily of the tubulin protein. The function of microtubules is the transportation of water, ions or small molecules, cytoplasmic streaming (cyclosis), and the formation of fibers or asters of the mitotic or meiotic spindle during cell division.

2-The thinnest are the microfilaments (7mm in diameter) which are solid and are solid and are principally formed of actin protein.

3-The fibers of middle order are called the intermediate filaments (Ifs) having a diameter of 10nm. They have been classified according to their constituent protein such as desmin filaments, keratin filament, Neurofilaments, vimentin and glial filaments.

(i) Cytoplasmic structures: In the cytoplasmic matrix certain non living and living structures remain suspended. The non living structures are called paraplasm and inclusion, while the living structures can be studied under the following headings:

Cytoplasmic Inclusion

The stored food and secretory substances of the cell remain suspended in the cytoplasmic matrix in the form of refractile granules forming the cytoplasmic inclusion. The cytoplasmic inclusion involves oil drops, yolk granules, triacylglyerol and starch grains.

Cytoplasmic Organelles

Besides the separates fibrous systems cytoplasm is coursed by a multitude of internal membranous structures, the organelles. Cytoplasmic organelles performed specialized tasks: Generation of energy in the form of ATP molecules in Mitochondria; formation and storage of

carbohydrates in plastids; protein synthesis in rough endoplasmic reticulum; lipid synthesis in soft endoplasmic reticulum; secretion by Golgi complex; Regulation of all cellular activities by nucleus.

A. Endoplasmic Reticulum (ER): Within the cytoplasm of most animals cells in an extensive network of membrane – limited channels, collectively called Endoplasmic reticulum. The outer surface of rough endoplasmic reticulum has attached ribosomes, where as smooth endoplasmic reticulum don't have. Functions of smooth ER include lipid metabolism (Both catabolism and anabolism) glycogenolysis (degradation of glycogen) and drug detoxification.

On their membranes, rough ER contain certain ribosome's – specific, transmembrane glycoprotein's called ribophorins I & II, to which are attached the ribosome's while engaged in polypeptide synthesis. Rough ER pinches off certain tiny protein- filled vesicles which ultimately get fused to Cis-Golgi. RER also synthesize membrane and glycoprotein's which are co translationally inserted into rough ER membranes. Thus ER is the site of biogenesis of cellular membranes'.

General features

1. The ER is a system of membranous tubules and sacs
2. The primary function of the ER is to act as an internal transport system, allowing molecules to move from one part of the cell to another
3. The quantity of ER inside a cell fluctuates, depending on the cell's activity. Cells with a lot include secretory cells and liver cells
4. The rough ER is studded with 80s ribosomes and is the site of protein synthesis. It is an extension of the outer membrane of the nuclear envelope, so allowing mRNA to be transported swiftly to the 80s ribosomes, where they are translated in protein synthesis
5. The smooth ER is where polypeptides are converted into functional proteins and where proteins are prepared for secretion. It is also the site of lipid and steroid synthesis, and is associated with the Golgi apparatus. Smooth ER has no 80s ribosomes and is also involved in the regulation of calcium levels in muscle cells, and the breakdown of toxins by liver cells
6. Both types of ER transport materials throughout the cell.

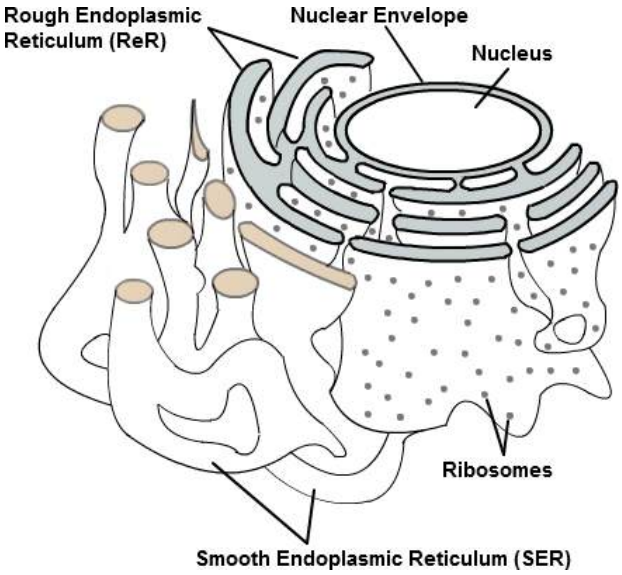


Fig.1.10- Ultra structure of Endoplasmic reticulum

B. Golgi Apparatus: It is cup shaped organelle which is located near the nucleus in many type of cells. Golgi apparatus consists of a set of smooth cisternae (i.e. close fluid –filled flattened membranous sacs or vesicles) which often are stacked together in parallel rows. It is surrounded by spherical membrane bound vesicles which appear to transport proteins to and from it.

Golgi apparatus consist of at least three distinct classes of cisternae: Cis Golgi, Median Golgi and Trans Golgi. Synthesized proteins appear to move in following direction: Rough ER → Cis Golgi→Median Golgi→Trans Golgi→Secretory Vesicles→Cortical Granules.

Plant cells may contain freely distributed subunits of Golgi apparatus, called Dictyosome.

Generally, Golgi apparatus perform the following functions:

1. Packaging of secretory materials.
2. Synthesis of certain polysaccharides and glycolipids.
3. Formation of acrosome of the spermatozoa.

General features

1. The Golgi apparatus is the processing, packaging and secreting organelle of the cell, so it is much more common in glandular cells.
2. The Golgi apparatus is a system of membranes, made of flattened sac-like structures called cisternae.
3. It works closely with the smoother, to modify proteins for export by the cell.

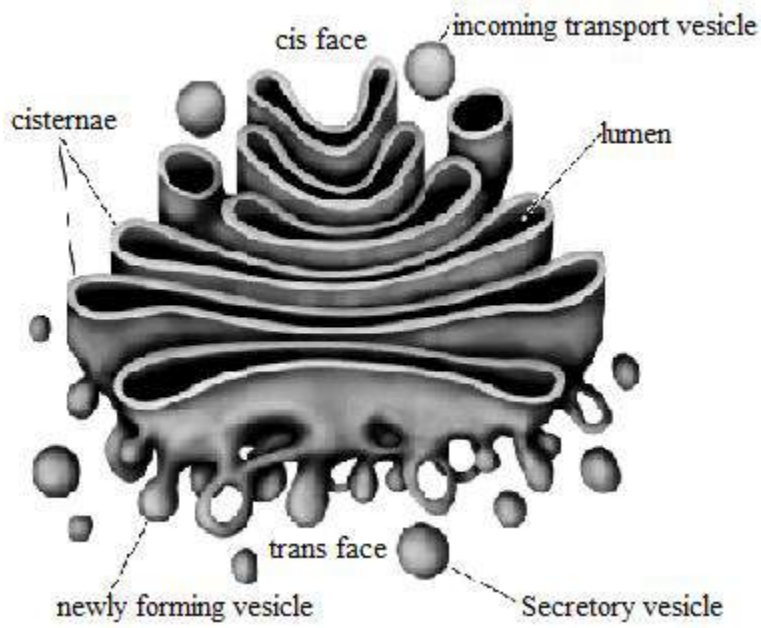


Fig.1.11- Structure of Golgi apparatus

C. Lysosomes: The cytoplasm of animals cells contains many tiny, spherical or irregular shape, membrane bounded vesicles known as lysosomes. They digest the material taken in by endocytosis, part of cells and extra cellular substances. Lysosomes have a high acidic medium (pH 5) and its acidification depends upon ATP dependent proton pumps which are present in lysosomes membrane. Lysosomes exhibits great polymorphism i.e. there is following four types of lysosomes: primary lysosomes (storage granules) secondary lysosomes (digestive vacuoles); residual bodies. The lysosomes of plant cells are membrane bounded storage granules containing hydrolytic digestive enzymes' large vacuoles of parenchymatous cells of corn seedlings, proteins or aleurone bodies and other seeds.

General features

1. Lysosomes are small spherical organelles that enclose hydrolytic enzymes within a single membrane
2. Lysosomes are the site of protein digestion – thus allowing enzymes to be re-cycled when they are no longer required. They are also the site of food digestion in the cell, and of bacterial digestion in phagocytes
3. Lysosomes are formed from pieces of the Golgi apparatus that break off
4. Lysosomes are common in the cells of Animals, Protoctista and even Fungi, but rare in plants.

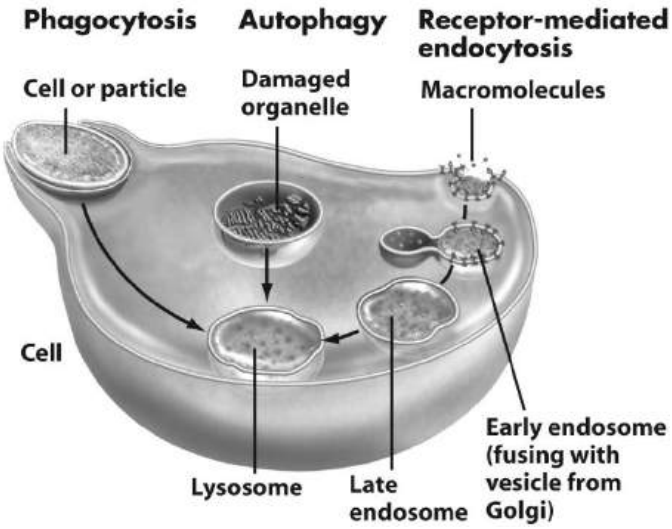


Fig.1.12- Type of formations of Lysosomes

D. Cytoplasmic Vacuoles: The cytoplasm of many plant and some animals' cell contain numerous small or large-sized hollow, liquid-filled structures, the vacuoles .The vacuoles of animals are bounded by a lipoproteinous membrane and their function is the storage, transmission of the materials and the maintenance of internal pressure of cell. The vacuoles of plants are bounded by a single, semi permeable membrane known as Tonoplast.

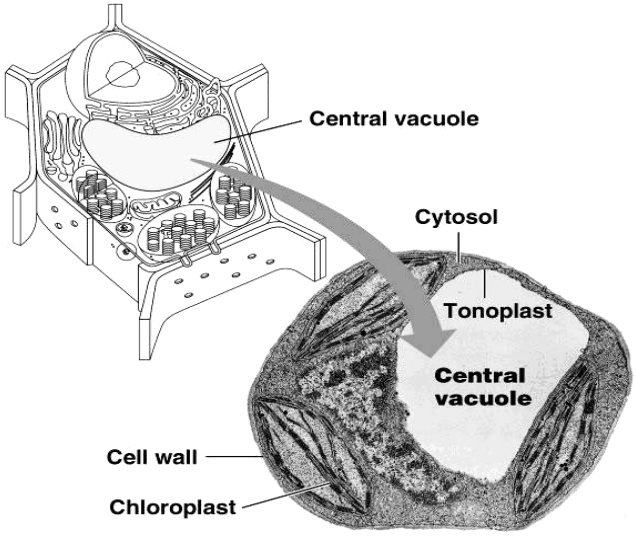


Fig.1.13- Structure of Vacuole

E. Peroxisomes: These are tiny circular membrane bound organelles containing a crystal core of enzymes. These enzymes are required by peroxisomes in detoxification activity. i.e., in the metabolism or production and decomposition, of hydrogen peroxide or H_2O_2 molecules which

are produced during neutralization of certain superoxides- the end products of mitochondrial or cytosolic reactions. In green leaves of plants peroxisomes carry out the process of photorespiration.

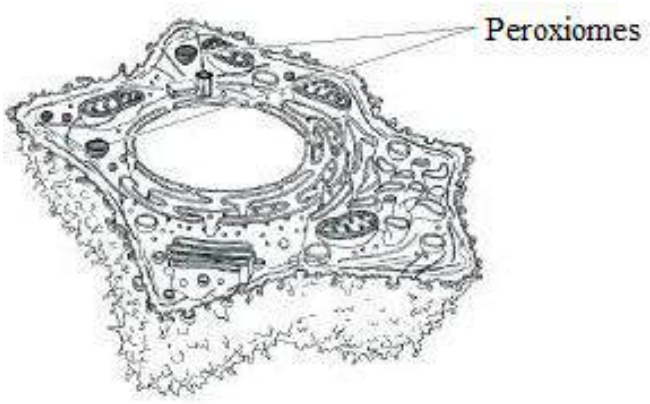


Fig.1.14- Structure of cell showing Peroxisomes

F. Glyoxysomes: These organelles develop in a germinating plant seed to utilize stored fat of the seed. Glyoxysomes consist of an amorphous protein matrix surrounded by a limited membrane. The membrane of Glyoxysomes originates from the ER and their enzymes are synthesized in the free ribosomes in the cytosol, Enzymes of Glyoxysomes are used to transform the fat stores of the seed into carbohydrates by the way Glyoxylate cycle.

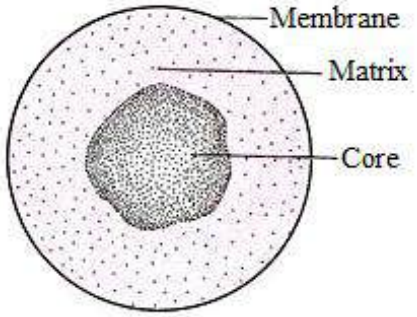


Fig.1.15- Structure of Glyoxysomes

G. Mitochondria: Mitochondria are oxygen consuming ribbon shaped cellular organelles of immense importance. Each Mitochondria is bounded by two unit membranes, the outer Mitochondria membrane resembles more with the plasma membrane in structure and chemical composition. It contains Porins, proteins that render the membrane permeable to molecule having molecular weight as high as 10,000. Inner Mitochondrial membrane is rich in many enzymes, coenzymes another component of electron transport chain. It also contain proton pumps and many Permease proteins for the transport of various molecules so such as citrates, ADP, Phosphate & ATP.

Inner Mitochondria membrane gives out finger-like out growths (Cristae) towards the lumen of Mitochondrion. Mitochondrial matrix which is the liquid (colloidal) area in circled by the inner membrane contains the soluble enzyme of Krebs cycle which completely oxidized the acetyl-CoA to produce CO_2, H_2O, hydrogen ions. Hydrogen ions reduce the molecules of NAD and FAD, both of which can pass on hydrogen ions to reparatory or electron transport chain where oxidative phosphorylation takes place to generate energy rich molecules. Mitochondria act as the **"Power House of Cells"**. Since Mitochondria can synthesize 10% of their proteins in their own protein –synthetic machinery, they are considered as semi autonomous organelles.

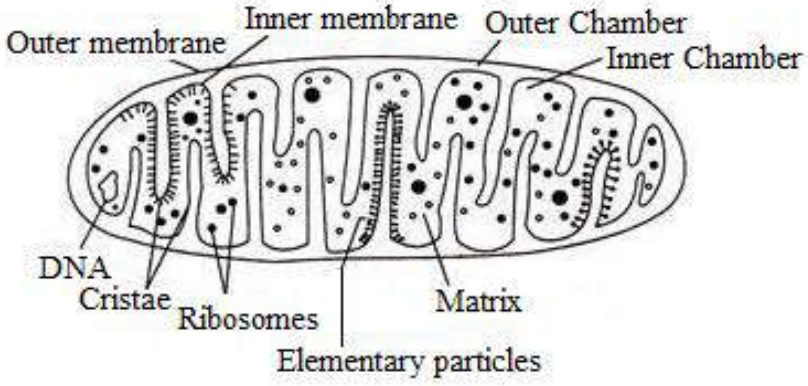


Fig.1.16 - Structure of Mitochondria

General features

1. Mitochondria are found scattered throughout the cytosol, and are relatively large organelles (second only to the nucleus and chloroplasts).
2. Mitochondria are the sites of aerobic respiration, in which energy from organic compounds is transferred to ATP. For this reason they are sometimes referred to as the 'powerhouse' of the cell.
3. ATP is the molecule that most cells use as their main energy 'currency'.
4. Mitochondria are more numerous in cells that have a high energy requirement - our muscle cells contain a large number of mitochondria, as do liver, heart and sperm cells.
5. Mitochondria are surrounded by two membranes, indicating that they were once free-living organisms that have become mutualistic and then a part of almost every eukaryotic cell (not RBC's and xylem vessels).

a) The smooth outer membrane serves as a boundary between the mitochondria and the cytosol.
b) The inner membrane has many long folds, known as Cristae, which greatly increase the surface area of the inner membrane, providing more space for ATP synthesis to occur.

6. Mitochondria have their own DNA, and new mitochondria arise only when existing ones grow and divide. They are thus "*semi-autonomous organelles*".

H. Plastids: Plastids are double membrane organelles which are found in plant cells only. They are usually spherical or discoidal in shape and their average size is 4 to 6 µm. A plastid shows two distinct regions-grana and stroma. Grana are stacks of membrane-bound, flattened, discoid

sacs containing chlorophyll molecules. These molecules are responsible for the production of food by the process of photosynthesis.

They are, therefore, called "*Kitchen of the cell*". They are the main functional units of the chloroplast. The homogenous matrix in which Grana are embedded is known as Stroma. A variety of photosynthetic enzymes and starch grains are present in the Stroma. The Stroma is colourless, whereas the Grana contain the pigments. Plastids are living and multiply by division of the pre-existing plastids called Proplastids.

Plastids are of three types:

1. **Leucoplasts** are colorless plastids. They store the food of the plant body in the form of starch, protein and lipids. They occur most commonly in the storage cells of roots and underground stems.
2. **Chloroplasts** are green plastids because of the presence of chlorophyll. Chloroplasts occur abundantly in green leaves, and also to some extent in green parts of the shoot.
3. **Chromoplasts** are variously colored plastids. They are mostly present in flowers and fruits.

One form of plastid can change into another. For example, leucoplasts can change into chloroplasts when the former are exposed to light for a long period.

Functions

1. By trapping solar energy, green plastids manufacture food through photosynthesis.
2. Chromoplasts provide colored to various flowering parts.
3. Leucoplasts help in storage of protein, starch and oil.

I. Ribosomes: They are dense, rounded, granular and smallest known electron microscopic Ribonucleoprotein (RNP) particles attached either on RER or floating freely in the cytoplasm. These are the site of protein synthesis. They may exist either in free state in cytosol or attached to RER. Ribosomes in eukaryotes have sedimentation coefficient of about 80S and are composed of 2 subunits namely 40S and 60S, and prokaryotes have 70S and are composed of 2 subunits namely 30S and 50S.

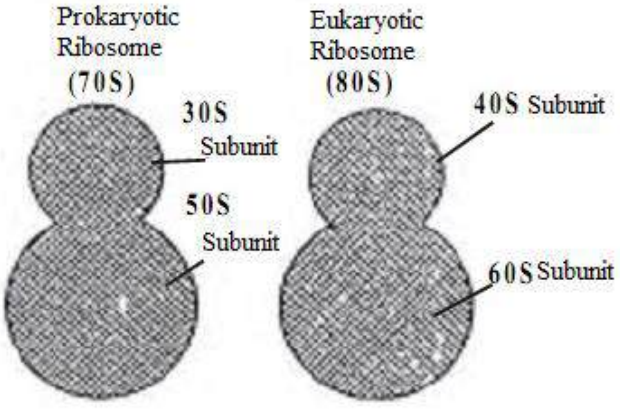


Fig.1.17- Ribosomes, Prokaryotic and Eukaryotic

General features

1. Unlike most other organelles, ribosomes are not surrounded by a membrane.
2. Ribosomes are the site of protein synthesis in a cell.
3. They are the most common organelles in almost all cells.
4. Some are free in the cytoplasm (Prokaryotes) others line the membranes of rough endoplasmic reticulum (rough ER).
5. They exist in two sizes: 70S are found in all Prokaryotes, chloroplasts and mitochondria, suggesting that they have evolved from ancestral Prokaryotic organisms. They are free-floating. While 80S found in all eukaryotic cells – attached to the rough ER (they are rather larger). Here "S" stand as a *Svedberg coefficient* or unit.
6. Groups of 80s ribosomes, working together, are known as a Polysome.

J. Cytoskeletal Structures: Many eukaryotes have long slender motile cytoplasmic projections, called flagella, or similar structures called cilia. Flagella and cilia are sometimes referred to as undulipodia, and are variously involved in movement, feeding, and sensation. They are composed mainly of tubulin. These are entirely distinct from prokaryotic flagella. They are supported by a bundle of microtubules arising from a basal body, also called a Kinetosome or Centrioles, characteristically arranged as nine doublets surrounding two singlets. Flagella also may have hairs, or Mastigoneme, and scales connecting membranes and internal rods. Their interior is continuous with the cell's cytoplasm.

Microfilament structures composed of actin and actin binding proteins, e.g., α-actinin, fimbrin, filamin are present in submembranous cortical layers and bundles, as well. Motor proteins of microtubules, e.g., dynein or kinesin and actin, e.g., myosins provide dynamic character of the network.

Centrioles are often present even in cells and groups that do not have flagella, but conifers and flowering plants have neither. They generally occur in groups of one or two, called kinetids that give rise to various microtubular roots. These form a primary component of the cytoskeletal structure, and are often assembled over the course of several cell divisions, with one flagellum retained from the parent and the other derived from it. Centrioles may also be associated in the formation of a spindle during nuclear division.

The significance of cytoskeletal structures is underlined in the determination of shape of the cells, as well as their being essential components of migratory responses like chemo-taxis and Chemokinesis. Some protists have various other microtubule-supported organelles. These include the radiolaria and heliozoa, which produce axopodia used in flotation or to capture prey, and the haptophytes, which have a peculiar flagellum-like organelle called the haptonema.

General features

1. Just as your body depends on your skeleton to maintain its shape and size, so a cell needs structures to maintain its shape and size.

2. In animal cells, which have no cell wall, an internal framework called the cytoskeleton maintains the shape of the cell, and helps the cell to move.
3. The cytoskeleton consists of two structures: a) microfilaments (contractile). They are made of actin, and are common in motile cells. b) microtubules (rigid, hollow tubes – made of tubulin).
4. Microtubules have three functions:
   (a) To maintain the shape of the cell.
   (b) To serve as tracks for organelles to move along within the cell.
   (c) They form the Centrioles.

K. Nucleus: The nucleus is a membrane-enclosed organelle found in eukaryotic cells. Eukaryotes usually have a single nucleus, but a few cell types, such as mammalian red blood cells, have no nuclei, and a few others have many.

Cell nuclei contain most of the cell's genetic material, organized as multiple long linear DNA molecules in complex with a large variety of proteins, such as histones, to form chromosomes. The genes within these chromosomes are the cell's nuclear genome and are structured in such a way to promote cell function. The nucleus maintains the integrity of genes and controls the activities of the cell by regulating gene expression—the nucleus is, therefore, the control center of the cell. The main structures making up the nucleus are the nuclear envelope, a double membrane that encloses the entire organelle and isolates its contents from the cellular cytoplasm, and the nuclear matrix (which includes the nuclear lamina), a network within the nucleus that adds mechanical support, much like the cytoskeleton, which supports the cell as a whole.

Because the nuclear membrane is impermeable to large molecules, nuclear pores are required that regulate nuclear transport of molecules across the envelope. The pores cross both nuclear membranes, providing a channel through which larger molecules must be actively transported by carrier proteins while allowing free movement of small molecules and ions. Movement of large molecules such as proteins and RNA through the pores is required for both gene expression and the maintenance of chromosomes. Although the interior of the nucleus does not contain any membrane-bound sub compartments, its contents are not uniform, and a number of sub-nuclear bodies exist, made up of unique proteins, RNA molecules, and particular parts of the chromosomes. The best-known of these is the nucleolus, which is mainly involved in the assembly of ribosomes. After being produced in the nucleolus, ribosomes are exported to the cytoplasm where they translate mRNA.

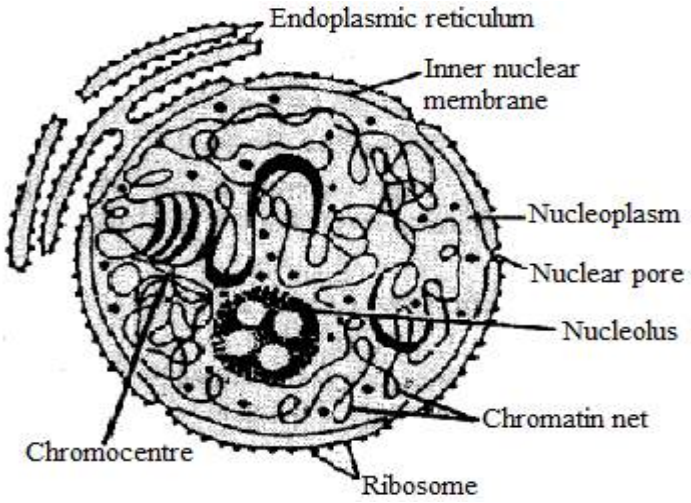


Fig.1.18- Eukaryotic Nucleus

General features

1. The Nucleus is normally the largest organelle within a Eukaryotic cell.
2. Prokaryotes have no nucleus, having a nuclear body instead. This has no membrane and a loop of DNA (and no chromatin proteins).
3. The nucleus contains the cell's chromosomes (human 46, fruit fly 6, fern 1260) which are normally uncoiled to form a chromatin network, which contain both linear DNA and proteins, known as histones. These proteins coil up (dehydrate) at the start of nuclear division, when the chromosomes first become visible.
4. Whilst most cells have a single nucleus some cells (macrophages, phloem companion cells) have more than one.
5. Fungi have many nuclei in their cytoplasm, they are coenocytic (common cytoplasm throughout).
6. The nucleus is surrounded by a double membrane called the nuclear envelope.
7. The nuclear envelope has many nuclear pores through which mRNA, and proteins can pass.
8. Most nuclei contain at least one nucleolus (plural, nucleoli). The nucleoli are, where ribosomes are synthesized. Ribosomes translate mRNA into proteins.
9. When a nucleus prepares to divide, the nucleolus disappears.

The following table featuring different organelles of cell with their location, description and functions.

ORGANELLE	LOCATION	DESCRIPTION	FUNCTION
Cell wall	Plant, not animal	Outer layer rigid, strong, stiff made of cellulose	Support (grow tall) protection allows H2O, O2, CO2 to pass into and out of cell

Cell membrane	Both plant/animal	Plant - inside cell wall animal - outer layer; cholesterol selectively permeable	Support protection controls movement of materials in/out of cell barrier between cell and its environment maintains homeostasis
Nucleus	Both plant/animal	Large, oval	Controls cell activities
Nuclear membrane	Both plant/animal	Surrounds nucleus selectively permeable	Controls movement of materials in/out of nucleus
Cytoplasm	Both plant/animal	Clear, thick, jellylike material and organelles found inside cell membrane	Supports /protects cell organelles
Endoplasmic reticulum (E.R.)	Both plant/animal	Network of tubes or membranes	Carries materials through cell
Ribosome	Both plant/animal	Small bodies free or attached to E.R.	Produces proteins
Mitochondrion	Both plant/animal	Bean-shaped with inner membranes	Breaks down sugar molecules into energy
Vacuole	Plant - few/large animal - small	Fluid-filled sacs	Store food, water, waste (plants need to store large amounts of food)
Lysosome	Plant - uncommon animal - common	Small, round, with a membrane	Breaks down larger food molecules into smaller molecules digests old cell parts
Chloroplast	Plant, not animal	Green, oval usually containing chlorophyll (green pigment)	Uses energy from sun to make food for the plant (photosynthesis)

Differences among Eukaryotic Cells

There are many different types of eukaryotic cells, though animals and plants are the most familiar eukaryotes, and thus provide an excellent starting point for understanding eukaryotic structure. Fungi and many protists have some substantial differences, however.

Animal Cell

All animals consist of eukaryotic cells. Animal cells are distinct from those of other eukaryotes, most notably plants, as they lack cell walls and chloroplasts and have smaller vacuoles. Due to the lack of a cell wall, animal cells can adopt a variety of shapes. A phagocytic cell can even

engulf other structures. There are many other types of cell. For instance, there are approximately 210 distinct cell types in the adult human body.

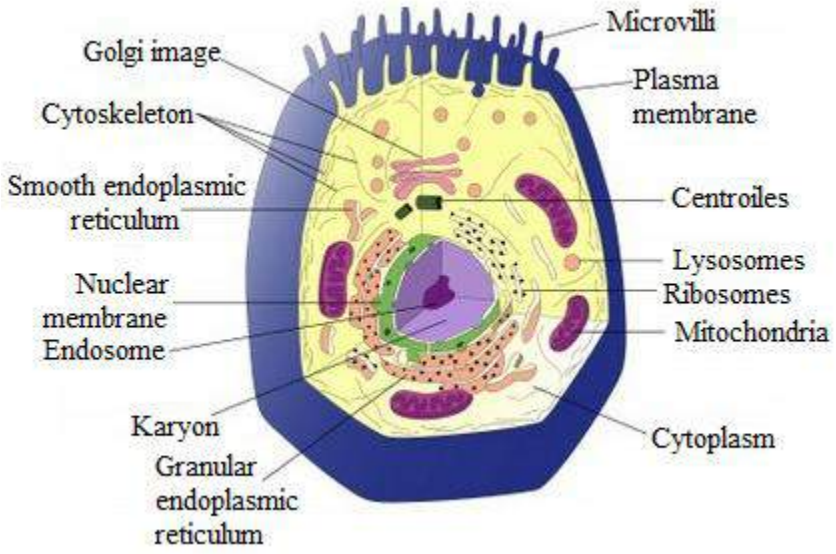


Fig.1.19 Structure of a typical animal cell

Plant Cell

1. Most of the organelles and other parts of the cell are common to all Eukaryotic cells. Cells from different organisms have an even greater difference in structure
2. Plant cells have three additional structures not found in animal cells:
   (a) Cellulose cell walls
   (b) Chloroplasts (and other plastids)
   (c) A Central Vacuole

Cellulose cell wall

1. One of the most important features of all plants is presence of a cellulose cell wall.
2. Fungi such as Mushrooms and Yeast also have cell walls, but these are made of chitin.
3. The cell wall is freely permeable (porous), and so has no direct effect on the movement of molecules into or out of the cell.
4. The rigidity of their cell walls helps both to support and protect the plant.
5. Plant cell walls are of two types:

(a) Primary (cellulose) cell wall - While a plant cell is being formed, a middle lamella made of pectin, is formed and the cellulose cell wall develops between the middle lamella and the cell membrane. As the cell expands in length, more cellulose is added, enlarging the cell wall. When the cell reaches full size, a secondary cell wall may form.

(b) Secondary (lignified) cell wall - The secondary cell wall is formed only in woody tissue (mainly xylem). The secondary cell wall is stronger and waterproof and once a secondary cell wall forms, a cell can grow no more – it is dead.

Vacuoles

1. The most prominent structure in plant cells is the large vacuole.
2. The vacuole is a large membrane-bound sac that fills up much of most plant cells.
3. The vacuole serves as a storage area, and may contain stored organic molecules as well as inorganic ions.
4. The vacuole is also used to store waste. Since plants have no kidney, they convert waste to an insoluble form and then store it in their vacuole - until autumn.
5. The vacuoles of some plants contain poisons (e.g. tannins) that discourage animals from eating their tissues.
6. Whilst the cells of other organisms may also contain vacuoles, they are much smaller and are usually involved in food digestion.

Chloroplasts (and other plastids)

1. A characteristic feature of plant cells is the presence of plastids that make or store food.
2. The most common of these are chloroplasts – the site of photosynthesis.
3. Each chloroplast encloses a system of flattened, membranous sacs called thylakoids, which contain chlorophyll.
4. The thylakoids are arranged in stacks called Grana.
5. The space between the Grana is filled with cytoplasm-like Stroma.
6. Chloroplasts contain cp DNA and 70S ribosomes and are semi-autonomous organelles.
7. Other plastids store reddish-orange pigments that colour petals, fruits, and some leaves.

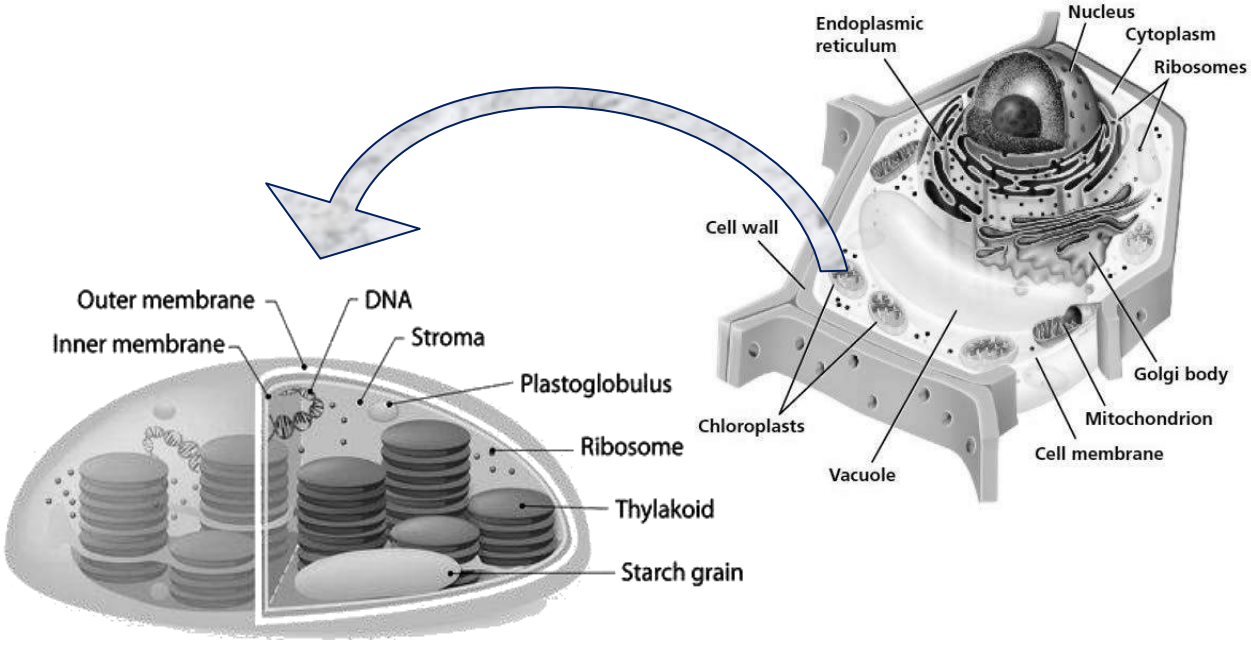


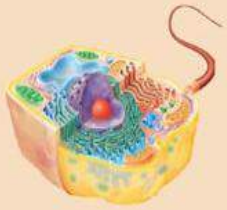
Fig.1.20- Structure of a Chloroplast and a typical plant cell

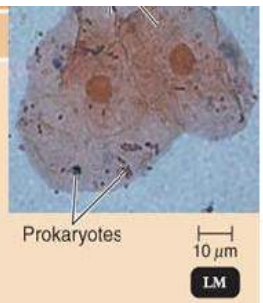
Plant cells are quite different from the cells of the other eukaryotic organisms. Their distinctive features are:

1. A large central vacuole (enclosed by a membrane, the Tonoplast), which maintains the cell's turgor and controls movement of molecules between the cytosol and sap.
2. A primary cell wall containing cellulose, hemicellulose and pectin, deposited by the protoplast on the outside of the cell membrane; this contrasts with the cell walls of fungi, which contain chitin, and the cell envelopes of prokaryotes, in which peptidoglycans are the main structural molecules.
3. The plasmodesmata, linking pores in the cell wall that allow each plant cell to communicate with other adjacent cells; this is different from the functionally analogous system of gap junctions between animal cells.
4. Plastids, especially chloroplasts that contain chlorophyll, the pigment that gives plants their green color and allows them to perform photosynthesis.
5. Bryophytes and seedless vascular plants lack flagella and centrioles except in the sperm cells. Sperm of cycads and Ginkgo are large, complex cells that swim with hundreds to thousands of flagella.
6. Conifers (Pinophyta) and flowering plants (Angiospermae) lack the flagella and centrioles that are present in animal cells.

1.7 SUMMARY

1-All living organisms are made up of one or numerous coordinated compartments called cells.
2- Robert Hooke (1665) was first to discover and use the term cell.
3- Cell Theory was proposed by M.J Schleiden and T. Schwann in 1838.
4- PPLO is the smallest cell.
5- Viruses do not have cellular structures.
6-Nucleolus is absent in prokaryotic cells.
7-Complexcity of cell increases from prokaryotes to eukaryotes.
8-Nuclear material of bacteria is also known as Bacterial chromosome.
9-Difference between prokaryotic and eukaryotic cell:

Characteristic	Prokaryotic	Eukaryotic
		
Size of Cell	Typically 0.2–2.0 μm in diameter	Typically 10–100 μm in diameter
Nucleus	No nuclear membrane or nucleoli	True nucleus, consisting of nuclear membrane and nucleoli
Membrane-Enclosed Organelles	Absent	Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria, and chloroplasts
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack a cell wall
Cell Wall	Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple (includes cellulose and chitin)
Plasma Membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors
Cytoplasm	No cytoskeleton or cytoplasmic streaming	Cytoskeleton; cytoplasmic streaming
Ribosomes	Smaller size (70S)	Larger size (80S); smaller size (70S) in organelles
Chromosome (DNA)	Usually single circular chromosome; typically lacks histones	Multiple linear chromosomes with histones
Cell Division	Binary fission	Involves mitosis
Sexual Recombination	None; transfer of DNA only	Involves meiosis



10. Difference between animal and plant cell:

S.No	Animal cell	Plant cell
1.	Animal cells are generally small in size.	Plant cells are larger than animal cells.
2.	Cell wall is absent.	The plasma membrane of plant cells is surrounded by a rigid cell wall of cellulose.
3.	Except the protozoan *Euglena* no animal cell possesses plastids.	Plastids are present.
4.	Vacuoles in animal cells are many and small.	Most mature plant cells have a large central sap vacuole.
5.	Animal cells have a single highly complex Golgi	Plant cells have many simpler units of and prominent Golgi apparatus. apparatus, called dictyosomes.
6.	Animal cells have centrosome and centrioles.	Plant cells lack centrosome and centrioles.

1.8 GLOSSARY

Aleurone: Protein stored as granules in the cells of plant seeds.
Autotroph: Any organism capable of self-nourishment by using inorganic materials as a source of nutrients and using photosynthesis or chemosynthesis as a source of energy, as most plants and certain bacteria and protists.
Cell Biology: Sub-discipline of biology that focuses on the study of the basic unit of life, the cell.
Cell theory: All organisms consist of one or more cells, the cell is the smallest unit of organization still displaying the properties of life, and life's continuity arises directly from growth and division of single cells.
Cell wall: Of many cells (not animal cells), a semi-rigid but permeable structure that surrounds the plasma membrane; helps a cell retain its shape and resist rupturing.
Cell: Smallest unit that still displays the properties of life; it has the capacity to survive and reproduce on its own.
Central vacuole: In many mature, living plant cells, an organelle that stores amino acids, sugars, and some wastes; when it enlarges during growth, it forces the cell to enlarge and increase its surface area.
Chemokinesis: Increased activity of an organism due to a chemical substance.
Chloroplast: A plastid containing chlorophyll.

Chromoplast: A plastid containing coloring matter other than chlorophyll.

Chromosome: A long, stringy aggregate of genes that carries heredity information (DNA) and is formed from condensed chromatin.

Cilium: A cilium (plural cilia) is an organelle found in eukaryotic cells. It is a hair-like extensions from the cell membrane that allow some cells to "*sweep*" materials across their surfaces.

Cisternae: A cisterna (plural cisternae) is a flattened membrane disk of the endoplasmic reticulum and Golgi apparatus.

Colicin: Any bacteriocin produced by certain strains of Escherichia coli and having a lethal effect on strains other than the producing strain.

Cristae: Each of the partial partitions in a mitochondrion formed by infolding of the inner membrane.

Cytoplasm: Consists of all of the contents outside of the nucleus and enclosed within the cell membrane of a cell.

Cytoskeleton: A network of fibers throughout the cell's cytoplasm that helps the cell maintain its shape and gives support to the cell.

Cytosol: Semi-fluid component of a cell's cytoplasm.

Detoxification: The metabolic process by which toxins are changed into less toxic or more readily excretable substances.

Dictyosome: The set of flattened membranes in a Golgi body, resembling a stack of plates.

Ectoplasm: The outer portion of the cytoplasm of a cell.

Endocytosis: The taking in of matter by a living cell by invagination of its membrane to form a vacuole.

Endoplasm: The more fluid, granular inner layer of the cytoplasm in amoeboid cells.

Endoplasmic reticulum: The endoplasmic reticulum is a type of organelle in eukaryotic cells that forms an interconnected network of flattened, membrane-enclosed sacs or tube-like structures known as cisternae.

Eukaryote: Any organism whose cells have a cell nucleus and other organelles enclosed within membranes. Eukaryotes belong to the domain Eukaryota, and can be single-celled or multicellular.

Flagella: A long, lash-like appendage serving as an organ of locomotion in protozoa, sperm cells, etc.

Glyoxysomes: Are specialized peroxisomes found in plants (particularly in the fat storage tissues of germinating seeds) and also in filamentous fungi.

Golgi Bodies: A complex of vesicles and folded membranes within the cytoplasm of most eukaryotic cells, involved in secretion and intracellular transport.

Granum: One of the structural units of a chloroplast in vascular plants, consisting of layers of thylakoids.

Heredity: It is the process by which features and characteristics are passed on from parents to their children before the children are born.

Heterotrophic: Capable of utilizing only organic materials as a source of food.

Histone: Any of a group of five small basic proteins, occurring in the nucleus of eukaryotic cells, that organize DNA strands into nucleosomes by forming molecular complexes around which the DNA winds.

Homeostasis: It refers to the ability of the body to maintain a stable internal environment despite changes in external conditions.

Kinetosome: A structure in some flagellate protozoans which forms the base of the flagellum, consisting of a circular arrangement of microtubules.

Leucoplast: A colourless organelle found in plant cells, used for the storage of starch or oil.

Lysosome: An organelle in the cytoplasm of eukaryotic cells containing degradative enzymes enclosed in a membrane.

Mastigoneme: They are lateral "hairs" found covering the flagella of heterokont and cryptophyte algae.

Mycoplasma: It is a genus of bacteria that lack a cell wall around their cell membrane. Without a cell wall, they are unaffected by many common antibiotics such as penicillin or other β-lactam antibiotics that target cell wall synthesis.

Nucleoid: The nucleoid (meaning nucleus-like) is an irregularly shaped region within the cell of a prokaryote that contains all or most of the genetic material, called genophore.

Nucleus: A dense organelle present in most eukaryotic cells, typically a single rounded structure bounded by a double membrane, containing the genetic material.

Parasite: An organism that lives on or in an organism of another species, known as the host, from the body of which it obtains nutriment.

Parenchyma: The fundamental tissue of plants, composed of thin-walled cells able to divide.

Pectin: Pectin is a structural hetero-polysaccharide contained in the primary cell walls of terrestrial plants.

Phloem: The part of a vascular bundle , in vascular plants, phloem is the living tissue that transports the soluble organic compounds made during photosynthesis, in particular the sugar sucrose, to parts of the plant where needed.

Photorespiration: The oxidation of carbohydrates in many higher plants in which they get oxygen from light and then release carbon dioxide, somewhat different from photosynthesis.

Plasmid: A plasmid is a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA. Plasmids naturally exist in bacterial cells, and they also occur in some eukaryotes.

Plasmodesma: Any of many minute strands of cytoplasm that extend through plant cell walls and connect adjoining cells.

Plastid: The plastid is a major double-membrane organelle found in the cells of plants, algae, and some other eukaryotic organisms. Plastids are the site of manufacture and storage of important chemical compounds used by the cell.

Polymorphism: The existence of an organism in several form or color varieties.

Porins: They are beta barrel proteins that cross a cellular membrane and act as a pore, through which molecules can diffuse.

Prokaryote: A prokaryote is a unicellular organism that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle.

Proteins: Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues. It performs a vast array of functions within organisms, including catalyzing metabolic reactions, DNA replication, responding to stimuli, and transporting molecules from one location to another.

Ribosome: A tiny, somewhat mitten-shaped organelle occurring in great numbers in the cell cytoplasm either freely, in small clusters, or attached to the outer surfaces of endoplasmic reticulum, and functioning as the site of protein manufacture.

Rotifers: The rotifers (wheel animals) make up a phylum of microscopic and near-microscopic pseudo-coelomate animals.

Saprophytes: Any organism that lives on dead organic matter, as certain fungi and bacteria.

Spermatozoa: Spermatozoa (sperm) are the male sex cells that carry a man's genetic material.

Stroma: The matrix of a chloroplast, containing various molecules and ions. The supporting framework or matrix of a cell.

Superoxide: A compound containing the univalent ion O_2^-.

Thylakoid: A flattened sac or vesicle lined with a pigmented membrane that is the site of photosynthesis, in plants and algae occurring in interconnected stacks constituting a granum of the chloroplast, and in other photosynthesizing organisms occurring either singly or as part of the cell membrane or other structure.

Tonoplast: A membrane separating a vacuole from the surrounding cytoplasm in a plant cell.

Unicellular: Having or consisting of a single cell.

Vacuole: A membrane-bound cavity within a cell, often containing a watery liquid or secretion.

Vesicle: It is a small structure within a cell, or extracellular, consisting of fluid enclosed by a lipid bilayer. Vesicles form naturally during the processes of secretion (exocytosis), uptake (endocytosis) and transport of materials within the cytoplasm.

Xylem: A compound tissue in vascular plants that helps provide support and that conducts water and nutrients upward from the roots, consisting of tracheids, vessels, parenchyma cells, and woody fibers.

1.9 SELF ASSESSMENT QUESTIONS

1.9.1 Very short answer type:

1. What is the function of chloroplast in plant cell?
2. Who gave the term protoplast?
3. An undefined fibrillar nucleus is seen in which type of cell?
4. Which organelle is known as the power house of the cell?

5. Which type of cell possesses centrioles?
6. The thread-like structure present in the nucleus?
7. The unit of measurement used for expressing dimension (size) of cell?
8. The jelly-like fluid substance present in the cell called?
9. A suitable term used for various components of cell is?
10. Who proposed the fluid mosaic model of plasma membrane?

1.9.2 Objective type Questions:

1. Cells originate from-
   (a) Bacterial fermentation (b) Pre existing cells
   (c) Abiotic materials (d) Regeneration of old cells

2. The smallest size of cell that can be seen with an unaided eye is-
   (a) 1 micron (b) 10 micron
   (c) 100 micron (d) 1000 micron

3. The term cell was first used by-
   (a) Robert Hooke (b) Harvey
   (c) Hopkins (d) Fleming

4. What Robert Hooke had discovered in the thin section of cork as a cell was really a-
   (a) Cellulose (b) Nuclei
   (c) Protoplasm (d) Cell walls

5. Which one of these is agent of flow of extrinsic information to cell-
   (a) DNA (b) RNA
   (c) Hormone (d) Protein

6. The prokaryotic cells are characterized by
   (a) A distinct nuclear membrane (b) Distinct chromosomes
   (c) Absence of chromatin material (d) Absence of nuclear membrane

7. Animal and plant cell differ in their
   (a) Movement (b) Growth
   (c) Nutrition (d) Respiration

8. Protoplasm is a
   (a) Liquid (b)Solid
   (c) Colloidal solution (d) Crystalloid colloidal

9. Prokaryotes are classified as belonging to two different domains. What are the domains?
   (a) Bacteria and Eukarya (b) Archaea and Monera

(c) Eukarya and Monera (d) Bacteria and Archaea

10. Which of the following is a common feature of all cells?
    (a)Intracellular organelles (b) A lipid cell membrane
    (c) A nuclear membrane (d) An RNA genome

11. Which Eukaryotic cellular organelles are believed to have evolved from symbiotic bacteria?
    (a) ER and Golgi apparatus (b) Lysosomes
    (c) Mitochondria and Chloroplast (d) Peroxisomes

12. What is the main difference between prokaryotes and eukaryotes?
    (a) Prokaryotes can't undergo cell division (b) Prokaryotes have no internal membranes
    (c) Prokaryotes have no DNA (d) Prokaryotes have no cytosol

13. A cell has circular DNA, is small and simple and no nucleus or membrane bound organelles. What type of cell is this?
    (a) Plant (b) Eukaryotic
    (c) Animal (d) Prokaryotic

14. Prokaryotic cells have a specialized material with them called as?
    (a) Peptidoglycan (b) Pectin
    (c) Peptidoaminase (d) Peptidoglucose

15. Organism which lack mitosis division and use binary fission method for cell division are known as?
    (a) Eukaryotes (b) Prokaryotes
    (c) Yeast (d) Fungi

16. The nucleus was first described by
    (a) Robert Brown (b) Nageli
    (c) Weismann (d) Robert Hook

17. Select one which is not true for Ribosome
    (a) Made up of two subunits (b) Form polysomes
    (c) May attached to mRNA (d) Have role in protein synthesis

18. Which one of these is not a eukaryote?
    (a) *Euglena* (b) *Anabaena*
    (c) *Spirogyra* (d) *Agaricus*

19. Which of the following dyes is best suited for staining chromosomes?
    (a) Basic Fuchsin (b) Safranin
    (c) Methylene blue (d) Carmine

20. What is a Tonoplast?
    (a) Outer membrane of mitochondria
    (b) Inner membrane of chloroplast
    (c) Membrane boundary of the vacuole of plant cells
    (d) Cell membrane of a plant cell

21. Which of the following is not true of a eukaryotic cell?
    (a) It has 80S type of ribosome present in the mitochondria
    (b) It has 80S type of ribosome present in the cytoplasm
    (c) Mitochondria contain circular DNA
    (d) Membrane bound organelles are present

22. Plastid differs from mitochondria on the basis of one of the following features. Mark the right answer.
    (a) Presence of two layers of membrane
    (b) Presence of ribosome
    (c) Presence of chlorophyll
    (d) Presence of DNA

23. Which of the following is not a function of cytoskeleton in a cell?
    (a) Intracellular transport
    (b) Maintenance of cell shape and structure
    (c) Support of the organelle
    (d) Cell motility

24. The stain used to visualize mitochondria is
    (a) Fast green (b) Safranin
    (c) Acetocarmine (d) Janus green

25. Which of the following statements is not true for plasma membrane?
    (a) It is present in both plant and animal cell
    (b) Lipid is present as a bilayer in it
    (c) Proteins are present integrated as well as loosely associated with the lipid bilayer
    (d) Carbohydrate is never found in it

1.9.3 Fill up the blanks:

1. Living cells can be studied by _____
2. The bacteria that has smallest cell is _____
3. Eukaryotic cells have true _____
4. A plant cell consists of _____cell wall.
5. Chromatin with histones is absent in _____

6. _____ is the process in which ingestion of material by the cell is done through plasma membrane.
7. _____ is an organelle which serves as a primary packaging area for molecules that will be distributed throughout the cell.
8. The jelly-like substance present inside the cell is known as_____
9. _____ is the outermost boundary of cell.
10. _____ discovered cell in the year 1665.

1.9.1Answers Key: 1.- Photosynthesis, 2.- Purkinje 3.- Prokaryotes 4.- Mitochondria 5.- Animal cell. 6- Chromosomes, 7-Micrometer, 8-Cytoplasm, 9- Cell organelles 10. Singer and Nicolson

1.9.2 Answer key: 1- (b), 2- (c), 3-(a), 4-(d), 5-(c), 6- (d), 7-(c), 8-(c), 9-(d), 10-(b), 11-(c), 12-(b), 13-(d), 14-(a), 15-(b), 16-(d), 17-(d), 18-(b), 19-(d), 20-(c), 21-(a), 22-(c), 23-(a), 24-(d), 25-(d)

1.9.3 Answer key: 1-Phase contrast microscope, 2-PPLO, 3-Nucleus, 4-cellulose, 5-Prokaryotes, 6- Endocytosis, 7-Golgi apparatus, 8- Cytoplasm, 9- Plasma membrane, 10-Robert Hooke

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1.12 TERMINAL QUESTIONS

1.12.1 Long Answers Questions:

1. Why is cell considered as unit of life? Briefly describe the cell theory.
2. Differentiate between Rough Endoplasmic Reticulum (RER) and Smooth Endoplasmic Reticulum (SER).
3. Briefly give the contributions of the following scientists in formulating the cell theory
   a. Robert Virchow
   b. Schleiden and Schwann
4. Enumerate the cell organelles present in an eukaryotic cell. Give description and function of each.
5. Structure and function are correlatable in living organisms. Can you justify this by taking plasma membrane as an example?
6. Eukaryotic cells have organelles which may
   a. not be bound by a membrane
   b. bound by a single membrane
   c. bound by a double membrane
   Group the various sub-cellular organelles into these three categories.

7. What is the significance of vacuole in a plant cell? Compare the plant cell to that of animal cell.
8. Write the functions of the following
   a. Centromere
   b. Cell wall
   c. Smooth ER
   d. Golgi Apparatus
   e. Centrioles
9. Discuss briefly the role of nucleolus in the cells actively involved in protein synthesis.
10. What does 'S' refer in a 70S & an 80S ribosome? Bring out the difference between prokaryotes and eukaryotes.

UNIT-2 STRUCTURES AND FUNCTIONS OF CELL ORGANELLES

2.1 OBJECTIVE

Main objective of this unit is to provide student sufficient knowledge about the cell so that student will be able to-

- Define Cytology and Organelles very well.
- Able to identify the parts of a cell from models or diagrams given and will get to know their general function.
- Know about Organelle such as Endoplasmic reticulum (SER and RER), Chloroplast, Mitochondrion
- Know about Nucleus & Nuclear membrane (envelope), Nucleolus

2.2 INTRODUCTION

Our natural world originated the principle of form following function, especially in cell biology, and this will become clear as we explore eukaryotic cells. Unlike prokaryotic cells, eukaryotic cells have: (1) a membrane-bound nucleus; (2) numerous membrane-bound organelles—such as the endoplasmic reticulum, Golgi apparatus, chloroplasts, mitochondria, and others; and (3) several, rod-shaped chromosomes. Because a eukaryotic cell's nucleus is surrounded by a membrane, it is often said to have a "*true nucleus*". The word "*organelle*" means "*little organ*," and, organelles have specialized cellular functions, just as the organs of your body have specialized functions.

Cells are the smallest units of life. They are a closed system, can self-replicate, and are the building blocks of our bodies. In order to understand how these tiny organisms work, we will look at a cell's internal structures. We will focus on eukaryotic cells, cells that contain a nucleus. A cell consists of two major regions, the cytoplasm and the nucleus. The nucleus is surrounded by a nuclear envelope and contains DNA in the form of chromosomes. The cytoplasm is a fluid matrix that usually surrounds the nucleus and is bound by the outer membrane of the cell. Organelles are small structures within the cytoplasm that carry out functions necessary to maintain homeostasis in the cell. They are involved in many processes, for example energy production, building proteins and secretions, destroying toxins, and responding to external signals.

Organelles are considered either membranous or non-membranous. Membranous organelles possess their own plasma membrane to create a lumen separate from the cytoplasm. This may be the location of hormone synthesis or degradation of macromolecules. Non-membranous organelles are not surrounded by a plasma membrane. Most non-membranous organelles are part of the cytoskeleton, the major support structure of the cell. These include: filaments, microtubules, and centrioles.

Ribosomes, as a site for turning RNA code into protein sequences, and *chromosomes*, the DNA storage complex, are examples of non-membrane organelles. These non-membranous organelles are commonly *molecular complexes.* They may have complex functions, but the processes by which those functions are done are usually localized to the surfaces of the complex. They neither require specific isolation nor a large working surface of membrane. Some functional parts of a eukaryote cell are types of extensions of the external membrane. They will be treated here as *cell extension organelles,* although they are not always called "organelles" in some biology books.

The "soup" inside a cell, often so thick that it becomes a gel, has various names. In prokaryotes, its *protoplasm.* In eukaryotes, the material between the cell membrane and the nuclear envelope is usually called *cytoplasm,* which sometimes is further divided as *cytosol* is considered to be just outside the organelles. The material inside the nucleus is usually called *nucleoplasm.* All these organelles along with their structures and functions have been discussed in this unit.

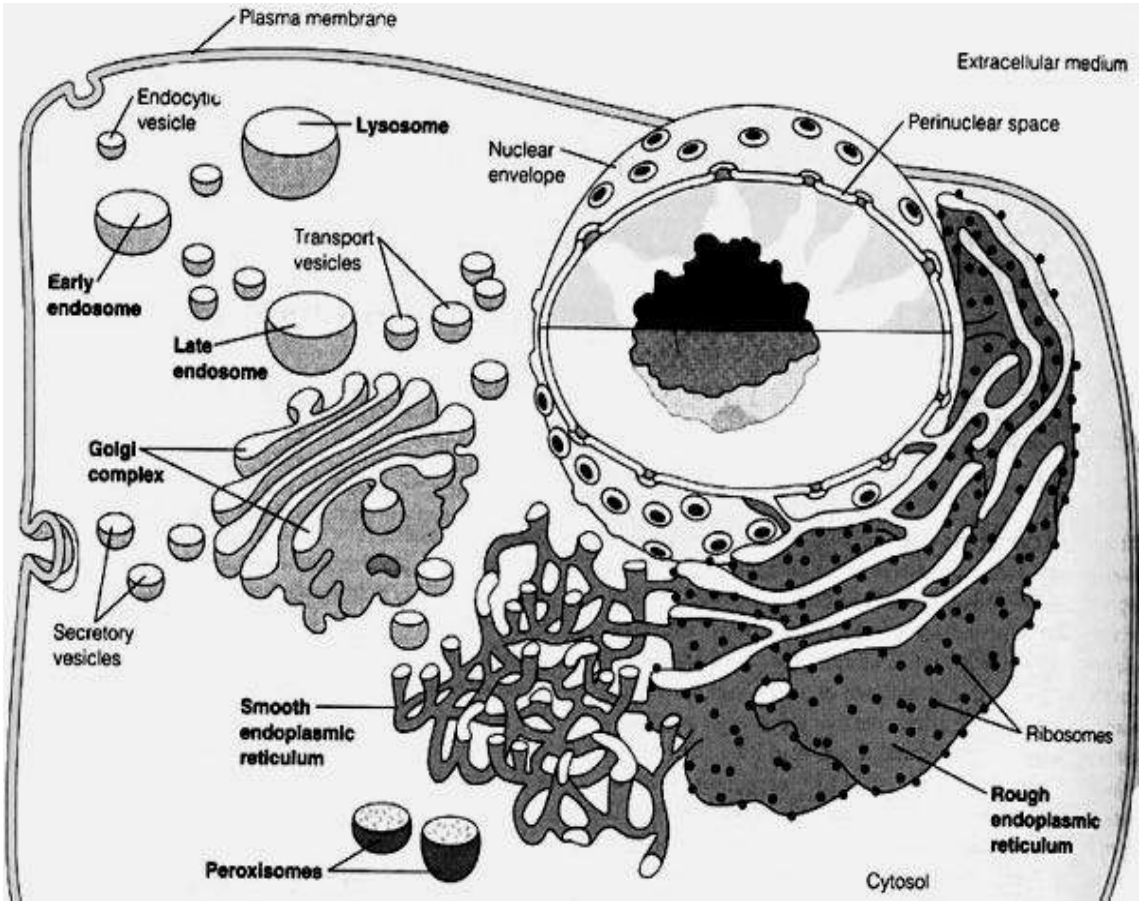

Fig.2.1 Typical Cell Membrane Bound Compartments

2.3 NUCLEUS

Nucleus the most prominent organelle of the cell. The number of nuclei may vary, they may be uninucleate (single nucleus), binucleate (two nuclei) or even multi-nucleate. Certain eukaryotic cells such as the mature sieve tubes of higher plants and mammalian erythrocytes contain no nucleus. Prokaryotic cells lack nucleus and is complemented by nucleoid.

The contents of the nucleus are DNA genome, RNA synthetic apparatus, and a fibrous matrix. It is surrounded by two membranes, each one a phospholipid bilayer containing many different types of proteins. The inner nuclear membrane defines the nucleus itself. In most cells, the outer nuclear membrane is continuous with the rough endoplasmic reticulum, and the space between the inner and outer nuclear membranes is continuous with the lumen of the rough endoplasmic reticulum. The two nuclear membranes appear to fuse at nuclear pores, the ring-like complexes composed of specific membrane proteins through which material moves between the nucleus and the cytosol. It contains cell's genetic material, organized as multiple long linear DNA molecules in complex with histones, to form chromosomes. The genes within these chromosomes are the cell's nuclear genome. The function is to maintain the integrity of the genes that controls the activities of the cell by regulating gene expression.

History

Nucleus was the first cell organelle to be discovered. **Antonie von Leeuwenhoek** (1632 - 1723) observed lumen (nucleus) in the red blood cells of salmon. The nucleus was also described in 1804 by **Franz Bauer** (14 March 1758 – 11 December 1840) an Austrian microscopist & botanical artist, and, in more detail in 1831 by Scottish botanist **Robert Brown (21 December 1773 – 10 June 1858)** in a talk at the *Linnean Society of London*. Brown was studying orchids under microscope when he observed an opaque area, which he called the "*areola*" or "*nucleus*", in the cells of the flower's outer layer. It was discovered and named by Robert Brown in 1833 in the plant cells and is recognized as a constant feature of all animal and plant cells.

Nucleus Definition

In cell biology, the nucleus (plural-nuclei; from Latin *nucleus* or *nuculeus*, meaning kernel or seed) is a membrane-enclosed organelle found in eukaryotic cells. Eukaryotes usually have a single nucleus, but a few cell types, such as mammalian red blood cells, have no nuclei, and a few others have many. Human skeletal muscle cells have more than one nucleus, as do eukaryotes like fungi. Cell nuclei contain most of the cell's genetic material, organized as multiple long linear DNA molecules in complex with a large variety of proteins, such as histones, to form chromosomes. The genes within these chromosomes are the cell's nuclear genome and are structured in such a way to promote cell function. The nucleus maintains the integrity of genes and controls the activities of the cell by regulating gene expression—the nucleus is, therefore, the control center of the cell.

2.3.1- Origin

A study of the comparative genomics, evolution and origins of the nuclear membrane led to the proposal that the nucleus emerged in the primitive eukaryotic ancestor (the "prekaryote"), and was triggered by the archaeo-bacterial symbiosis. Several ideas have been proposed for the evolutionary origin of the nuclear membrane. These ideas include the invagination of the plasma membrane in a prokaryote ancestor, or the formation of a genuine new membrane system following the establishment of proto-mitochondria in the archaeal-host. The adaptive function of the nuclear membrane may have been to serve as a barrier to protect the genome from reactive oxygen species (ROS) produced by the cells' pre-mitochondria.

2.3.2- Nucleus Structure

The nucleus is the largest organelle of the cell. It occupies about 10% of the total volume of the cell. In mammalian cells the average diameter of the nucleus is approximately 6 micrometers. The viscous liquid within it is called nucleoplasm (karyolymph), and is similar in composition to the cytosol found outside the nucleus. Generally there is a single nucleus per cell (Mononucleate conditions), but more than one nucleus (Polynucleate condition) may be found in certain special cases. There are many nuclei in a syncytium which is formed due to fusion of cells. A similar multinucleate situation is found in coenocytes commonly found in plants. A coenocyte results by repeated nuclear divisions without cytokinesis.

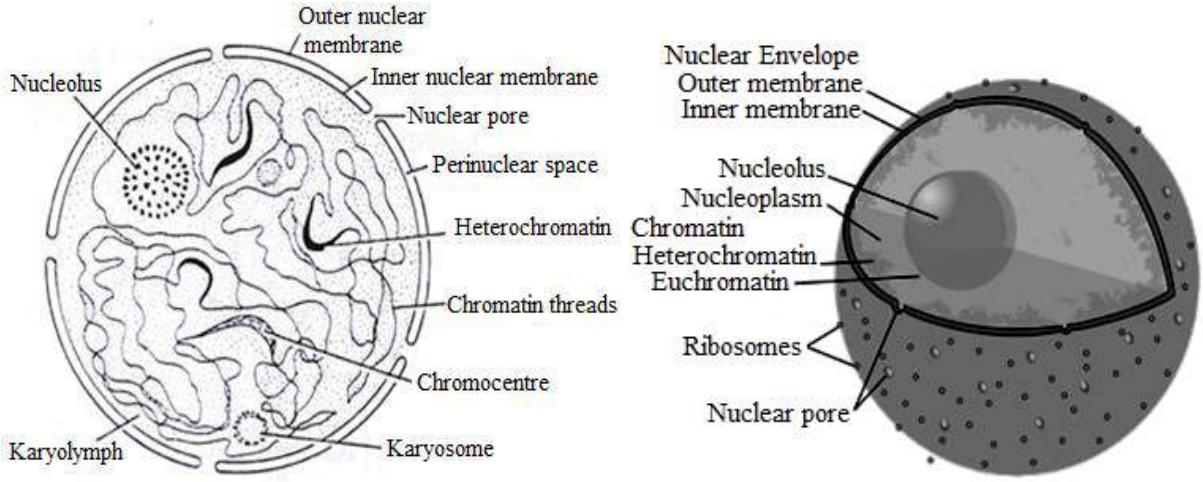

Fig.2.2 The schematic representation of nucleus

There are also variations with respect to shape and size of nucleus. It may be spherical oval to flattened lobe or irregular in shape. Shape of nucleus also depends on the cell. The spheroid, cuboid or polyhedral cells, nucleus is usually spheroid. In cylindrical, prismatic or fusiform cells, nucleus is ellipsoid.

Nuclear Envelope

1. The nuclear envelope is also known as the nuclear membrane.

2. It is made up of two membranes the outer membrane and the inner membrane.
3. The outer membrane of the nucleus is continuous with the membrane of the rough endoplasmic reticulum.
4. The space between these layers is known as the Perinuclear space.
5. The nuclear envelope encloses the nucleus and separates the genetic material of the cell from the cytoplasm of the cell.
6. It also serves as a barrier to prevent passage of macro-molecules freely between the nucleoplasm and the cytoplasm.

Nuclear Pore

1. The nuclear envelope is perforated with numerous pores called nuclear pores.
2. The nuclear pores are composed of many proteins known as nucleoproteins.
3. The nuclear pores regulate the passage of the molecules between the nucleus and cytoplasm.
4. The pores allow the passage of molecules of only about 9nm wide. The larger molecules are transferred through active transport.
5. Molecules like of DNA and RNA are allowed into the nucleus. But energy molecules (ATP), water and ions are permitted freely.

Chromosomes (Chromatin structure)

1. The nucleus of the cell contains majority of the cells genetic material in the form of multiple linear DNA molecules.
2. These DNA molecules are organized into structures called chromosomes.
3. The DNA molecules are in complex with a large variety of proteins (histones) which form the chromosome.
4. In the cell they are organized in a DNA-protein complex known as chromatin.
    a. Chromatin = DNA + Histone + DNA binding proteins.
    b. Two type of chromatin are present.
        (i) Euchromatin
        (ii) Heterochromatin
5. During cell-division the chromatin forms well-defined chromosomes.
6. The genes within the chromosomes consists of the cells nuclear genome.
7. Mitochondria of the cell also contains a small fraction of genes.
8. Human cells has nearly 6 feet of DNA, which is divided into 46 individual molecules.

Nucleolus

1. The nucleolus is not surrounded by a membrane, it is a densely stained structure found in the nucleus.
2. The nucleoli are formed around the nuclear organizer regions.
3. It synthesizes and assembles ribosomes and r-RNA.

4. The number of nucleoli is different from species to species but within a species the number is fixed.
5. During cell division, the nucleolus disappears.
6. Studies suggest that nucleolus may be involved in cellular aging and senescence.

In the nucleolus seems to proceed from center to periphery 3 distinct region are:
i) **Fibriller Center (FC):** Where r-RNA genes of nucleolus organizer region (NOR) are located, the transcription of r-RNA genes also takes place in this region.
ii) **Dense Fibriller Component (DFC):** Which surround the fibriller genes and where RNA synthesis progress. The 80S ribosomal proteins also bind to the transcripts in this region.
iii) **Cortical Granular Component(CGC):** It is the inner-most region and where processing and maturation of pre ribosomal particles occurs.
Therefore, these region roles in ―ribosome formation.

2.3.3- Functions of the Nucleus

Speaking about the functions of a cell nucleus, it controls the hereditary characteristics of an organism. This organelle is also responsible for the protein synthesis, cell division, growth, and differentiation. Some important functions carried out by a cell nucleus are:

1. Storage of hereditary material, the genes in the form of long and thin DNA (deoxyribonucleic acid) strands, referred to as chromatins.
2. Storage of proteins and RNA (ribonucleic acid) in the nucleolus.
3. It is responsible for protein synthesis, cell division, growth and differentiation.
4. Nucleus is a site for transcription in which messenger RNA (mRNA) are produced for the protein synthesis.
5. It controls the heredity characteristics of an organism. Exchange of hereditary molecules (DNA and RNA) between the nucleus and rest of the cell.
6. During the cell division, chromatins are arranged into chromosomes in the nucleus.
7. Production of ribosomes (protein factories) in the nucleolus.
8. Selective transportation of regulatory factors and energy molecules through nuclear pores.
9. It also regulates the integrity of genes and gene expression.

Animal Cell Nucleus

Animal cell nucleus is a membrane bound organelle. It is surrounded by double membrane. The nucleus communicates with the surrounding cell cytoplasm through the nuclear pores. The DNA in the nucleus is responsible for the hereditary characteristics and protein synthesis. The active genes on the DNA are similar, but some genes may be turned on or off depending on the specific cell type. This is the reason why a muscle cell is different from a liver cell. Nucleolus is a prominent structure in the nucleus. This aids in ribosomes production and protein synthesis.

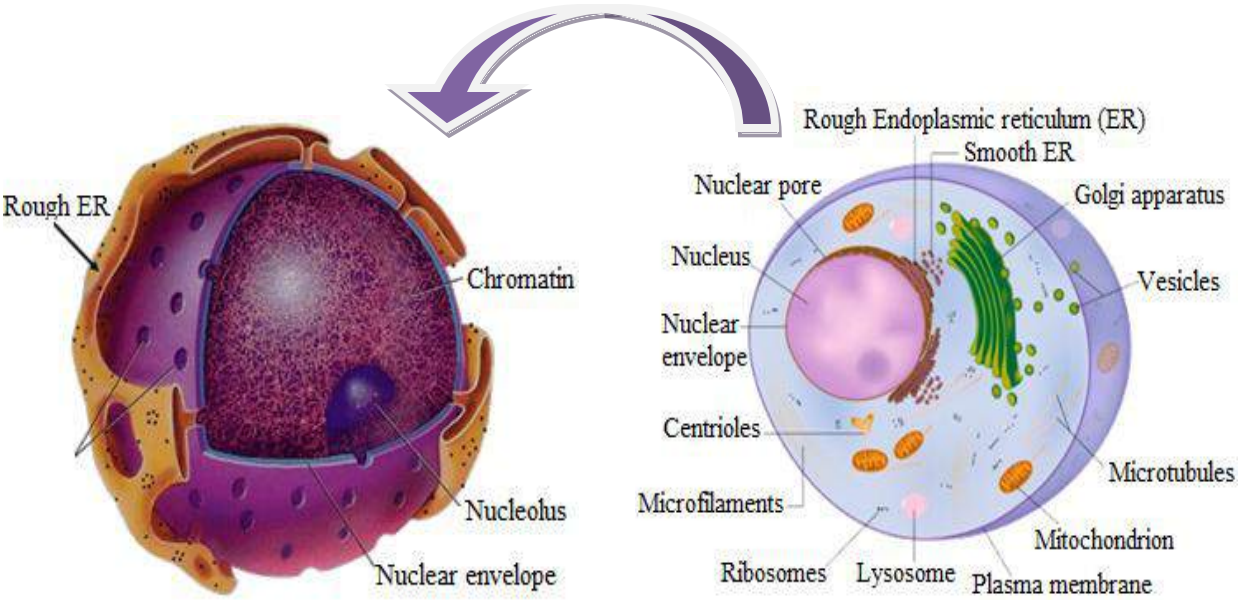

Fig. 2.3 Structure of a typical Animal cell nucleus

Plant Cell Nucleus

Plant cell nucleus is a double-membrane bound organelle. It controls the activities of the cell and is known as the master mind or the control center of the cell. The plant cell wall has two layers - the outer membrane and the inner membrane, which encloses a tiny space known as perinuclear space. The nucleus communicates to the cell cytoplasm through the nuclear pores present in the nuclear membrane. The nuclear membrane is continuous with the endoplasmic reticulum. The DNA is responsible for cell division, growth and protein synthesis.

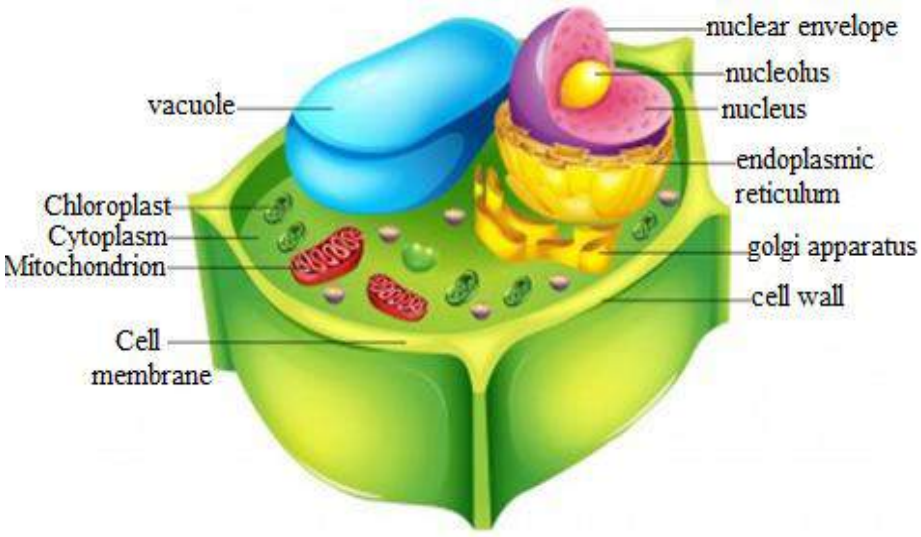

Fig. 2.4 Structure of typical Plant cell with nucleus

Bacterial Cell Nucleus

Bacteria are minute single-celled microorganisms under the domain Prokaryota. Interestingly, they are believed to be the direct descendants of the first ever organisms that thrive on Earth about 3.5 billion years ago. While they seem to be invisible with the naked eye, under powerful microscopes, the structures within bacteria can be observed.

The bacterial cell does not contain any nucleus. The bacterial chromosome is not enclosed in a membrane bound nucleus. The bacterial chromosome is circular and located in the cytoplasm.

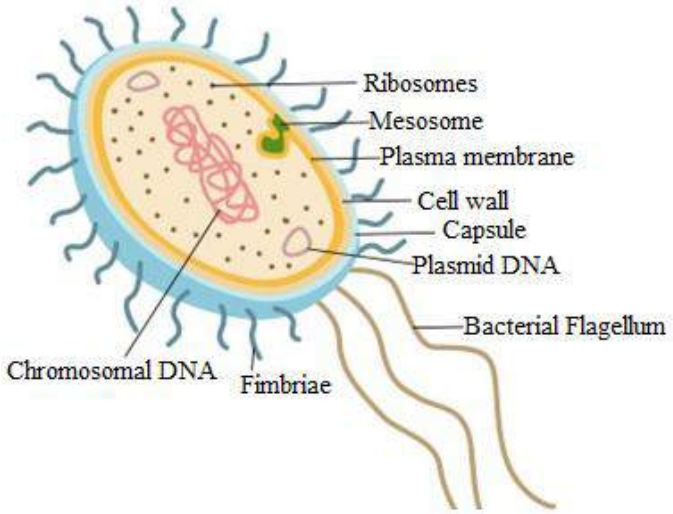

Fig. 2.5 Structure of a typical bacterial cell

2.4 RIBOSOME

Proteins are necessary for the cells to perform cellular functions. Ribosomes are the cellular components that make proteins from all amino acids. Ribosomes are made from complexes of RNAs and proteins. The number of ribosomes in a cell depends on the activity of the cell. Ribosomes are freely suspended in the cytoplasm or attached to the endoplasmic reticulum forming the rough endoplasmic reticulum. On an average in a mammalian cell there can be about 10 million ribosomes.

When the ribosomes are attached to the same mRNA strand, this structure is known as Polysome. The existence of ribosomes is temporary, after the synthesis of polypeptide the two sub-units separate and is reused or broken up. Amino acids are joined by the ribosomes at a rate of 200 per minute. Therefore small proteins can be made quickly but two or three hours are needed for proteins which are as large as 30,000 amino acids.

The ribosomes present in the prokaryotes function differently in protein production than the ribosomes of the eukaryote organisms. The ribosomes of bacteria, archaea and eukaryotes differ significantly from each other in structure and RNA sequences. The differences in the ribosomes allows the antibiotic to kill the bacterial ribosome by inhibiting the activity of the

bacterial ribosomes, the human ribosome remain unaffected. The ribosomes of the eukaryotic cells are similar to the ribosomes of the bacterial cells, showing the evolutionary origin of the organelle.

Ribosomes Definition

Ribosomes are small particles, present in large numbers in all the living cells. They are sites of protein synthesis. The ribosome word is derived - '*ribo'* from ribonucleic acid and '*somes*' from the Greek word '*soma*' which means '*body'*. The ribosomes link amino acids together in the order that is specified by the messenger RNA molecules.

The ribosomes are made up of two subunits - a small and a large subunit. The small subunit reads the mRNA while the large subunit joins the amino acids to form a chain of polypeptides. Ribosomal subunits are made of one or more rRNA (ribosomal RNA) molecules and various proteins. The ribosomes and associated molecules are also known as the translational apparatus.

Ribosomes were first observed in the mid-1950s by Romanian-American cell biologist **George Emil Palade**, using an electron microscope, as dense particles or granules. The term "ribosome" was proposed by scientist **Richard B. Roberts** in the end of 1950s.

Types of Ribosomes

Ribosomes are classified into two types based on their sedimentation coefficient, 70S and 80S. S stands for "Svedberg unit" and related to sedimentation rate (sedimentation depends on mass and size). Thus, the value before S indicates size of ribosome.

2.4.1- Origin

The ribosome may have first originated in an RNA world, appearing as a self-replicating complex that only later evolved the ability to synthesize proteins when amino acids began to appear. Studies suggest that ancient ribosomes constructed solely of rRNA could have developed the ability to synthesize peptide bonds. In addition, evidence strongly points to ancient ribosomes as self-replicating complexes, where the rRNA in the ribosomes had informational, structural, and catalytic purposes because it could have coded for tRNAs and proteins needed for ribosomal self-replication.

In the prokaryotes, the ribosome originates in the cytoplasm as there is no nucleolus, but in eukaryotes, the ribosome is partly nucleolar (rRNA) and partly cytoplasmic (proteins) in origin.

2.4.2- Structure

1. Ribosomes are tiny particles about 200 Ã. Ribosomes in a cell are located in two regions of the cytoplasm. They are found scattered in the cytoplasm and some are attached to the endoplasmic reticulum.

2. When the ribosomes are bound to the ER there are known as the Rough Endoplasmic Reticulum (RER). The bound and the free ribosomes are similar in structure and are involved in protein synthesis.
3. Ribosomes are composed of both RNA and proteins. About 37 - 62% of RNA are made up of RNA and the rest is proteins.
4. Ribosome is made up of two subunits. The subunits of ribosomes are named according to their ability of sedimentation on a special gel which the Svedberg Unit.
5. Prokaryotes have 70S ribosomes, each subunit consisting of small subunit is of 30S and the large subunit is of 50S. Eukaryotes have 80S ribosomes, each consisting of small (40S) and large (60S) subunit.
6. The ribosomes found in the chloroplasts of mitochondria of eukaryotes consists of large and small subunits bound together with proteins into one 70S particle.
7. The ribosomes share a core structure which is similar to all ribosomes despite differences in its size.
8. The RNA is organized in various tertiary structures. The RNA in the larger ribosomes are into several continuous insertion as they form loops out of the core structure without disrupting or changing it.
9. The catalytic activity of the ribosome is carried out by the RNA, the proteins reside on the surface and stabilize the structure.
10. The differences between the ribosomes of bacterial and eukaryotic are used to create antibiotics that can destroy bacterial infection without harming human cells.

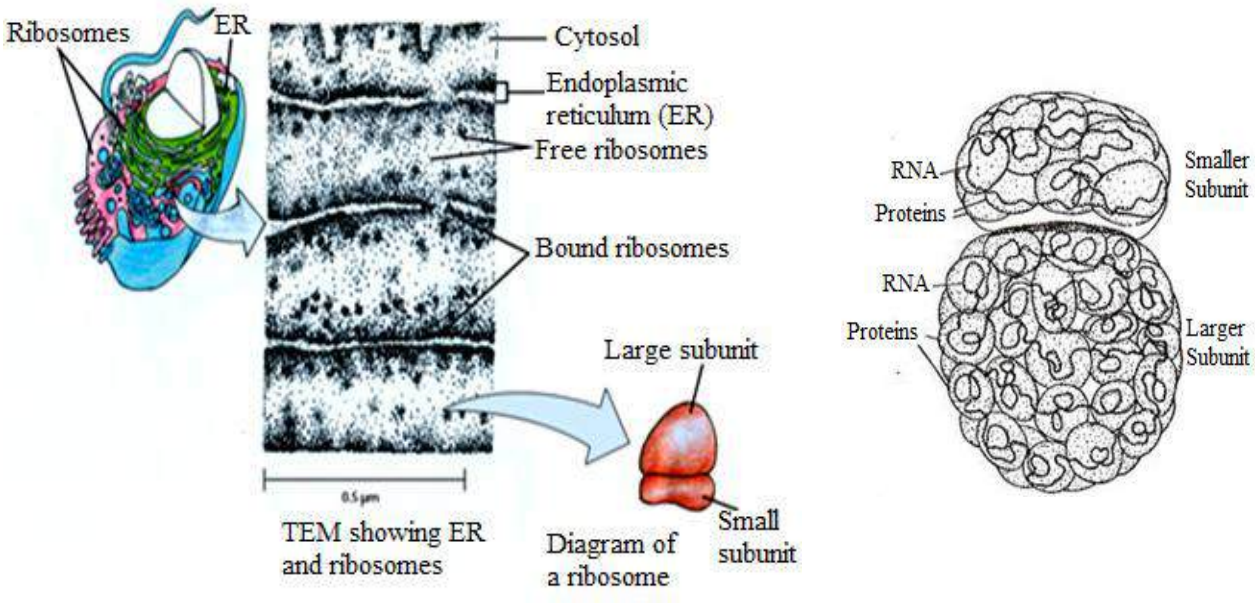

Fig: 2.6 Schematic representation of the ribosome

2.4.3-Function

1. Nearly all the proteins required by cells are synthesized by ribosomes. Ribosomes are found 'free' in the cell cytoplasm and also attached to rough endoplasmic reticulum.
2. Ribosomes receive information from the cell nucleus and construction materials from the cytoplasm.
3. Ribosomes translate information encoded in messenger ribonucleic acid (mRNA).
4. They link together specific amino acids to form polypeptides and they export these to the cytoplasm.
5. A mammalian cell may contain as many as 10 million ribosomes, but each ribosome has only a temporary existence.
6. Ribosomes can link up amino acids at a rate of 200 per minute.
7. Ribosomes are formed from the locking of a small sub-unit on to a large sub-unit. The sub-units are normally available in the cytoplasm, the larger one being about twice the size of the smaller one.
8. Each ribosome is a complex of Ribonucleoprotein with two-thirds of its mass is composed of ribosomal RNA and about one-third ribosomal protein.
9. Protein production takes place in three stages: (1) Initiation, (2) elongation, and (3) termination.
10. During peptide production the ribosome moves along the mRNA in an intermittent process called translocation.
11. Antibiotic drugs such as streptomycin can be used to attack the translation mechanism in prokaryotes. This is very useful. Unfortunately some bacterial toxins and viruses can also do this.
12. After they leave the ribosome most proteins are folded or modified in some way. This is called 'post translational modification'.

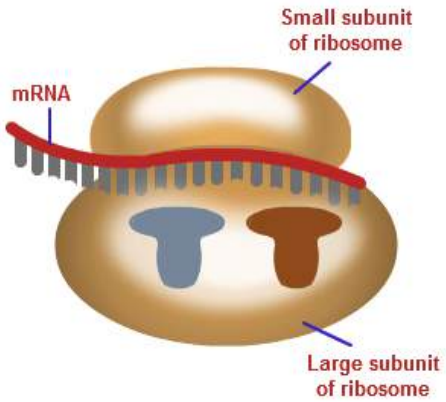

Fig. 2.7 Ribosome as a site of protein synthesis

2.5 NUCLEOPLASM

The nucleus of most cells contains a substance that suspends structures inside the nuclear membrane. Just like the cytoplasm found inside a cell, the nucleus contains nucleoplasm, also known as **Karyoplasm**.

The nucleoplasm is a type of protoplasm that is made up mostly of water, a mixture of various molecules, and dissolved ions. It is completely enclosed within the nuclear membrane or nuclear envelope. It is a highly gelatinous, sticky liquid that supports the chromosomes and nucleoli. The soluble, fluid component of the nucleoplasm is called the **Nucleosol** or **Nuclear Hyaloplasm**. The nucleoplasm includes the chromosomes and nucleoli. Many substances such as nucleotides (necessary for purposes such as the replication of DNA) and enzymes (which direct activities that take place in the nucleus) are dissolved in the nucleoplasm.

The term "*nucleoplasm*" was coined by embryologist, cytologist and marine biologist **Edouard van Beneden** (1875), while "*karyoplasm*" was by **Walther Flemming** (1878) a German biologist and a founder of Cytogenetics.

2.5.1- Origin

It arises from the nuclear content and chromatin content contained in a cell nucleus.

2.5.2- Structure

The nucleoplasm consists of a viscous mix of water, in which various substances and structures are dissolved or carried, and an underlying intranuclear ultrastructure. The nucleoplasm is especially rich in protein enzymes and protein constituents involved in the synthesis of deoxyribonucleic acid (DNA) and the various types of ribonucleic acid (RNA), the precursor molecules of RNA, and the nucleotides from which they are assembled. Some of these proteins direct initial transcription, while others function in the further modification of the RNA molecules for packaging and transport to the cytoplasm.

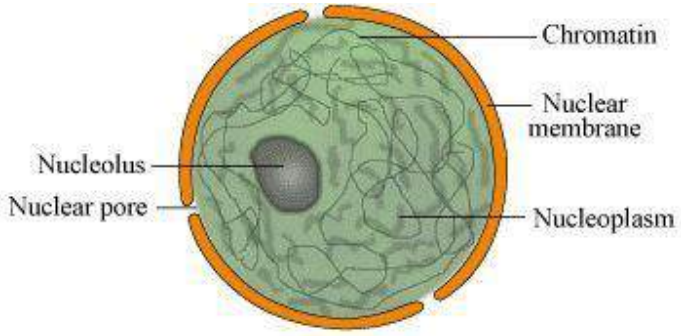

Fig. 2.8 Structure of Nucleus showing Nucleoplasm

Prominent structures located within the interphase nucleoplasm (the resting cell or the non replicating cell) include organelles called nucleoli and the unwound DNA, called chromatin. The

nucleoli resemble miniature nuclei and are the sites of synthesis of precursor RNA molecules and their assembly.

The other major components in nucleoplasm include the DNA chromosomes seen during mitosis. During cell interphase most of the DNA chromosomes exist as unwound chromatin that extend through the nucleoplasm. Two distinct types of chromatin are recognized. Diffuse, or uncondensed, chromatin is called Euchromatin and exists as thin threads that extend throughout much of the nucleoplasm.

2.5.3- Function

1. The nucleoplasm acts as a suspension medium for components of the nucleus including the nucleolus, and chromatin.
2. Nucleotides required for DNA replication and enzymes involved in other nuclear processes are also found dissolved within the nucleoplasm.
3. The nucleoplasm plays a role in the maintenance of the shape and structure of the nucleus.
4. The nuclear matrix is present within the nuclear hyaloplasm, the liquid component of the nucleoplasm.
5. One other function is that it is responsible for the transport of materials that are vital to metabolism and cell function.

2.6 MITOCHONDRIA

Mitochondria are well-defined cytoplasmic organelles of the cell which take part in a variety of cellular metabolic functions. Survival of the cells requires energy to perform different functions. The mitochondria are important as the fact that these organelles supply all the necessary biological energy of the cell, and they obtain this energy by oxidizing the substrates of the Krebs cycle. Energy of the cell is got from the enzymatic oxidation of chemical compounds in the mitochondria. Hence, the mitochondria re referred to as the "***power houses of the cell***". Almost all the eukaryotic cell have mitochondria, though they are lost in the later stages of development of cell like in the red blood cells or in elements of phloem sieve tube.

In 1890, mitochondria were first described by **Richard Altmann** (12 March 1852 – 8 December 1900) an German pathologist and histologist, and he called them as "*bioblasts*". **Carl Benda,** another German scientist (one of the first microbiologists) in the year 1897 coined the term "*mitochondrion"*. In the 1920s, a biochemist Warburg found that oxidative reactions takes place in most tissues in small parts of the cell.

Mitochondria Definition

Mitochondria is a membrane bound cellular structure and is found in most of the eukaryotic cells. The term 'mitochondrion' is derived from a Greek word *mitos* which means "thread" and *chondrion* which means "granule" or "grain-like". Mitochondria are commonly between 0.75

and 3 μm in diameter but vary considerably in size and structure. The mitochondria are sometimes described as power plants of the cells. These organelles generate most of the energy of the cell in the form of adenosine triphosphate (ATP) and it is used a source of chemical energy. The mitochondria also involved in other cellular activities like signaling, cellular differentiation, cell senescence and also control of cell cycle and cell growth. Mitochondria also affect human health, like mitochondrial disorder and cardiac dysfunction and they also play important role in the aging process.

2.6.1- Origin

There are two hypotheses about the origin of mitochondria: endosymbiotic and autogenous. The endosymbiotic hypothesis suggests that mitochondria were originally prokaryotic cells, capable of implementing oxidative mechanisms that were not possible for eukaryotic cells; they became endosymbionts living inside the eukaryote. In the autogenous hypothesis, mitochondria were born by splitting off a portion of DNA from the nucleus of the eukaryotic cell at the time of divergence with the prokaryotes; this DNA portion would have been enclosed by membranes, which could not be crossed by proteins. Since mitochondria have many features in common with bacteria, the endosymbiotic hypothesis is more widely accepted.

Unlike any other organelle, except for chloroplasts, mitochondria appear to originate only from other mitochondria. They contain their own DNA, which is circular as is true with bacteria, along with their own transcriptional and translational machinery. Mitochondrial ribosomes and transfer RNA molecules are similar to those of bacteria, as are components of their membrane.

2.6.2- Structure

A mitochondrion contains outer and inner membranes composed of phospholipid bilayers and proteins. The two membranes have different properties. Because of this double-membraned organization, there are five distinct parts to a mitochondrion.

1. Outer mitochondrial membrane,
2. Intermembrane space (the space between the outer and inner membranes),
3. Inner mitochondrial membrane,
4. Cristae space (formed by infoldings of the inner membrane), and
5. Matrix (space within the inner membrane).

   Mitochondria stripped of their outer membrane leaving the inner membrane intact are called Mitoplasts.

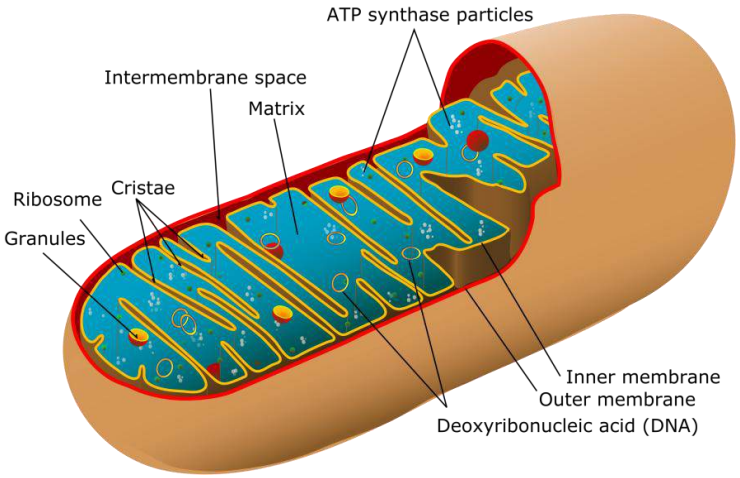

Fig.2.9 Structure of Mitochondria

Outer Membrane

1. It is smooth and is composed of equal amounts of phospholipids and proteins.
2. It has a large number of special proteins known as the Porins.
3. The Porins are integral membrane proteins and they allow the movement of molecules that are of 5000 daltons or less in weight to pass through it.
4. The outer membrane is freely permeable to nutrient molecules, ions, oxygen, pyruvate, energy molecules like the ATP and ADP molecules.

Intermembrane Space

1. It is the space between the outer and inner membrane of the mitochondria, it has the same composition as that of the cell's cytoplasm.
2. It has a high proton concentration. This is due to the electron transport system of the inner mitochondrial membrane.

Inner Membrane

1. The inner membrane of mitochondria is more complex in structure.
2. It has many invaginations and is known as the Cristae.
3. This folding help to increase the surface area inside the organelle.
4. Many of the chemical reactions that take place within mitochondria occur on the inner membrane. It contains the electron transport system and the ATPase complex:
   (i) Electron transport system - generates a proton gradient.
   (ii) ATPase complex - uses proton gradient to produce adenosine triphosphate (ATP) from adenosine diphosphate (ADP).
5. Hence the inner mitochondrial membrane is the site of oxidative phosphorylation.

6. Unlike the outer membrane, the inner membrane is strictly permeable, it is permeable only to oxygen, ATP and it also helps in regulating transfer of metabolites across the membrane.

Cristae Space

The inner mitochondrial membrane is compartmentalized into numerous Cristae, which expand the surface area of the inner mitochondrial membrane, enhancing its ability to produce ATP. Cristae are covered with many tiny "*stalked particles*" called inner membrane spheres that are also known as simply "*spheres*" or "*knobs*".

Matrix Space

The matrix of the mitochondria is a complex mixture of proteins and enzymes. These enzymes are important for the synthesis of ATP molecules, mitochondrial ribosomes, tRNAs and mitochondrial DNA.

Plant Cell Mitochondria

Like in other eukaryotic cells, the mitochondria in plants play an important role in the production of ATP via the process of oxidative phosphorylation. Mitochondria also play essential roles in other aspects of plant development and performance. It also has various properties which allows the mitochondria to interact with special features of metabolism in plant cell.

Animal Cell Mitochondria

Mitochondria are known as "power houses" of the cells, they are unusual organelles and are surrounded by a double membrane. These organelles have their own small genome. They divide independently by simple fission. The division of the mitochondria is a result of the energy demand, so the cells with high need of energy have greater number of mitochondria. A typical animal cell may have about 1000 to 2000 mitochondria. The process creating energy for the cell is known as cellular respiration. Most of the chemical reactions of this process happen in the mitochondria.

2.6.3- Function

Functions of mitochondria depend on the cell type in which they are present.

1. The most important function of the mitochondria is to produce energy. The simpler molecules of nutrition are sent to the mitochondria to be processed and to produce charged molecules. These charged molecules combine with oxygen and produce ATP molecules. This process is known as oxidative phosphorylation.
2. Mitochondria help the cells to maintain proper concentration of calcium ions within the compartments of the cell.
3. The mitochondria also help in building certain parts of blood and hormones like testosterone and estrogen.
4. The liver cells mitochondria have enzymes that detoxify ammonia.

5. The mitochondria also play important role in the process of apoptosis or programmed cell death. Abnormal death of cells due to the dysfunction of mitochondria can affect the function of organ.

2.7 CHLOROPLAST

The word chloroplast is derived from the Greek word *chloros* meaning "green" and *plastes* meaning "the one who forms". Chloroplasts are organelles, specialized compartments, in plant and algal cells. Their discovery inside plant cells is usually credited to Julius von Sachs (1832–1897), an influential botanist and author of standard botanical textbooks -sometimes called "The Father of Plant Physiology".

Chloroplasts are organelles present in plant cells and some eukaryotic organisms. Chloroplasts are the most important plastids found in plant cells. It is the structure in a green plant cell in which photosynthesis occurs. Chloroplast is one of the three types of plastids. The chloroplasts take part in the process of photosynthesis and it is of great biological importance. Animal cells do not have chloroplasts. All green plant take part in the process of photosynthesis which converts energy into sugars and the byproduct of the process is oxygen that all animals breathe. This process happens in chloroplasts. The distribution of chloroplasts is homogeneous in the cytoplasm of the cells and in certain cells chloroplasts become concentrated around the nucleus or just beneath the plasma membrane. A typical plant cell might contain about 50 chloroplasts per cell.

2.7.1- Origin

Chloroplasts are unique organelles and are said to have originated as endosymbiotic bacteria. They develop from colourless precursors, called Proplastids or Eoplasts. They are semi autonomous in nature and arise from pre existing chloroplast as they have their own machinery to synthesize the required proteins. This is very clear in algae, where one chloroplast divides into two during cell division. In higher plants, the division of chloroplasts is very difficult to observe as. the number of chloroplast is very high. Still, some-times the dividing chloroplast is seen under the phase contrast microscope as in Spinach.

2.7.2- Structure

1. Chloroplasts found in higher plants are generally biconvex or planoconvex shaped. In different plants chloroplasts have different shapes, they vary from spheroid, filamentous saucer-shaped, discoid or ovoid shaped.
2. They are vesicular and have a colorless center. Some chloroplasts are in shape of club, they have a thin middle zone and the ends are filled with chlorophyll. In algae a single huge chloroplast is seen that appears as a network, a spiral band.

3. The size of the chloroplast also varies from species to species and it is constant for a given cell type. In higher plants, the average size of chloroplast is 4-6 microns in diameter and 1-
    - microns in thickness.

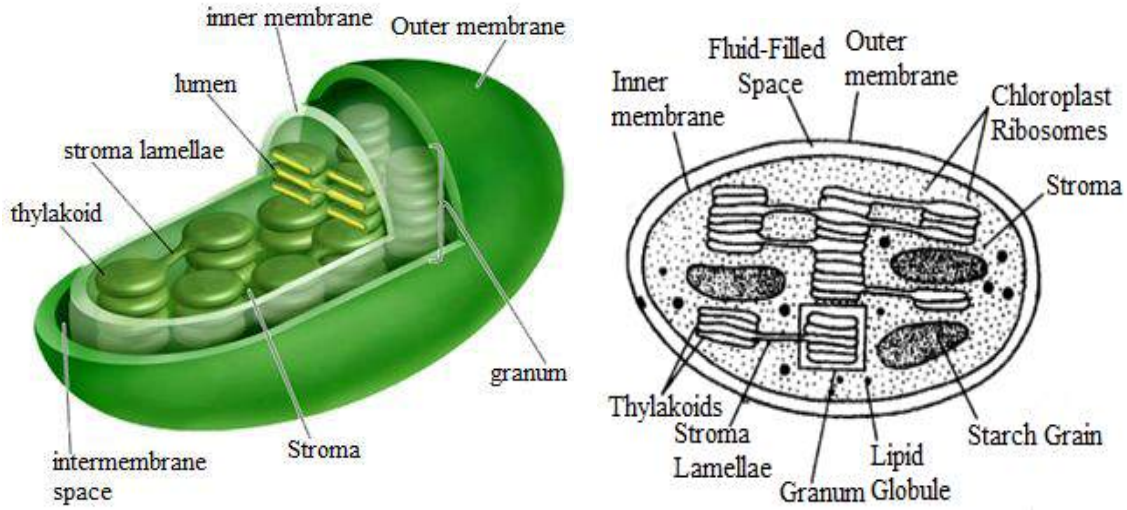

Fig.2.10 Structure of Chloroplast

4. The chloroplasts are double membrane bound organelles and are the site of photosynthesis. The chloroplasts have a system of three membranes: The Outer Membrane, The Inner Membrane and The Thylakoid system. The outer and the inner membrane of the chloroplast enclose a semi-gel-like fluid known as the Stroma. This stroma makes up much of the volume of the chloroplast, the thylakoids system floats in the stroma.

Components of Chloroplast

Outer Membrane: It is a semi-porous membrane and is permeable to small molecules and ions, which diffuses easily. The outer membrane is not permeable to larger proteins.
Intermembrane Space: It is usually a thin intermembrane space about 10-20 nanometers and it is present between the outer and the inner membrane of the chloroplast.

Inner Membrane: The inner membrane of the chloroplast forms a border to the stroma. It regulates passage of materials in and out of the chloroplast. In addition of regulation activity, the fatty acids, lipids and carotenoids are synthesized in the inner chloroplast membrane.

Stroma: Stroma is a alkaline, aqueous fluid which is protein rich and is present within the inner membrane of the chloroplast. The space outside the thylakoid space is called the stroma. The chloroplast DNA, chloroplast ribosomes and the thylakoid system, starch granules and many proteins are found floating around the stroma.

Thylakoid System: It is suspended in the stroma. The Thylakoid system is a collection of membranous sacks called thylakoids. The chlorophyll is found in the thylakoids and is the sight

for the process of light reactions of photosynthesis to happen. The thylakoids are arranged in stacks known as Grana. Each granum contains around 10-20 thylakoids.

General Features of Thylakoid System

1. Thylakoids are interconnected small sacks, the membranes of these thylakoids is the site for the light reactions of the photosynthesis to take place. The word thylakoid is derived from the Greek word "*thylakos"* which means 'sack'.
2. Important protein complexes which carry out light reaction of photosynthesis are embedded in the membranes of the thylakoids. The Photosystem I and the Photosystem II are complexes that harvest light with chlorophyll and carotenoids, they absorb the light energy and use it to energize the electrons.
3. The molecules present in the thylakoid membrane use the electrons that are energized to pump hydrogen ions into the thylakoid space, this decrease the pH and become acidic in nature. A large protein complex known as the ATP synthase controls the concentration gradient of the hydrogen ions in the thylakoid space to generate ATP energy and the hydrogen ions flow back into the stroma.
4. Thylakoids are of two types - Granal Thylakoids and Stromal Thylakoids. Granal thylakoids arranged in the grana, are pancake shaped circular discs, which are about 300-600 nanometers in diameter. The Stromal thylakoids are in contact with the stroma and are in the form of helicoid sheets.
5. The Granal thylakoids contain only Photosystem II protein complex, this allows them to stack tightly and form many granal layers with granal membrane. This structure increases stability and surface area for the capture of light.
6. The Photosystem I and ATP synthase protein complexes are present in the stroma. These protein complexes act as spacers between the sheets of Stromal thylakoids.

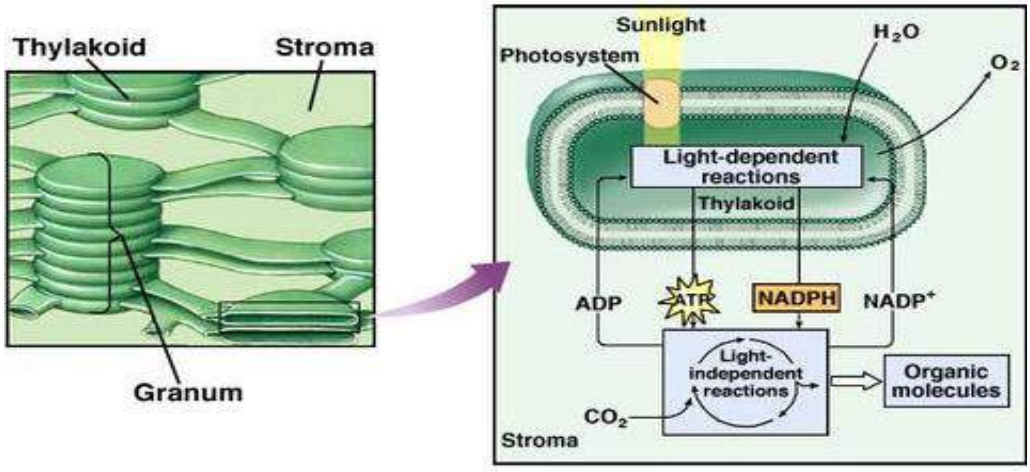

Fig. 2.11 Light reaction of photosynthesis in thylakoid

Transport of proteins into Chloroplast

1. For import of protein across double membrane, the chloroplast seems to employ ATP hydrolysis.
2. Signal peptide on chloroplast, proteins can be recognized by receptor on chloroplast membrane.
3. In first step the protein passes through chloroplast double membrane to reach stroma.
4. From where in the second step they are transported to Thylakoid space.
5. After the protein reaches the stroma, the chloroplast signal peptide cleaved by Stromal peptidase which facilitates transport to Thylakoid.

2.7.3- Functions of Chloroplast

1. In plants all the cells participate in plant immune response as they lack specialized immune cells. The chloroplasts with the nucleus and cell membrane and ER are the key organelles of pathogen defense.
2. The most important function of chloroplast is absorption of light energy and conversion of it into biological energy, making food by the process of photosynthesis. Food is prepared in the form of sugars. The chloroplast is very important as it is the cooking place for all the green plants.
3. During the process of photosynthesis sugar and oxygen are made using light energy, water, and carbon dioxide. Conversion of PGA (phosphoglyceric acid) into different sugars and store as starch.
4. Like the mitochondria, Chloroplasts use the potential energy of the H+ ions or the hydrogen ion gradient to generate energy in the form of ATP.
5. Light reactions takes place on the membranes of the thylakoids. Production of $NADPH_2$ and evolution of oxygen through the process of photolysis of water.
6. The dark reactions also known as the Calvin cycle takes place in the stroma of chloroplast.
7. Enzymes for carbon dioxide fixation and other dark reactions are present in the stroma and the enzymes for light reactions are present in the thylakoids. Two separate ways for carbon dioxide fixation are observed in higher plants which are broadly classified into C3 and C4 plants.
8. Breaking of 6-carbon atom compound into two molecules of phosphoglyceric acid by the utilization of assimilatory powers ($NADPH_2$ and ATP).

2.8 TYPES OF PLASTID

Plastids are double membraned organelles which are found in plant cells only. They are usually spherical or discoidal in shape and their average size is 4-6 µm. A plastid shows two distinct regions- Grana and Stroma. Grana are stacks of membrane-bound, flattened, discoid sacs containing chlorophyll molecules. These molecules are responsible for the production of food by

the process of photosynthesis. They are, therefore, called "*Kitchen of the cell"*. They are the main functional units of the chloroplast. The homogenous matrix in which grana are embedded is known as Stroma. A variety of photosynthetic enzymes and starch grains are present in the stroma. The stroma is colourless, whereas the grana contain the pigments. Plastids are living and multiply by division of the pre-existing plastids called Proplastids.

Types of Plastids

1. Leucoplasts: These are colorless plastids. They store the food of the plant body in the form of starch, protein and lipids. They occur most commonly in the storage cells of roots and underground stems.

2. Chloroplasts: These are green plastids because of the presence of chlorophyll. Chloroplasts occur abundantly in green leaves, and also to some extent in green parts of the shoot.

3. Chromoplasts: These are variously colored plastids. They are mostly present tin flowers and fruits.

One form of plastid can change into another. For example, leucoplasts can change into chloroplasts when the former are exposed to light for a long period.

Functions of Plastids:

1. By trapping solar energy, green plastids manufacture food through photosynthesis
2. Chromoplasts provide colored to various flowering parts.
3. Leucoplasts help in storage of protein, starch and oil.

On the basis of presence of pigments, the plastids are of two types:

1. Chromoplasts: The chromoplasts may be further divided on the basis of colour of the pigment and these are of the following types-

A. Chloroplasts: It is the most common plastid which contains chlorophyll a and b pigments, and DNA and RNA. Chloroplasts are found mainly in the cells of the leaves of higher plants and algae. It is the most biologically important plastid. By the process of photosynthesis, they produce oxygen and the most of the chemical energy used by living organisms.

B. Phaeoplast: These are yellow or brown plastids found in brown algae, diatoms and dinoflagellates. Fucoxanthin is a carotenoid pigment which masks the colour of chlorophyll a, which is also present. It also absorbs light and transfer the energy to chlorophyll a.

C. Rhodoplasts: These are red coloured plastids. It is found in red algae and its red colour is due to phycoerythrin. It also absorbs light.

D. Chromatophores: These are present in the blue-green algae. The term chromatophore is used instead of plastid, since the pigments are not organized within a discrete plastid body but are often arranged on lamellar structures in concentric rings or plates within algal cell. Blue-green

colour of this algae is due to phycocyanin and phycobilins. These accessory pigments do not participate in photosynthesis.

Non-photosynthetic chromoplasts:

(i) A variety of accessory pigments is also found which do not appear to be directly involved in photosynthesis or energy transfer.

(ii) Chromoplasts may develop from chloroplasts by accumulation of non-photosynthetic pigments, e.g., red carotenoid, Lycopene in tomatoes. Genes for synthesis of pigments lie in the nucleus.

2. Leucoplasts: These plastids are devoid of pigment and are membranous structures. They serve to store starches, oils and proteins. These are of the following types-

A. Amyloplasts: These are food storage cells and store starch. These are generally found in storage tubers, cotyledons and endosperm. These are found in regions of little or no illumination. Amyloplasts have nucleoids and ribosomes.

B. Elaioplasts: These are found in certain monocotyledons and their function is to store oils.

C. Proteinoplasts: Also known as Aleuroneplasts. These are found in seeds of Ricinus and Brazil nut, and store proteins. Epidermal cells of Helleborus also possess Proteinoplast.

- Plastid differentiation depends upon the metabolic requirements of the cell. The chloroplasts may develop from leucoplasts, and chromoplasts, which are considered end forms of plastid differentiation, may develop from either leucoplasts or chloroplasts.
- Proplastids can differentiate into one of three types of plastids and since, in certain cases, one type of plastid can differentiate into another, it has been generally assumed that all plastids are essentially the same in structure, having the ability to differentiate in various ways, depending upon the requirements of the cells.

2.9 GOLGI COMPLEX

Golgi apparatus or Golgi complex is a cytoplasmic organelle of smooth membranes sac or cisternae, tubules and vesicles. It was identified in 1897 by the Italian scientist **Camillo Golgi,** in the nerve cells of barn owl and cat by means of impregnation method, and named after him in 1898. With the aids of special staining techniques the Golgi bodies were seen as densely stained region of the cytoplasm under the optical microscope. Under the electron microscope the Golgi apparatus is seen to be composed of stacks of flattened structures which contains numerous vesicles containing secretory granules.

The Golgi apparatus is the processing, packaging and secretion organelle of the cell. It is found in all eukaryotic cells with the exception of mammalian erythrocytes, sieve tube elements. Prokaryotic cell do not contain the apparatus. In plants Golgi apparatus is formed of a number of unconnected units called Dictyosomes. The newly synthesized proteins, found in the channels of

the rough endoplasmic reticulum are moved to the Golgi body where the carbohydrates are added to them and these molecules are enveloped in a part of the Golgi membrane and then the enveloped molecules leave the cell. The Golgi apparatus hence acts as the assembly factory of the cell where the raw materials are directed to the Golgi apparatus before being passed out from the cell.

Golgi apparatus Definition

An organelle, consisting of layers of flattened sacs, that takes up and processes secretory and synthetic products from the endoplasmic reticulum and then either releases the finished products into various parts of the cell cytoplasm or secretes them to the outside of the cell. The Golgi complex is responsible inside the cell for packaging of the protein molecules before they are sent to their destination. This organelles helps in processing and packaging the macromolecules like proteins and lipids that are synthesized by the cell, sometimes referred as "*post office"* of the cell.

Origin

The intracellular origin of Golgi bodies has been a hotly debated subject for many years. Among the proposed sources of new Golgi bodies are:

(i) Vesicles dis-patched from the endoplasmic reticulum,

(ii) Vesicles dispatched from the outer membrane of the nuclear envelope,

(iii) Vesicles formed by invaginations of the plasma membrane, and

(iv) Division of Golgi bodies al-ready present in the cell.

The most widely accepted view is that Golgi bodies are formed from vesicles dis-patched from the ER. These vesicles are called transi-tion vesicles Transition vesicles migrate to the forming face of the Golgi body, fuse there with existing cisterna membranes, and in so doing contribute to the organelle's growth.

Structure

Shape and size of Golgi complex is largely dependent upon type of cell and its physiological state. It is small in muscle cell but it is well developed in secretary cells. Further, it can be compact stack of fenestrated saccules or a diffuse network of lamellae. It posses four types of components: cisternae, tubules, vesicles and vacuoles.

1. The Golgi apparatus is a major organelle in most of the eukaryotic cells. They are membrane bound organelles, which are sac-like. They are found in the cytoplasm of plant and animal cells.

2. The Golgi complex is composed of stacks of membrane-bound structures, these structures are known as the cisternae. An individual stack of the cisternae is sometimes referred as Dictyosome.

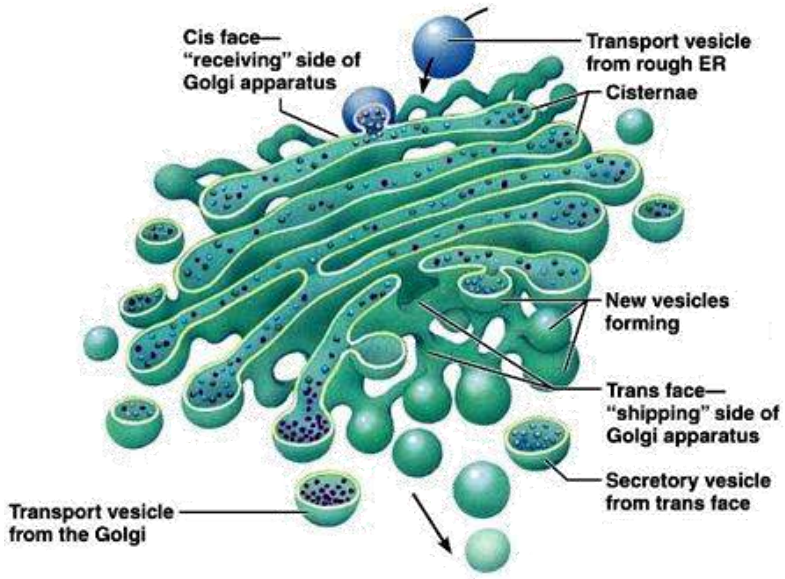

Fig.2.12 Structure of Golgi complex

3. In a typical animal cell, there are about 40-100 stacks. In a stack there are about four to eight cisternae. Each cisternae is a disc enclosed in a membrane, it possess special enzymes of the Golgi which help to modify and transport of the modified proteins to their destination.
4. The flat sacs of the cisternae are stacked and is bent and semicircular in shape. Each group of stacks is membrane bound and its insides are separated from the cytoplasm of the cell. The interaction in the Golgi membrane in responsible for the unique shape of the apparatus.
5. The Golgi complex is polar in nature. The membranes of one end of the stack is different in composition and thickness to the membranes at the other end.
6. One end of the stack is known as the **Cis-face**, it is the "receiving department" while the other end is the **Trans-face** and is the "shipping department". The Cis-face of the Golgi apparatus is closely associated with the endoplasmic reticulum.

Golgi apparatus Function

1. The cell synthesizes a huge amount of variety of macromolecules. The main function of the Golgi apparatus is to modify, sort and package the macromolecules that are synthesized by the cells for secretion purposes or for use within the cell.
2. It is involved in the formation of lysosomes and other enzyme-containing cellular inclusions, and in the formation of secretory granules in cells such as those found in the pancreas, pituitary and mammary glands, and mucous-secreting glands of the intestine and in many other cell types.
3. They are also involved in the transport of lipid molecules around the cell. The Golgi complex is thus referred as post office where the molecules are packaged, labeled and sent to different parts of the cell.

4. It mainly modifies the proteins that are prepared by the rough endoplasmic reticulum. The enzymes in the cisternae have the ability to modify proteins by the addition of carbohydrates and phosphate by the process of glycosylation and phosphorylation respectively.
5. Carbohydrates are synthesized in the Golgi body. The process of carbohydrate synthesis involves production of polysaccharides and glycosaminoglycans (GAGs).
6. The long, unbranched polysaccharides and GAGs are attached to proteins in order to form proteoglycans, the molecules that are present in the extracellular matrix of the animal cells.
7. Sulfation process of certain molecules is an important task carried out by the Golgi body. The sulfating of substances passing through the lumen of Golgi body is carried out with the help of sulfotransferases.
8. To carry out the glycosylation and phosphorylation processes, nucleotide sugars and ATP are imported by the Golgi apparatus from cytosol.
9. Golgi apparatus plays an important role in the prevention of destruction of cells (or apoptosis). The Bcl-2 genes present in the Golgi are used for this purpose.

Functions (Secretion) of Golgi complex in different types of cells

S.No.	Cell Type	Golgi body Functions
1	Plant cells	Secretion of protein and cellulose.
2	Exocrine cells of Pancreas	Secretion of Zymogen (Digestive enzymes-protease, lipase)
3	Goblet cells of intestinal mucosa	Secretion of mucus and Zymogens
4	Paneth cells of intestine	Secretion of proteins
5	Brunner's gland cell or duodenum and ileum	Secretion of mucopolysaccharides (Glycosaminoglycan)
6	Hepatic cells of liver	Transformation and secretion of lipids
7	Follicle cells of thyroid gland	Prothyroglobulins
8	Plasma cells of blood	Immunoglobulins
9	Cells of alveolar epithelium (mammary glands)	Secretion of milk proteins

2.10 ENDOPLASMIC RETICULUM

Endoplasmic reticulum is a continuous membrane, which is present in both plant cells, animal cells and absent in prokaryotic cells. It is the membrane of network tubules and flattened sacs, which serves a variety of functions within the cell. The space, which is present in the endoplasmic reticulum, is called as the Lumen.

The word reticulum, which means "*network*", was applied to describe the fabric of membranes. It can be defined as a eukaryotic organelle, which forms a network of tubules,

vesicles and cisternae within the cells. There are two regions of the Endoplasmic reticulum, which differ in both structure and function. One region is called as Rough Endoplasmic Reticulum, as it contains ribosome attached to the cytoplasmic side of the membrane and they are the series of flattened sacs. The other region is called as Smooth Endoplasmic Reticulum as it lacks the attached ribosome and they are tubule network.

The electron microscope reveals an extensive membrane system in the cytoplasm called Endoplasmic reticulum (ER). It was first reported by **Keith R. Porter** (a Canadian-American cell biologist) in 1945. This continuous membrane system joins the nuclear membrane on one end and the cell membrane on the other.

Types of Endoplasmic Reticulum

Two types of ER, such as smooth walled and rough walled, have been recognized. They may be present in the same or different types of cells.

(i) Smooth Endoplasmic Reticulum (SER): The surface of this type of reticulum is smooth as ribosomes not attached. Smooth ER is present in cells, which are actively engaged in steroid synthesis, carbohydrate metabolism, pigment production etc.

(ii) Rough Endoplasmic Reticulum: The rough ER have ribosomes attached throughout the surface. These are present in cells, which are active in protein synthesis.

Plant Cell Endoplasmic Reticulum

In plant cell, the endoplasmic reticulum acts as a port for the entry of proteins into the membrane. It also plays a vital role in the biosynthesis and storage of lipids. There are number of soluble membrane, which are associated with the enzymes and the molecular chaperones. The general functions of the endoplasmic reticulum in plant cell are protein synthesis and maturation. Endoplasmic reticulum of plant cell possesses some additional functions, which is not found in animal cells. The additional function involves cell to cell communication between specialized cells and also it serves as a storage site for proteins. Endoplasmic reticulum of plant cell contains enzymes and structural proteins, which are involved in the process of oil body biogenesis and lipid storage. In plants, the endoplasmic reticulum is connected between the cells via the plasmodesmata.

Animal Cell Endoplasmic Reticulum

In animal cells, the endoplasmic reticulum is a network of sacs, which play a vital role in manufacturing, processing and transporting different types of chemical compounds for use of both inside and outside of the cell. It is connected to the double-layered nuclear envelope, which provides the pipeline between the nucleus and the cytoplasm of a cell. In animal cells, the endoplasmic reticulum is a multifunctional organelle, which synthesis the membrane lipids, proteins and also regulates the intracellular calcium.

Endoplasmic Reticulum Structure

1. Endoplasmic reticulum is an extensive membrane network of cisternae (sac-like structures), which are held together by the cytoskeleton. The phospholipid membrane encloses a space, the lumen from the cytosol, which is continuous with the Perinuclear space.
2. The surface of the rough endoplasmic reticulum is studded with the protein manufacturing ribosome, which gives it a rough appearance. Hence it is referred as a rough endoplasmic reticulum.
3. The smooth endoplasmic reticulum consists of tubules, which are located near the cell periphery. This network increases the surface area for the storage of key enzymes and the products of these enzymes.
4. Rough endoplasmic reticulum synthesizes proteins, while smooth endoplasmic reticulum synthesizes lipids and steroids. It also metabolizes carbohydrates and regulates calcium concentration, drug detoxification, and attachment of receptors on cell membrane proteins.
5. Endoplasmic reticulum varies extensive extending from the cell membrane through the cytoplasm and forming a continuous connection with the nuclear envelope.

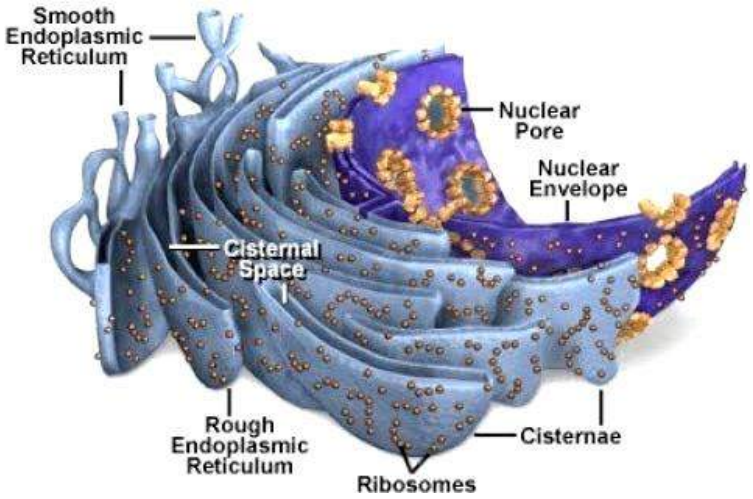

Fig.2.10 Structure of Endoplasmic reticulum

The Major Functions of Endoplasmic reticulum

(1)Common to both Endoplasmic Reticulum:

(i) Forms the skeletal framework.
(ii) Active transport of cellular materials.
(iii) Metabolic activities due to presence of different enzymes.
(iv) Provides increased surface area for cellular reactions.
(v) Formation of nuclear membrane during cell division.

(2) Function of Smooth Endoplasmic Reticulum:

(i) Lipid synthesis.

(ii) Glycogen synthesis.
(iii) Steroid synthesis like cholesterol, progesterone, testosterone, etc.
(iv) Metabolism of carbohydrates.
(v) Detoxification function.
(vi) Major storage and released site of inter cellular calcium ions.

(3) Function of Rough Endoplasmic Reticulum:

(i) It provides site for protein synthesis.
(ii) Protein translocation, folding and transport of protein.
(iii) Glycosylation (this is the relation of a saccharides group with a hydroxyl or amino functional group to form a glucoside).
(iv) Disulfide bond formation (disulfide bonds stabilize the tertiary and quaternary structures of many proteins).
(v) Membrane synthesis.

2.11 SUMMARY

A cell is enclosed by a plasma membrane, which forms a selective barrier that allows nutrients to enter and waste products to leave. The interior of the cell is organized into many specialized compartments, or organelles, each surrounded by a separate membrane. One major organelle, the **nucleus**, contains the genetic information necessary for cell growth and reproduction. Each cell contains only one nucleus, whereas other types of organelles are present in multiple copies in the cellular contents, or cytoplasm.

Organelles include **mitochondria**, which are responsible for the energy transactions necessary for cell survival; **lysosomes**, which digest unwanted materials within the cell; and the **endoplasmic reticulum** and the **Golgi apparatus**, which play important roles in the internal organization of the cell by synthesizing selected molecules and then processing, sorting, and directing them to their proper locations. In addition, plant cells contain **chloroplasts**, which are responsible for photosynthesis, whereby the energy of sunlight is used to convert molecules of carbon dioxide (CO2) and water (H2O) into carbohydrates. Between all these organelles is the space in the cytoplasm called the **cytosol**. The cytosol contains an organized framework of fibrous molecules that constitute the **cytoskeleton**, which gives a cell its shape, enables organelles to move within the cell, and provides a mechanism by which the cell itself can move. The cytosol also contains more than 10,000 different kinds of molecules that are involved in cellular biosynthesis, the process of making large biological molecules from small ones.

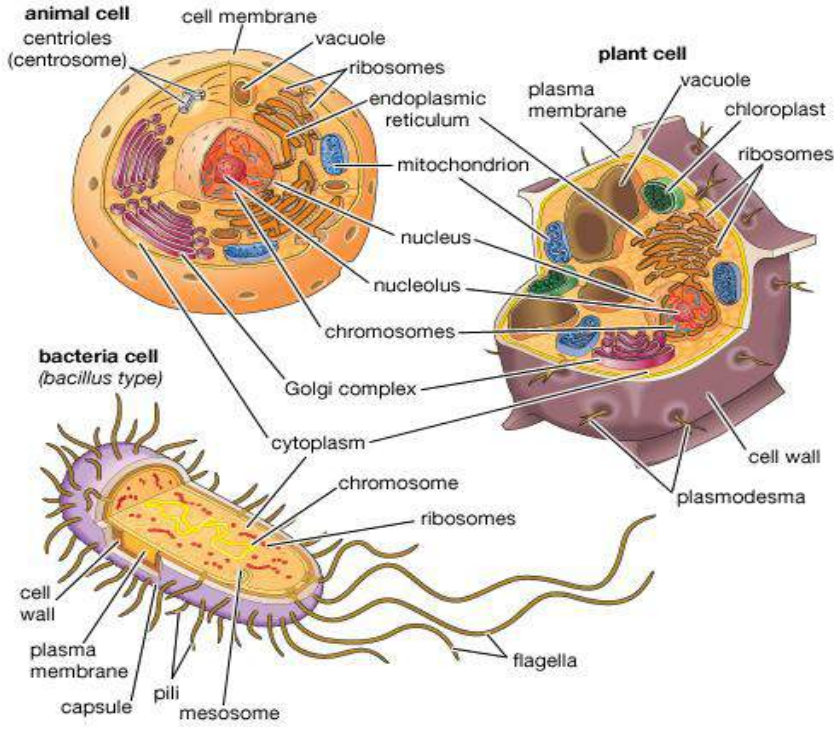

Fig.2.11 Structure of some typical cells

2.12 GLOSSARY

Aleurone: Protein stored as granules in the cells of plant seeds.

Carotenoid: Any of a group of red and yellow pigments, chemically similar to carotene, contained in animal fat and some plants.

Cell Biology: Sub-discipline of biology that focuses on the study of the basic unit of life, the cell.

Cell wall: Of many cells (not animal cells), a semi-rigid but permeable structure that surrounds the plasma membrane; helps a cell retain its shape and resist rupturing.

Cell: Smallest unit that still displays the properties of life; it has the capacity to survive and reproduce on its own.

Central vacuole: In many mature, living plant cells, an organelle that stores amino acids, sugars, and some wastes; when it enlarges during growth, it forces the cell to enlarge and increase its surface area.

Centrioles: a small, cylindrical cell organelle, seen near the nucleus in the cytoplasm of most eukaryotic cells, that divides in perpendicular fashion during mitosis, the new pair of centrioles moving ahead of the spindle to opposite poles of the cell as the cell divides: identical in internal structure to a basal body.

Chloroplast: A plastid containing chlorophyll. Organelle of photosynthesis in plants and algae.

Chromatin: All of the DNA molecules and associates proteins in a nucleus.

Chromatin: The readily stainable substance of a cell nucleus, consisting of DNA, RNA, and various proteins, that forms chromosomes during cell division.

Chromoplast: A plastid containing coloring matter other than chlorophyll.

Chromosome: A long, stringy aggregate of genes that carries heredity information (DNA) and is formed from condensed chromatin.

Cisternae: A cisterna (plural cisternae) is a flattened membrane disk of the endoplasmic reticulum and Golgi apparatus.

Coenocytes: An organism made up of a multinucleate, continuous mass of protoplasm enclosed by one cell wall, as in some algae and fungi.

Cristae: Each of the partial partitions in a mitochondrion formed by infolding of the inner membrane.

Cytokinesis: The division of the cell cytoplasm that usually follows mitotic or meiotic division of the nucleus.

Cytoplasm: Consists of all of the contents outside of the nucleus and enclosed within the cell membrane of a cell.

Cytoskeleton: A network of fibers throughout the cell's cytoplasm that helps the cell maintain its shape and gives support to the cell.

Cytosol: Semi-fluid component of a cell's cytoplasm.

Detoxification: The metabolic process by which toxins are changed into less toxic or more readily excretable substances.

Dictyosome: The set of flattened membranes in a Golgi body, resembling a stack of plates.

Dictyosomes: Are stacks of flat, membrane-bound cavities (cisternae) that together comprise the Golgi apparatus.

Endocytosis: The taking in of matter by a living cell by invagination of its membrane to form a vacuole.

Endo-membrane system: Endoplasmic reticulum, Golgi bodies, and transport vesicles concerned with modification of many new proteins, lipid assembly, and their transport within the cytoplasm or to the plasma membrane for export.

Endoplasm: The more fluid, granular inner layer of the cytoplasm in amoeboid cells.

Endoplasmic reticulum: The endoplasmic reticulum is a type of organelle in eukaryotic cells that forms an interconnected network of flattened, membrane-enclosed sacs or tube-like structures known as cisternae.

Endosymbiosis: Symbiosis in which one symbiont lives within the body of the other.

Euchromatin: The part of a chromosome that condenses maximally during metaphase and contains most of the genetically active material.

Eukaryote: Any organism whose cells have a cell nucleus and other organelles enclosed within membranes. Eukaryotes belong to the domain Eukaryota, and can be single-celled or multicellular.

Flagella: A long, lash-like appendage serving as an organ of locomotion in protozoa, sperm cells, etc.

Genomics: The study of genomes.

Glyoxysomes: Are specialized peroxisomes found in plants (particularly in the fat storage tissues of germinating seeds) and also in filamentous fungi.

Golgi Bodies: A complex of vesicles and folded membranes within the cytoplasm of most eukaryotic cells, involved in secretion and intracellular transport.

Granum: One of the structural units of a chloroplast in vascular plants, consisting of layers of thylakoids.

Heredity: It is the process by which features and characteristics are passed on from parents to their children before the children are born.

Homeostasis: The tendency of a system, especially the physiological system of higher animals, to maintain internal stability, owing to the coordinated response of its parts to any situation or stimulus that would tend to disturb its normal condition or function.

Hyaloplasm: The clear fluid portion of cytoplasm as distinguished from the granular and netlike components. Also called ground substance

Invagination: The action or process of being turned inside out or folded back on itself to form a cavity or pouch.

Leucoplast: A colourless organelle found in plant cells, used for the storage of starch or oil.

Lumen: The cavity that the cell walls enclose.

Lysosome: An organelle in the cytoplasm of eukaryotic cells containing degradative enzymes enclosed in a membrane.

Mitoplast: A mitochondrion that has been stripped of its outer membrane leaving the inner membrane intact.

Mycoplasma: It is a genus of bacteria that lack a cell wall around their cell membrane. Without a cell wall, they are unaffected by many common antibiotics such as penicillin or other β-lactam antibiotics that target cell wall synthesis.

Nuclear Envelope: A double membrane that is the other boundary of the nucleus.

Nucleoid: The nucleoid (meaning nucleus-like) is an irregularly shaped region within the cell of a prokaryote that contains all or most of the genetic material, called genophore.

Nucleolus: In an interphase nucleus, a mass of material from which RNA and proteins are assembled into the subunits of ribosomes.

Nucleoplasm: The protoplasm of the nucleus of a cell.

Nucleus: A dense organelle present in most eukaryotic cells, typically a single rounded structure bounded by a double membrane, containing the genetic material.

Organelles: One of the membrane- bound compartments that carry out specialized metabolic functions in eukaryotic cells; e.g. a nucleus, mitochondria.

Perinuclear space: The space between the membranes.

Phospholipid: Any of a group of fatty compounds, as lecithin, composed of phosphoric esters, and occurring in living cells.

Photolysis: The decomposition or separation of molecules by the action of light.

Photorespiration: The oxidation of carbohydrates in many higher plants in which they get oxygen from light and then release carbon dioxide, somewhat different from photosynthesis.

Photosystems: Are functional and structural units of protein complexes involved in photosynthesis that together carry out the primary photochemistry of photosynthesis: the absorption of light and the transfer of energy and electrons.

Plasmid: A plasmid is a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA. Plasmids naturally exist in bacterial cells, and they also occur in some eukaryotes.

Plastid: The plastid is a major double-membrane organelle found in the cells of plants, algae, and some other eukaryotic organisms. Plastids are the site of manufacture and storage of important chemical compounds used by the cell.

Polysome: A complex of ribosomes strung along a single strand of messenger RNA that translates the genetic information coded in the messenger RNA during protein synthesis.

Porins: Are beta barrel proteins that cross a cellular membrane and act as a pore, through which molecules can diffuse.

Porins: They are beta barrel proteins that cross a cellular membrane and act as a pore, through which molecules can diffuse.

Prokaryote: A prokaryote is a unicellular organism that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle.

Proteins: Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues. It perform a vast array of functions within organisms, including catalyzing metabolic reactions, DNA replication, responding to stimuli, and transporting molecules from one location to another.

Ribosome: A tiny, somewhat mitten-shaped organelle occurring in great numbers in the cell cytoplasm either freely, in small clusters, or attached to the outer surfaces of endoplasmic reticulum, and functioning as the site of protein manufacture.

Sedimentation: The deposition or accumulation of sediment.

Senescence: No longer capable of dividing but still alive and metabolically active.

Stalked particles: On the inner surface of cristae contain the enzymes required to make ATP from ADP and phosphate.

Stroma: The matrix of a chloroplast, containing various molecules and ions. The supporting framework or matrix of a cell.

Symbiosis: The living together of two dissimilar organisms, as in mutualism, commensalism, amensalism, or parasitism.

Syncytium: A multinucleate mass of cytoplasm that is not separated into cells.

Thylakoid: A flattened sac or vesicle lined with a pigmented membrane that is the site of photosynthesis, in plants and algae occurring in interconnected stacks constituting a granum of the chloroplast, and in other photosynthesizing organisms occurring either singly or as part of the cell membrane or other structure.

Tonoplast: A membrane separating a vacuole from the surrounding cytoplasm in a plant cell.
Vacuole: A membrane-bound cavity within a cell, often containing a watery liquid or secretion.
Vesicle: It is a small structure within a cell, or extracellular, consisting of fluid enclosed by a lipid bilayer. Vesicles form naturally during the processes of secretion (exocytosis), uptake (endocytosis) and transport of materials within the cytoplasm.

2.13 SELF ASSESSMENT QUESTION

2.13.1 Very short questions:

1. What are infoldings of inner membrane of mitochondria known as?
2. What are flattened or tubular Golgi bodies known as?
3. Which organelle is known as semiautonomous organelle?
4. Which organelle contains both DNA and RNA?
5. Plastids are present in which type of cells?
6. Which cell organelle plays a crucial role in detoxifying many poisons and drugs in a cell?
7. Smooth endoplasmic reticulum is the site of ?
8. Ribosomes are composed of?
9. The cell organelle involved in forming complex sugars from simple sugars are ?
10. The proteins and lipids, essential for building the cell membrane, are manufactured by ?

2.13.2 Objective type Questions:

1- The chloroplast thylakoids are in the form of
(a) Interconnected sacs (b) Interconnected tubule
(c) Independent discs (d) Stacked discs

2. Golgi bodies in cell arise from
(a) Plasma membrane (b) Endoplasmic reticulum
(c) Nuclear membrane (d) Chloroplast

3. Ribosomes are present in
(a) E.R (b) Mitochondria
(c) Chloroplast (d) All of the above

4. Cristae represent
(a) Outer membrane of mitochondria (b) Inner membrane of mitochondria
(c) Outer membrane of chloroplast (d) Inner membrane of chloroplast

5. Endoplasmic reticulum arises from
(a) Mitochondria (b) De novo synthesis
(c) Pre existing reticulum (d) Chloroplast

6. Two sub units of 80S ribosomes are
(a) 40S+40S (b) 50S+30S
(c) 60S+20S (d) 60S+40S

7. The type of plastid that store starch are
(a) Leucoplast (b) Amyloplast
(c) Elaioplast (d) Aleuroplast

8. Ribosomes were first discovered by
(a)Claude in 1945 (b) Claude in 1965
(c) Palade in 1955 (d) Palade in 1970

9. Golgi complex plays a major role in
(a) Protein synthesis
(b) Glycosylation of lipids and proteins
(c) Removal of sulphate from the carbohydrate moiety of glycolipids
(d) formation of secondary lysosomes

10. Function of Golgi apparatus in animals cells include
(a) Sorting and packaging (b) Exocytosis of melanin granules
(c) Exocytosis of thyroxine hormone (d) All of these

11. Golgi apparatus is often seen associated with
(a) Mitochondria (b) RER
(c) Lysosomes (d) None of these

12. Golgi apparatus is absent in
(a) Higher (b) Yeast
(c) Bacteria and Blue green algae (d) None

13. Golgi complex was first recognized
(a) Blood cell (b) Root cell
(c) Nerve cell (d) None

14. Which of the following organelle is called as the "Traffic Police" of the cell?
(a) Lysosome (b) SER
(c) Golgi apparatus (d) RER

15. The simplest unit, the saucer-like closed compartments of Golgi apparatus is called
(a) Tubules (b) Vesicles
(c) Cristae (d) Cisternae

16. In chloroplast, photosynthesis reactions occur in

(a) Thylakoid membrane (b) Thylakoid lumen
(c) Stroma (d) Inner chloroplast membrane

17. In the presence of light, pH of lumen of Thylakoid
(a) Increases (b) Decreases
(c) Remain same (d) None

18. If the Thylakoid are treated with detergents to solubilize lipids and then proteins gently isolated, the following complexes will be observed, except
(a) ATP synthesis (b) Cytochrome oxidase
(c) Light harvesting complex (d) Pheophytin

19. Which of the following protein targeting mechanisms is common for mitochondria and chloroplast organelles?
(a) Co-translational (b) Post-translational
(c) Co-translational and post-translational (d) De novo protein synthesis

20. The place in the mitochondria where pH is lowest during aerobic respiration
(a) Matrix (b) Cytosol
(c) Intermembrane space (d) Mitochondria ribosome

21. The DNA molecules within mitochondria and chloroplast have
(a) Only linear form (b) Covalently closed circular form
(c) DNA having covalently sealed end (d) Linear dsDNA with single chain breaks

22. The Nucleosome
(a) Contains DNA and non-histone proteins
(b) Has a core of histones with DNA wound around it
(c) Is fully responsible for DNA packaging into chromosomes
(d) Surrounds nuclear pores

23. The three DNA sequences, which define a chromosome, include the following except
(a) Centromere (b) Enhancer
(c) Origin of DNA replication (d) Telomere

24. Nucleolus Organizing Region (NOR) occurs in the region of
(a) Secondary constriction (b) Primary constriction
(c) Telomere (d) Centromere

25. Which of the following is a double membrane bound organelles

(a) Mitochondria (b) Chloroplast
(c) Nucleus (d) All of these

26. Plastids are absent in
(a) Animals and plants (b) Fungi and animals
(c) Animals, bacterium and fungi (d) None of these

27. All are colorless plastids (leucoplasts) except
(a) Elaioplast (b) Amyloplast
(c) Proteinoplast (d) Rhodoplast

28. Chloroplast is similar to mitochondria in having
(a) Double layered membrane (b) Circular DNA
(c) 70s Ribosomes (d) All of these

29. Nucleoplasm is found inside
(a) Hemoglobin (b) Nucleus
(c) Cell membrane (d) Nucleoli

30. Which of the following pigment is most abundant in green plants
(a) Chlorophyll a (b) Chlorophyll b
(c) Carotene (d) Xanthophyll

2.13.3 Fill in the blanks:

1. Enzymes located in the _____that are needed to carry out the light-independent reactions.
2. Organelle without a cell membrane is_____
3. Plant cell wall mainly composed of_____
4. Ribosomes are responsible for assembling the_____ of the cell.
5. There are two places that ribosomes usually exist in the cell, suspended in the_____ and bound to the_____
6. In higher plants, the shape of the chloroplast is_____
7. _____ is also known as Kitchen of the cell.
8. The only cell organelle seen in prokaryotic cell is_____
9. The undefined nuclear region of prokaryotes are also known as_____
10. _____controls most of the cell processes and contains the hereditary information of DNA.

2.13.1 Answers Key: (1) Cristae, (2) Cisternae, (3) Mitochondria, (4) Chloroplast, (5) Plant cells, (6) Smooth endoplasmic reticulum, (7) Lipid synthesis, (8) RNA & Proteins, (9) Golgi apparatus, (10) Rough Endoplasmic Reticulum

2.13.2. Answers Key: 1-(d), 2-(d), 3-(d), 4-(b), 5-(c), 6-(d), 7-(b), 8-(c), 9-(b), 10-(d), 11-(b), 12-(c), 13-(c), 14-(c), 15-(d), 16-(a), 17-(a), 18-(b), 19-(b), 20-(c), 21-(b), 22-(b), 23-(b), 24-(a), 25-(d), 26-(d), 27-(d), 28-(d), 29-(b), 30-(a)

2.13.3 Answers Key: 1- Stroma of the Chloroplast, 2- Ribosome, 3- Cellulose, 4- Proteins, 5- Cytoplasm, Endoplasmic Reticulum, 6- Discoid, 7- Golgi apparatus, 8- Ribosomes, 9- Nucleoid, 10. Nucleus

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2.16 TERMINAL QUESTIONS

2.16.1 Short answer type Questions:

a) Give the principle roles of nucleus.
b) List the main functions of chloroplast.
c) Give the origin of mitochondria.
d) List the types and subunits of ribosomes.
e) Give a general account of nuclear pore.

2.16.2 Long answer type Questions:

1. Give an account of the structure of nucleus. Discuss the structure and function of different components of nucleus.

2. What are ribosomes? What is meant by 70S and 80S ribosomes? Also highlight the function of ribosomes.
3. Explain why mitochondria are considered as semiautonomous organelles. What are the characteristic features of mitochondria that aid in their identification?
4. What are chloroplasts? What is the main function of chloroplasts?
5. Draw a neat diagram of plant cell and label any three parts which differentiate it from animal cell.
6. Discuss the structure of chloroplast giving details of structure of thylakoids. How are these organized in different plants?
7. Give a detailed account on types of plastids and their functions.
8. Write short notes on
   (i) Lysosomes (ii) Golgi apparatus (iii) Ribosome
9. Write an essay on Golgi complex and its function in cell.
10. Give an account of structure and function of endoplasmic reticulum. What is the difference between the smooth and rough endoplasmic reticulum?

UNIT-3 STRUCTURE AND TYPES OF CHROMOSOMES

3.1 OBJECTIVES

After reading this unit student will be able:

- To study about their shape, size, nature and function of chromosomes
- To study about structure of chromosomes
- To learn how does prokaryotic and eukaryotic chromosomes differ
- To understand how large sized chromosomes are packed into nucleus
- To study about special types of chromosomes

3.2 INTRODUCTION

All the living cells contains DNA as genetic material (viruses can have DNA or RNA as genetic material). Genetic material of all living organisms including viruses is made up of nucleoprotein because DNA (nucleic acid) is found to be associated with specific proteins hence called as **nucleoprotein**. It is actually this binding of DNA with proteins, due to which DNA exists in condensed state. Packaging of DNA helps to conserve space in the cells. Approximately, two meters of the human DNA can fit into a cell that is only a few micrometers wide. The chromosomes are found in the nucleus of the cell. In prokaryotic organisms, the DNA is not present in the nucleus; the DNA floats in the cytoplasm in an area called the **nucleoid**. Nuclear DNA of eukaryotes (plants and animals) is present in the form of **chromosomes**.

Chromosomes are made up of DNA segments and each chromosome comprises of single long duplex of DNA. Chromosomes carry all the information that helps a cell to grow, survive and reproduce. DNA segments with specific patterns are called genes. Chromosomes carry genes which transmit genetic information from one generation to another. Each chromosome can contain large number of genes. The position on which genes are present on chromosomes is called **locus**. Every species contains a fixed number of chromosomes pairs. However, chromosomes of eukaryotes are much more complex as compared to that of prokaryotes and viruses.

The chromosomes vary widely between different organisms. Eukaryotic cells have large number of linear chromosomes and cells of prokaryotes have smaller and circular DNA. Cells may contain more than one type of chromosome, like in most eukaryotic cells. The mitochondria and the chloroplasts in plant cells possess their own set of chromosomes. In nucleus of eukaryotic organism, the chromosomes are packed by proteins to form a compact structure called **chromatin.** This condensation allows long molecules of DNA to fit into the cell nucleus. Chromosomes are more condensed than the chromatin and they are essential for cell division. They are replicated, divided and passed on to the daughter cells, to ensure genetic diversity and survival of the progeny. Duplicated chromosomes contain two identical copies, known as **chromatids** or sister chromatids; they are joined by a **Centromere**. Compaction of the chromosomes during the cell division process, results in the four-arm structure. Recombination of chromosome plays a vital role in genetic diversity. Incorrect multiplication of the

chromosomes may lead to mitotic failure or death of the cell; it may lead to apoptosis and sometimes may be cancerous.

3.3 CHROMOSOMES

A chromosome can be considered a stainable threadlike nuclear component having special organization, individuality and function. Their presence was first demonstrated in the eukaryotic cell in 1875 by **Eduard A. Strasburger** (1844-1912), a German plant cytologist and the founder of modern plant cell biology. These were first termed as chromosomes in 1888 by a German anatomist, **W. Waldeyer** (1836-1921). This term is actually taken from Greek word **chromosoma** which means "**coloured bodies**" (chroma = colour; soma = body) due to their marked affinity for basic dyes as a consequence of which they are stained. This property is known as **Chromaticity**.

Staining the cell, with certain types of stain (e.g., Aceto-orcein, Acetocarmine, Feulgen's stain) shows that chromosomes are not visible in the interphase nucleus or metabolically active nucleus due to their high water content, but can be easily seen during cell division characteristics whether mitosis or meiosis. During cell division, the chromosome undergoes dehydration, spiralization and condensation. So they become progressively thicker and smaller and, accordingly, the stability of chromosome also increases. Hence the chromosome becomes readily observable under microscope. Staining of chromosomes is generally carried out to make them visible under light microscope.

Chromosomes are capable of **duplication** and maintaining their morphologic and physiologic properties through successive **cell divisions**. It has also been demonstrated that the chromosome contains DNA, which in turn, carries the genes and thus plays a major role in **heredity**. When reproduction of organism takes place, they are passed on to the next generation through the **gametes**. Besides, they play an important role in variation, mutation and evolution, and in their control of **morphogenesis**, multiplication and equilibrium of vital processes.

Table.1-Chemical composition of chromosome

Substance	Amount
DNA	40 % approx
Histone proteins	50 %
Non-Histone proteins	8.5 %
Metallic ions like Mg++, Ca++ etc.	In traces

The term chromosome is mainly used to describe the chromosome of eukaryotic cell. The naked DNA of prokaryotes and DNA or RNA of viruses is sometimes broadly called prokaryotic chromosome and viral chromosome, respectively, due to their similarity in fundamental properties with **eukaryotic chromosomes**. But the morphology and the organization of eukaryotic chromosome is much more complex. The morphology of chromosomes in all

eukaryotes is essentially similar-except some variations in number and size.

Most of the chromosomes in a eukaryotic cell are called **autosomes** which control all somatic characteristic of an organism. But, in addition, there are some other chromosomes which control some specialized characteristics of an organism and are called **allosomes**. **Sex chromosome** (X and Y) for determination of sex, **B-chromosomes**, **L-chromosomes**, **M-chromosomes**, **S-chromosomes** and **E-chromosomes** are examples of allosomes. Autosomes are universally present in all eukaryotic-organisms, but allosomes may or may not be present in all organisms.

3.3.1 Structure of Chromosome

Basic Structure of Chromosomes

1. Chromosomes are thread like bodies present in nuclei of animals and plants.
2. They are covered with a sheath made of proteins.
3. Inside this sheath is present granular matter referred as '**matrix**'.
4. Inside the matrix, there are two threads called '**Chromonemata**' which are the subunits of chromatids and are present during Prophase.
5. At Metaphase, the chromosome consists of two symmetrical strands called **Chromatids**.
6. Each chromosome possesses a distinct constriction called '**Centromere**' (Primary constriction), divides into two parts and it gets attached to the spindle network.
7. The ends of chromosome are termed as '**Telomeres**' and it protects from deterioration or from being destroyed.
8. Some chromosomes have another constriction called "**Secondary constriction**"

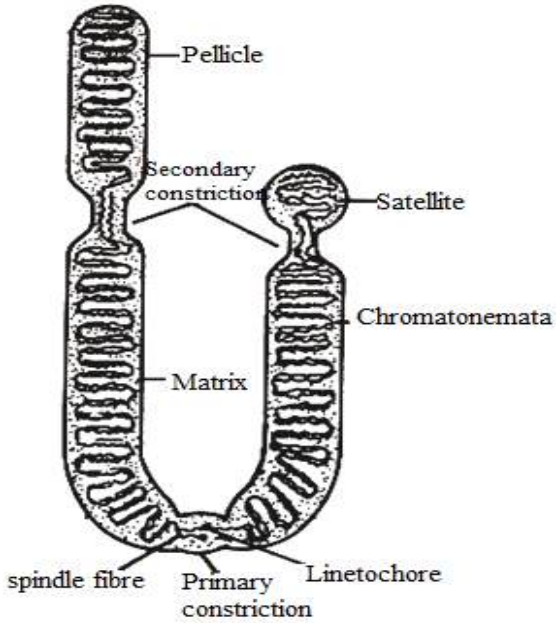

Fig. 3.1- Structure of a typical somatic chromosome at anaphase

3.3.1.1 Number, Size and Shape of Chromosome

(a) Chromosome Number: The number of chromosomes in a given species is usually constant containing diploid number of chromosomes in their somatic cells and haploid (gametic or reduced) number of chromosomes in their sex cells (sperms and ova). The number of chromosomes is variable from one to several hundred among different species. For example- In ***Ascaris megalocephala*** (horse roundworm) it is 2, while in certain protozoan's (group - ***Aggregata***), there are more than 300 chromosomes.

The chromosome numbers are also helpful for taxonomy. In the angiosperms, the most frequent haploid number is 12 and members of this group have a range from 3 to 16. Similarly, in fungi, haploid number ranges from 3 to 8. *Mucorheimalis* (bread mold fungus) chromosome number is 2 whereas; in *Ophioglossum* (Adder's tongue fern) it is 1262. In primates, this haploid number is from 16 to 30. The haploid set of chromosomes present in the nucleus of gametes is generally called **genome**. The somatic or body cells of most organisms contain two haploid set or genomes and are known as the **diploid cells**. The diploid cells achieve the diploid set of the chromosomes by the union of the haploid male and female gametes in the sexual reproduction.

In the radiolarian protozoan ***Aulacantha,*** is found a diploid number of approximately 1600 chromosomes. Among plants, chromosome number varies from 2n = 4 in ***Haplopappus gracilis*** (Asteraceae) to 2n = or > 1200 in some Pteridophytes. Chromosome number of few common animals and plants is given below:

Table-2: Chromosome numbers of various species of plants and animals

Species names of some Plant & Animals	Diploid (2N) Chromosome Number
Zea mays (Corn or maize)	20
Triticum vulgare (common wheat)	42
Ascaris lumbricoides (Giant roundworm)	48
Musca domestica (Housefly)	12
Drosophila melanogaster (fruit fly)	8
Homo sapiens (Man)	46
Macaca mulatta (Rhesus monkey)	42

(b) Chromosome size: The size of chromosomes varies greatly in size in organisms, it means that different organism have chromosomes of different size. Also, in same species, different chromosome pairs have different size. Chromosomes range, on an average from 0.5 to about 30µ in length and from 0.2 to 3µ in diameter. The relative number of chromosomes generally differs in the nucleus but at a time all chromosomes of a cell may be of the same size. Plant cells normally possess larger chromosomes than animal cells. *Trillium* spp. has chromosomes which may reach up to the length of 32µ at metaphase. Monocotyledon plants usually have larger

chromosomes than the dicotyledon which contain greater number of chromosomes. Among the animals, grasshoppers, crickets, mantids, newts and salamanders have large chromosomes.

(c) Chromosome Shape: The shape of the chromosomes is changeable from phase to phase in the continuous process of the cell growth and cell division. During cell division chromosomes may appear in different shapes, they can be rod shaped, twisted or spiral curved or filamentous. In the resting phase or interphase stage of the cell, the chromosomes occur in the form of thin, coiled, elastic and contractile, thread-like stainable structures, the chromatin threads. In the metaphase and the anaphase, the chromosomes become thick and filamentous. Based upon the position of centromere in anaphase, chromosomes may appear as rod-shaped, J-shaped and V-shaped. Each chromosome contains a clear zone, known as centromere or **kinetochore**, along their length. The centromere divides the chromosomes into two parts, each part is called **chromosome arm**.

3.3.1.2 Different region of Chromosome: There are different recognized regions of a chromosome.

(a) Centromere (Primary constriction): A metaphase chromosome has two identical sister chromatids, which are attached to each other at a point called centromere or primary constriction. At anaphase, the centromere splits the sister chromatids separate to become two anaphasic chromosomes. Therefore, anaphase chromosome is a half metaphase chromosome. The parts of chromosome on either side of centromere are called arms.

Thus metaphase chromosome has four arms while anaphase chromosome has two arms. The two arms are equal in isobrachial chromosomes and unequal in heterobrachial chromosomes. When the arms are unequal, the short arm is designated as ‘p’ and the long arm is designated as ‘q’.

Primary constriction is that part of chromosome which is comparatively narrow as compared to remaining part of chromosome. The position of primary constriction is fixed (constant) for each chromosome. Primary constriction divides the chromosome into two arms. Primary constriction is the region in which lies centromere.

Centromere is a highly condensed region which appears as a constriction in a chromosome. It is a region of chromosome to which spindle fibres attach during cell division (both mitosis and meiosis). The point of attachment of spindle fibres is called **kinetochore**. Also, through centromere region sister chromatids (formed as a result of DNA replication) are joined together.

Based on the position of centromere, chromosomes are called:

(1) **Telocentric** (centromere terminal) - When the position of centromere is terminal or proximal. There is no ‘p’ arm and chromosome is rod-shape at anaphase.

(2) **Acrocentric** (centromere sub-terminal)-When centromere is present at sub-terminal position on in a chromosome and capped by telomere. There is very small 'p' arm.

(3) **Submetacentric** (centromere sub-median) - In these chromosomes, centromere is slightly away from mid point. They are mainly of S shaped but sometimes, they are J-shaped or L-shaped. There is very little difference between 'p' and 'q' arm. **Example:** Mouse.

(4) **Metacentric** (centromere median). - When centromere lies in the middle of chromosome. They are also called V-shaped chromosome, where 'p' and 'q' arm have exactly same length. **Example:** Amphibians.

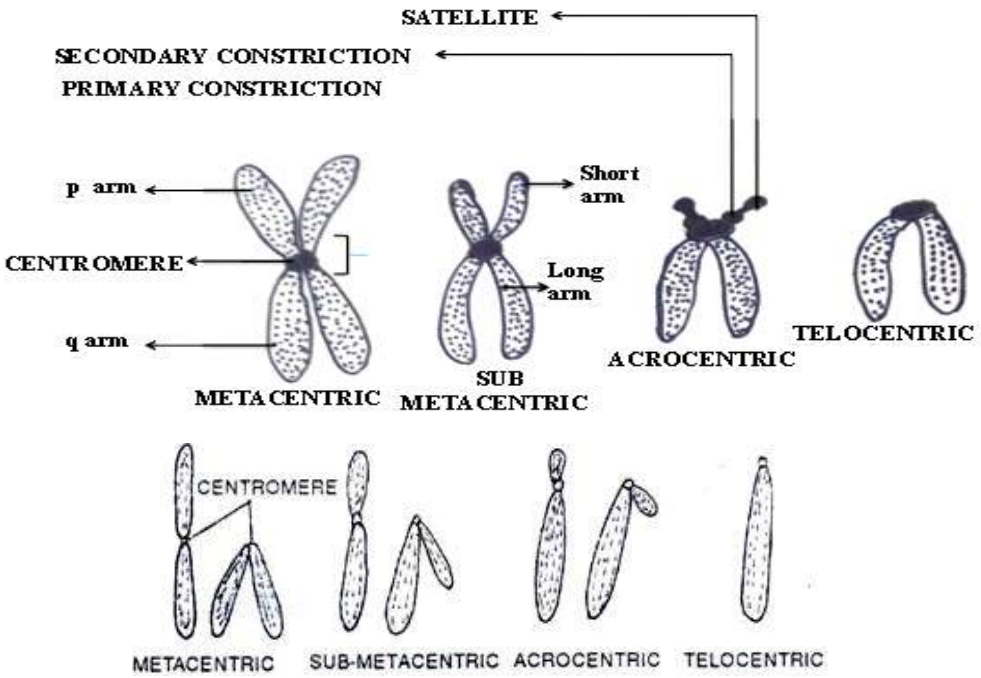

Fig. 3.2 - Types of chromosomes based upon position of centromere

Normally chromosomes are monocentric (one centromere); sometimes chromosomes may be dicentric (e.g. in wheat, maize etc.) or polycentric (e.g. *Luzula luzuloides* - family Juncaceae). However, in some insects, the centromere is diffused along the length of chromosome.

Table-3: Types of chromosomes based upon number of Centromere

Type of Chromosome	Number of Centromere
Monocentric	When one centromere is present
Dicentric	When two centromeres are present
Polycentric	Chromosome with more than two centromeres
Acentric	Chromatids without any centromere
Diffused	When the centromere is diffused throughout the length of chromosome

(b) Secondary Constriction: Any constricted or narrow region other than that of centromere or primary constriction is called as secondary constriction (Fig. 3.3.1). Secondary constriction is the area which is known to participate in the nucleolus formation. Hence secondary constriction is also known as **nucleolar organizer**. In humans, nucleolar organizer is found to be located in secondary constriction of chromosomes 13, 14, 15, 20 and 22.

It has constant position, and therefore, can be used as useful marker. It is generally found on the short arm of a chromosome, away from the centromere. But in some cases, it is located on the long arm. A chromosome segment separated from the main body of chromosome by one secondary constriction is known as **satellite**. A chromosome with secondary constriction is referred to as satellite chromosome or **Sat-chromosome**. The Sat-chromosomes are associated with nucleolar organizer.

(c) Tertiary constriction: Tertiary constriction is found to be present in all chromosomes and is utilized to distinguish between different chromosomes.

(d) Kinetochore: The surface of centromere bear a specialized multi-protein complex called kinetochore to which spindle fibers (microtubules) attach. The centromere of a metaphase chromosome contains two kinetochores facing in opposite direction. Kinetochore is trilaminar type in lower plants while ball and cup type in higher plants.

(e) Chromomeres: Sometimes along the entire length of interphase chromosomes appear beaded due to accumulation of chromatin. These '**bead-like**' structures are called chromomeres. At metaphase the chromomeres are tightly coiled and are no longer visible.

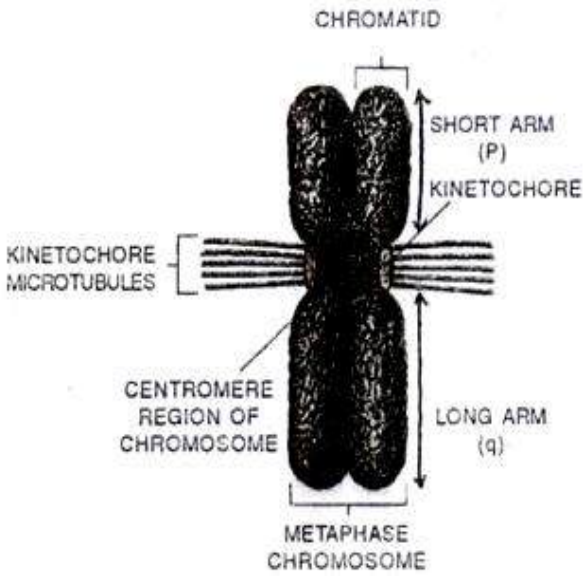

Fig. 3.3 Structure of a mitotic chromosome, Kinetochore microtubules are anchored to proteins at the centromere

(e) Telomere: The terminal region of a chromosome on either side is known as telomere. They provide stability and identity to each chromosome. Telomeric end prevent joining of two neighbouring chromosomes. These are not visible in the light or electron microscope, they are rather conceptual structures. Each chromosome has two telomeres. The telomere of one chromosome cannot unite with the telomere of another chromosome, due to polarity effect. In other words, translocations can occur when the ends of two chromosomes are damaged.

3.3.1.3 Chromatid and Chromatin

(i) Chromatids: At mitotic metaphase, each chromosome consists of two symmetrical structures called chromatids. One of the two distinct longitudinal subunits of a chromosome is called chromatid and each chromatid contains a single DNA molecule. These subunits of a chromosome get separated during anaphase. Chromatids are of two types, sister chromatids and non-sister chromatids. Sister chromatids are derived from one and the same chromosome, while non-sister chromatids originate from **homologous** chromosomes.

Chromatids are formed due to chromosome and DNA replications during interphase. Both chromatids are attached to each other only by the centromere and become separated at the beginning of anaphase, when the sister chromatids of a chromosome migrate to the opposite poles. After separation at anaphase each chromatid becomes a chromosome. Just before nuclear division takes place, the chromosomes coil up into shorter, thicker more compact structures and the chromatids become recognizable as separate structures. It is these structures that are much more visible under a microscope and therefore more commonly used when showing chromosomes.

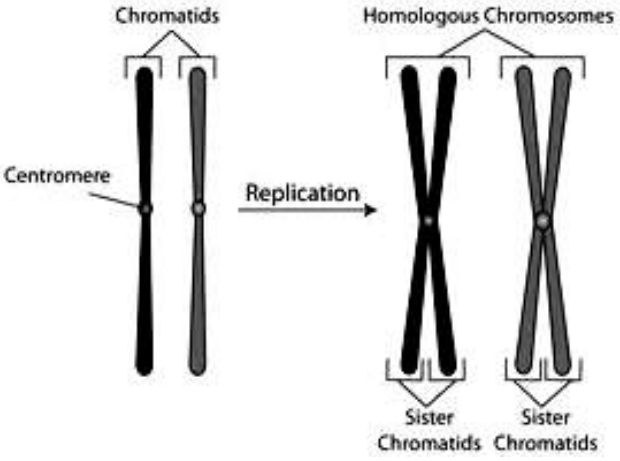

Fig. 3.4 Structure of sister chromatids

(ii) Chromatin: It is a complex of macromolecules composed of DNA, RNA, and protein, which is found inside the nucleus of eukaryotic cells. Chromatin exists in two forms, **heterochromatin** (condensed) and **euchromatin** (extended). Cytologically they are distinguished from one another based upon their staining pattern. The primary protein components of chromatin are histones that help to organize DNA into "bead-like" structures called nucleosomes by providing a base on which the DNA can be wrapped around.

A nucleosome consists of 147 base pairs of DNA that is wrapped around a set of 8 histones called an octamer. The nucleosome can be further folded to produce the chromatin fiber. Chromatin fibers are coiled and condensed to form chromosomes. Chromatin makes it possible for a number of cell processes to occur including DNA replication, transcription, DNA repair, genetic recombination, and cell division.

(a) Euchromatin: Portions of chromosomes that stain lightly, are only partially condensed that is highly rich in gene concentration and is often (but not always) under active transcription (formation of RNA from DNA). It represents most of the chromatin that disperse after mitosis has been completed. Euchromatin contains structural genes which replicate and transcribe during

G1 and **S phase** of interphase. Euchromatin is unfolded and elongated; enzymes such as *RNA polymerase* are able to bind to DNA due to unfolded structure of euchromatin.

Euchromatin comprises the most active portion of the genome within the cell nucleus. 92% of the human genome is euchromatic. It is considered genetically active chromatin, since it has a role in the phenotype expression of the genes. In euchromatin, DNA is found packed in 3 to 8 nm fibre.

(b) Heterochromatin: It is known to stain more deeply as compared to euchromatin. This is attributed due to tighter DNA packing in heterochromatin region. Presence of heterochromatin is a peculiar feature of eukaryotic DNA. It is generally found to be located on peripheral (outer) areas of nucleus. The main constituents of heterochromatin are inactive satellite sequences. The key roles of heterochromatin include gene regulation and protection of chromosomal integrity. Heterochromatin is classified into two groups:

(i) Constitutive heterochromatin remains permanently in the heterochromatic state, i.e., it does not revert to euchromatic state, e.g., centromeric regions. It contains short repeated sequences of DNA, called satellite DNA.

(ii) Facultative heterochromatin is essentially euchromatin that has undergone hetero-chromatinization which may involve a segment of chromosome, a whole chromosome (e.g. one X chromosome of human females and females of other mammals), or one whole haploid set of chromosomes (e.g., in some insects, such as mealy bugs).

Functions of chromatin

1. Packaging of large sized DNA into small volume nucleus.
2. Strengthening of DNA for mitosis and meiosis.
3. Protects DNA from damage.
4. Control of gene expression.

Table-4: Differences between Chromosomes and Chromatin

S.No.	Chromosomes	Chromatin
1-	In chromosomes DNA is tightly packed	Chromatin contains unwound DNA
2-	Chromosomes are found during cell division	Chromatin are found throughout interphase
3-	DNA is not used for macromolecule synthesis	DNA is utilized for macromolecule synthesis

Table-5: Differences between Euchromatin and Heterochromatin

S.NO.	Euchromatin	Heterochromatin
1	The chromatin fibres in this region are loosely coiled as compared with hetero chromatic regions	The chromatin fibres in this region are more tightly folded than euchromatic regions
2	Euchromatin is deeply stained in divisional cycle but less stained in interphase.	Heterochromatin is deeply stained in interphase but less stained in divisional cycle.
3	Due to addition or loss of this region phenotype is affected.	Addition or loss of this region does not affect phenotype
4	Euchromatic regions are able to synthesize mRNA *in vitro*	Heterochromatic regions are not able to synthesize mRNA *in vitro*
5	The crossover frequency is more in euchromatin	The crossover frequency is less in heterochromatin
6	Euchromatin does not show heteropycnosis	Heterochromatin shows heteropycnosis.
7	Euchromatin is less affected than hetero chromatin by temperature, sex, age, etc	Heterochromatin is more affected than euchromatin by temperature, sex, age of parents, proximity to the centromere

Chromomeres: The linearly arranged bead like structures found on the chromosomes is known as chromomeres. These are clearly visible in the polytene chromosomes. Available evidences indicate that chromomere represents a unit of DNA replication, chromosome coiling, RNA synthesis and RNA processing.

Chromonema: Under light microscope, thread-like coiled structures are found in the chromosomes and chromatids, which are called **Chromonema** (Plural Chromonemata). Chromonema is considered to be associated with three main functions. It controls size of chromosomes, results in duplication of chromosomes and is the gene bearing portion of chromosomes. Chromonema is a structure of sub-chromatid nature.

Matrix: A mass of acromatic material in which Chromonemata are embedded is called matrix. Matrix is enclosed in a sheath which is known as pellicle. Both matrix and pellicle are non-genetic materials.

3.3.1.4 Packaging of eukaryotic Chromosome

Eukaryotic chromosomes are made up of DNA-protein complex called chromatin. It consists of DNA, histone proteins, non-histone proteins, RNA, enzymes and metallic ions. The DNA protein complex is a compact structure and organized in a manner so as to accommodate large amount of DNA into small nucleus. DNA is organized into chromatin with the help of DNA binding proteins. In eukaryotes histone proteins play key role in packaging of DNA (Histone proteins are absent in prokaryotes). Histone proteins and DNA are always present in the ratio of 1: 1. Histone proteins are low molecular weight proteins and have large proportion of positively charged amino acids. The RNA and non-histone proteins vary in chromatin of various tissues. Enzymes include DNA polymerase and RNA polymerase.

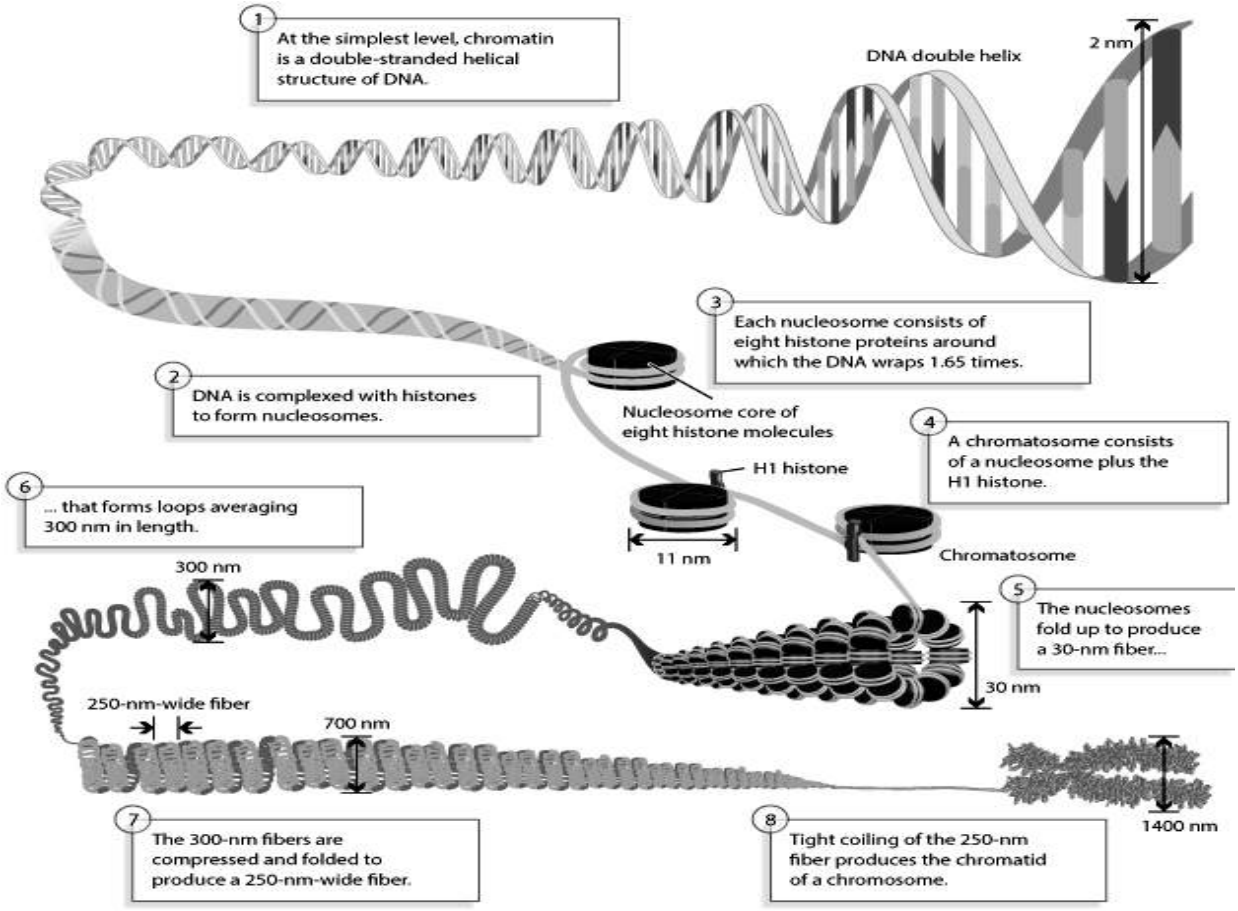

Fig. 3.5: Packaging of eukaryotic chromosome

There are 5 types of histones namely H2A, H2B, H3, H4 and H1 linker histone. Out of these H2A,H2B,H3,H4 are known an **nucleosomal proteins**, two molecules of each of these combine to form an octamer of nucleosome it means that nucleosome is made up of 2(H2A)+2(H2B)+2(H3)+2(H4). **Nucleosomes** are considered as fundamental unit of chromatin. Each nucleosome consists of core particle made up of octamer proteins and a spacer or **linker DNA** made up of histone H1. Generally these proteins are similar in different species. Each of

these proteins is made up of 102-135 amino acids and is rich in basic amino acids like lysine and arginin. Positively charged R group of amino acids of proteins binds to the negatively charged phosphate group of DNA in the ratio of 1: 1 to form deoxyribonucleic proteins (DNP). There are 5000 types of non-histone proteins. Fig. 3.3.1.4 shows packaging of eukaryotic DNA along with structure of single nucleosome.

If the DNA from all 46 chromosomes in a human cell nucleus was laid out end to end, it would measure approximately two meters; however, its diameter would be only 2 nm. Considering that the size of a typical human cell is about 10 μm (100,000 cells lined up to equal one meter), DNA must be tightly packaged to fit in the cell's nucleus. At the same time, it must also be readily accessible for the genes to be expressed. During some stages of the cell cycle, the long strands of DNA are condensed into compact chromosomes. There are a number of ways that chromosomes are compacted. In the first level of compaction, short stretches of the DNA double helix wrap around a core of eight histone proteins at regular intervals along the entire length of the chromosome. The **DNA-histone complex** is called chromatin. The bead-like, histone DNA complex is called a nucleosome, and DNA connecting the nucleosomes is called linker DNA. A DNA molecule in this form is about seven times shorter than the double helix without the histones, and the beads are about 10 nm in diameter, in contrast with the 2-nm diameter of a DNA double helix. The next level of compaction occurs as the nucleosomes and the linker DNA between them are coiled into a 30-nm chromatin fiber. This coiling further shortens the chromosome so that it is now about 50 times shorter than the extended form. In the third level of packing, a variety of fibrous proteins is used to pack the chromatin. These fibrous proteins also ensure that each chromosome in a non-dividing cell occupies a particular area of the nucleus that does not overlap with that of any other chromosome.

3.3.1.5 Karyotype

A karyotype is simply a pictorial presentation of all the chromosomes present in nucleus of a eukaryotic organism. In order to get this picture, the chromosomes are isolated, stained, and examined under the microscope. Most often, this is done using the chromosomes in the white blood cells. Then, the picture of the chromosomes is cut up and rearranged. A karyotype is prepared by arranging all the chromosome pairs according to their size and position of centromere (if size of chromosomes is same). A trained cytogeneticist can look for missing or extra pieces of chromosome. The chromosomes are lined up from largest to smallest. Karyotype describes the basic count of chromosomes in a species. Fig-3.3.1.5 depicts karyotype of human male in which 23 pair of chromosomes is arranged into different groups.

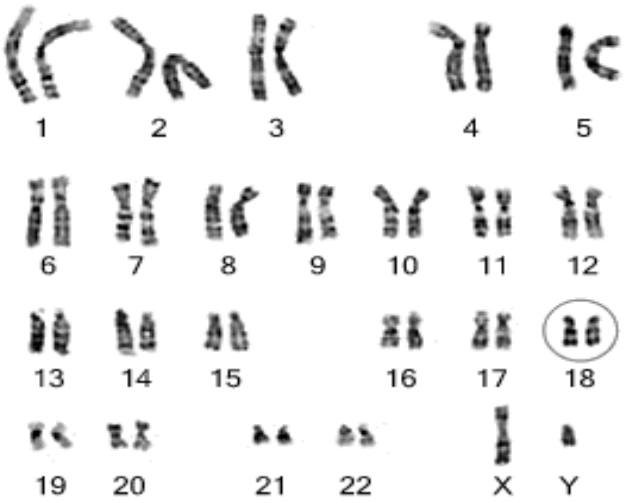

Fig 3.6 Organization of human karyotype

There are 22 numbered pairs of chromosomes called autosomes. The 23rd pair of chromosomes is the sex chromosomes. They determine an individual's gender. Females have two X chromosomes, and males have an X and a Y chromosome.

Karyotypes are generally utilized to observe and compare the following characteristics:

1- Difference in size of chromosome.

2- Difference in position of centromere.

3- Difference in number and position of satellite.

4- Difference in degree and distribution of heterochromatic region.

3.3.2 Types of chromosome

3.3.2.1 Prokaryotic and eukaryotic chromosome

Prokaryotic (particularly bacteria), have a single large circular double stranded DNA. It is more commonly known as **nucleoid**. Comparatively eukaryotes have two or more pair of chromosomes. Beside this bacterial chromosome is present free in cytoplasm i.e., it is not enclosed in nucleus whereas in eukaryotes a well defined nucleus is present inside which chromosomes are enclosed as chromatin. Although in bacteria histone protein are not found to be associated with chromosome however some RNA is found to be associated with bacterial chromosome. Eukaryotic chromosome contains histone proteins found to be associated with chromosomal DNA. In eukaryotes, beside nucleus DNA is also found to be present in mitochondria and chloroplast. In contrast to nuclear DNA, mitochondria and chloroplast contains circular chromosomes also called as extra chromosomal or **extra nuclear DNA**.

Table-6: Differences between prokaryotic and eukaryotic chromosome

S.No.	Prokaryotic chromosome	Eukaryotic chromosome
1	Prokaryotes have single circular chromosome	Eukaryotes have 2 or more pair of linear chromosomes
2	Prokaryotic chromosomes are found in cytoplasm	Eukaryotic chromosomes are found located in nucleus
3	Prokaryotic chromosomes are condensed in nucleoid through supercoiling and binding of various proteins	Eukaryotic chromosomes are packed into nucleus through histone proteins
4	Most prokaryotes contain only one copy of each gene	Eukaryotes generally contains two copies of each gene (i.e., they are diploid)
5	Prokaryotic chromosomes contain little repetitive DNA	Eukaryotic chromosomes contain large amount of non-coding and repetitive DNA
6	The types of proteins found in prokaryotic chromosomes, known as the nucleoid-associated proteins	The types of proteins found in eukaryotic chromosomes, known as the histone proteins

In viruses and bacteriophage, chromosome is made up of single molecule of DNA or single molecule of RNA. This DNA molecule may be single or double stranded. Viruses with DNA as viral chromosome can have two forms of chromosomes one linear and other circular. In most of the viruses with DNA as chromosome it occurs in the form of linear duplex. Ø174 bacteriophage is an exception possessing single strand circular DNA as chromosome. Viruses with RNA as viral chromosome are linear and generally made up of single strand of RNA. They are found in plant viruses and some animal viruses.

3.3.2.2 Autosomes and sex chromosomes

Chromosomes are grouped into two classes namely autosomes and sex chromosomes. Every species has a fixed number of chromosome pairs out of which the last pair is sex chromosome and rest of the chromosomes pairs are autosomes, e.g. in Humans, there is 23 pair of chromosomes out of which 22 pairs are autosomes and 23^{rd} (last) chromosome pair is sex chromosome. Similarly, in Fruit fly there are 4 pairs of chromosomes out of which first three pairs are autosomes and last pair (4^{th}) is sex chromosome. Autosomes are chromosomes which carry genes for phenotypic characters and physiological activity whereas sex chromosomes play key role in determination of sex of an individual (i.e., whether an organism will develop into male or female). However, beside sex determination sex chromosomes also carry genes for other characters.

(a) Autosomes: Non-sex chromosomes, which determine the trait of an organism is identified as autosomes. They are also known as **somatic chromosomes** since they determine the somatic characters of an individual. A genome mainly consists of autosomes. For example, human body contains 46 chromosomes within its genome and 44 chromosomes of them are autosomes. Autosomes exist as homologous pairs and 22 autosomes pairs can be identified in the human genome. Both autosomal chromosomes contain the same genes, which are arranged in the same order. But an autosomal chromosome pair differs from other autosomal chromosome pairs within the same genome. These pairs are labeled from 1 to 22, according to the base pair sizes contained in each chromosome.

(b) Sex chromosomes: In animals (particularly higher animals) there are two sex chromosomes designated as X and Y which determine whether an individual will develop into male or female. For example, In Human beings individuals with XX genetic combination (homogametic) are females and individuals with XY combination (heterogametic) are males. In most of the animals XX-XY pattern of sex determination is followed.

However, there are animals which follow alternate or different pattern of sex determination. For example in *Drosophila melanogaster* (Fruit fly), the ratio of autosomes to sex chromosomes play most important role in sex determination. In birds, fowl and fishes pattern of sex determination is just opposite to human beings. In these animals females are heterogametic (ZW) and males are homogametic (ZZ). Symbols 'Z' and 'W' are similar to X and Y, they are utilized to avoid confusion. XX-XY pattern of sex determination is common in animals but there is a plant, *Melandrium* which also follows similar pattern of sex determination.

Chromosomes are found in pairs. In each pair one chromosome is maternal and other is paternal. Autosomes are also called as **homologous** chromosomes because on both the chromosomes of each pair, same type of genes is present. However, both the sex chromosomes are **heterologous** in nature because different type of genes are present on X and Y chromosomes. For example testes determining factor is only found to be located on Y chromosome and not on X chromosome. So in males the sex chromosome is heterologous (XY) whereas in females even the sex chromosome (XX) is homologous just like autosomes.

Table:7- Difference between Autosomes and Sex Chromosomes

	Autosomes	**Sex Chromosomes**
Definition	Autosomes determine the trait. Males and females contain the same copy of autosomes.	It determines the gender. They are different in males and females by their size, form, and behavior.
Labeling	Autosomes are labeled with numbers, from 1 to 22.	They are labeled with letters as XY, ZW, XO and ZO.
Availability	Most of the chromosomes within a	A few of the chromosomes within a

	genome are autosomes.	genome are sex chromosomes.
Homogeneity	The 22 pairs of autosomes are homologous in humans.	Female sex chromosomes (XX) are homologous (homomorphic) while male sex chromosomes (XY) are non-homologous (heteromorphic).
Position of the Centromere	Since autosomes are homomorphic, the position of the centromere is identical.	Since the male sex chromosomes are heteromorphic, the position of the centromere is not identical. The position of the centromere in female sex chromosomes is identical.
Number of Genes	Autosomes contain the number of genes varying from 200 to 2000. Chromosome 1 which is the largest carries about 2800 genes in humans.	X chromosome contains more than 300 genes while Y chromosome contains only a few genes since it is small in size.
Genetic Disorders	Autosomal disorders show Mendelian inheritance.	Sex-linked disorders show Non-Mendelian inheritance.

Conclusion: Heteromorphic sex chromosomes inherit unequal times through offspring. Thus they do not have much implication in evolutionary processes such as mutation, selection, and genetic drift. But homomorphic chromosomes undergo evolutionary processes by homologous recombination and mutation. Thus, sex chromosomes are considered to be disproportionate in the Haldane's rule.

3.3.2.3 Special types of chromosomes

In some organisms (specifically in some tissues) are present specific types of chromosomes, which differ morphologically or functionally from normal chromosomes. Such chromosomes are called as special chromosomes.

1. **Giant Chromosomes:** Certain special chromosomes are greatly enlarged at a certain stage of their cell cycle. Two examples are Lampbrush chromosome and Polytene chromosome. They are fully extended chromosomes.

(a) Lampbrush chromosomes

They were first of all reported by **Walther Flemming** (German biologist and a founder of cytogenetics) in 1882 in Salamander (*Ambystoma mexicanum*). Ten years later they were described in the oocytes of a dogfish by **Ruckert** (1892) and named them lampbrush. Lampbrush chromosomes have been best characterized in tailed and tailless amphibians, insects and birds. Lampbrush chromosomes are found at diplotene stage of meiotic prophase in primary oocytes and also in spermatocytes of many invertebrates and vertebrates. In meiotic prophase, they are present in the form of bivalents in which the maternal and paternal chromosomes are held

together by Chiasmata and each bivalent has four chromatids. Beside animals lampbrush chromosomes are also found in few plants such as *Acetabularia.*

Lampbrush chromosomes of different species are almost similar in structure and function however loop length is species specific i.e., it may vary from species to species. Lateral loops give the appearance of a lampbrush. Lateral loops extrude in pairs from each sister chromatid. Loops are symmetrical; each loop is a single linear thread of DNA that runs through each chromatid. The loops are extruded part of DNA which is being actively transcribed, therefore they are surrounded by matrix of ribonucleoproteins (RNP). These chromosomes are quite long in length, their length may range between 800-1000µ. They are the largest known chromosomes. Later they revert back to their normal size.

Structure of lampbrush chromosomes

1. Each lampbrush chromosome consists of two homologous chromosomes. Each homologue has two chromatids.
2. Both the homologous chromosomes are joined to one another at several points called Chiasmata. Otherwise they are separated.
3. Lampbrush chromosomes appear brush like because of threads that loop out from two chromatids. These loops always occur in pairs.
4. Loops in lampbrush chromosomes are made up of DNA associated with RNA and ribosome and also act as site of transcription.
5. It has been experimentally proved that each loop is made up of single DNA double helix.
6. Chromosomal axis bears string of fine chromatin granules known as chromomeres. One or many pairs of loops arise from each chromomere.

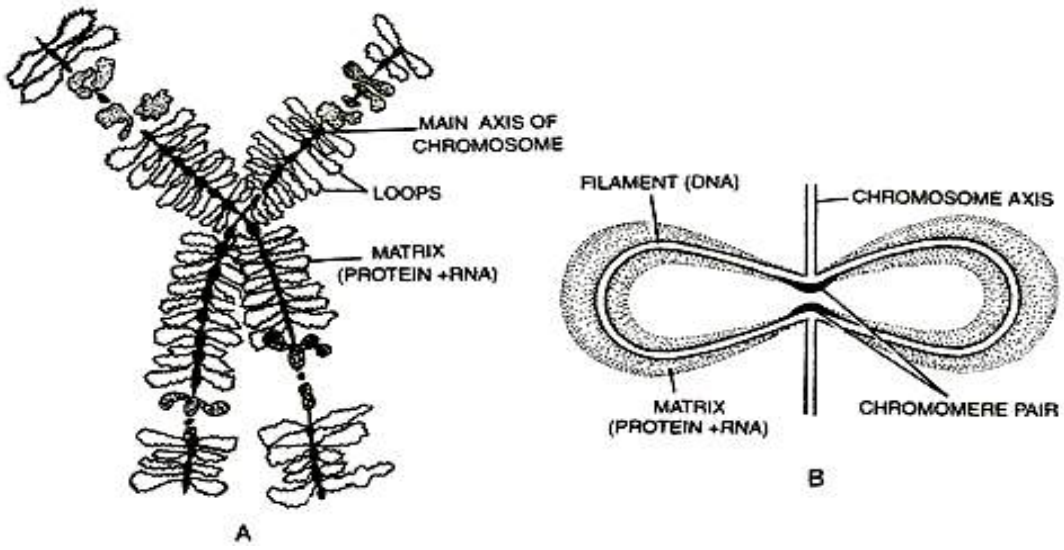

Fig.3.7: A - Lampbrush chromosome A. Enlarged view of a part of lampbrush chromosome, B. Single loop of lampbrush chromosome

Functions of Lampbrush chromosome

There are two main functions of lampbrush chromosomes one being synthesis of RNA and other formation of yolk material.

(i) RNA synthesis: Through the loop region of lampbrush chromosome, synthesis of RNA (transcription) occurs. One end of loop of lampbrush chromosome is thin while the other end is thick. Most of the RNA synthesis occurs at thin ends of loop however, there is little or no RNA synthesis at thicker end of loops.

(ii) Formation of yolk material: Lampbrush chromosome are believed to help in formation of yolk material in egg.

(b) Polytene Chromosomes

The salivary gland interphase chromosomes of *Drosophila melanogaster* and other dipterans like flies, larvae are greatly enlarged and are called polytene chromosomes. These multithreaded chromosomes were discovered by **E.G. Balbiani** (a French embryologist) in 1881 from the salivary glands of *chironomus* midges. The synapse diploid chromosomal pair undergoes repeated replication.

These chromosomes are like normal chromosomes but they undergo several round of DNA replication without undergoing cell division. This type of replication in which DNA keeps on replicating but the cell does not divides is known as **Endoreduplication** (or endoreplication) because normally after replication of DNA cell division takes places. The replicated threads are unable to separate as there is no nuclear division. As a result of this chromosomes become extensively banded. The resulting daughter chromatids do not separate and remain side by side attached to the chromocenter, which is formed by the fusion of centromeres. The number of threads may be in hundreds. Each thread represents a single 1C haploid chromosome.

Polytene chromosomes are unable to undergo mitosis and the cells ultimately die. Each haploid chromosome consists of series of dark bands consisting of mass of DNA. These bands can be darkly stained by Feulgen dye. In between these bands are lightly stained inter-bands. Some of these bands are further expanded to form reversible chromosomal puffs or **Balbiani rings**. These puffs are due to loose coiling of chromatids. These puffs are associated with intense metabolic activity and are sites of active RNA synthesis. Puffs have **RNA polymerase II** enzyme, which is involved in the process of transcription. The structure displayed by Lamp-brush and polytene chromosomes suggest that during transcription, the DNA is unpacked and uncondensed from its usual more tightly packed state.

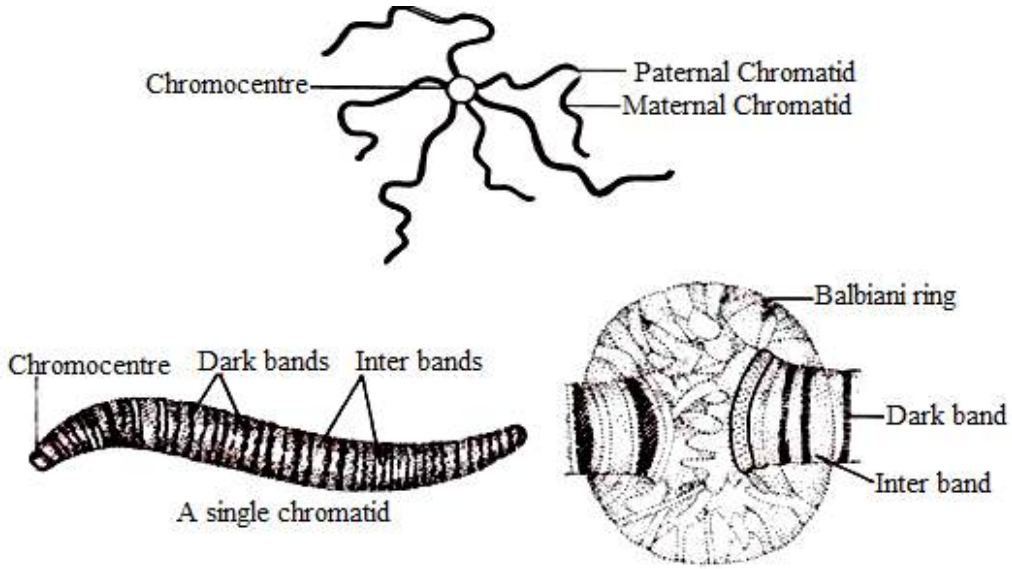

Fig.3.8- Polytene chromosome

Balbiani rings: At certain developmental stages regions of bands and interbands are significantly increases in diameter. The structures so produced as a result of this increase in diameter are called as puffs or Balbiani rings 3.3.3.3 (b). Such puffs are formed due to uncoiling of chromatin fibres, which extend out in form of loops. These puffs act as active site of RNA synthesis. The rate of RNA synthesis in these puffs is much higher than the rate of RNA synthesis in other parts of chromosome.

Functions of polytene chromosome

1-They carry genes involved in physiology of an organism
2- Multiple copies of genes permit higher level of gene expression.
3-They are also known to increase cell volume and cause cell expansion.

2. Supernumerary chromosomes

In 1905, **Wilson** (American zoologist and geneticist) discovered presence of supernumerary chromosomes in hemipteran insects Metapodius. These are additional chromosomes present in addition to normal chromosomes and can be one or more in number. These chromosomes are also called as accessory chromosomes or **B-chromosomes**. Supernumerary chromosomes are comparatively smaller in size and believed to be genetically inert. They have been largely reported in insects, higher plants and animals. Hence they produce little detectable phenotypic expression.

The origin of such chromosome is mostly unknown but in animal species they generally arise as a result of fragmentation of 'Y' chromosome. Generally, supernumerary chromosomes are mostly heterochromatic while in some species such as maize these chromosome are partly heterochromatic and partly euchromatic and in some other species like *Tradescantia*

(Spiderwort) they are completely euchromatic. Largely these chromosomes are morphologically similar to normal chromosomes. There number can vary among individuals of same species. Also such chromosomes may be lost or gained without any observable harmful or beneficial effect.

3.3.3 - Functions of chromosome

Chromosomes contain genes. All the hereditary information is located in the genes. They control the synthesis of structural proteins and thus help in cell division and cell growth. They also control cellular differentiation. By directing the synthesis of particular enzymes, chromosomes control cell metabolism. Sat-chromosomes produce nucleoli for synthesis of ribosome.

Their haploid or diploid number respectively brings about gametophytic and sporophytic characteristics to the individual. Some chromosomes called sex chromosomes (e.g., X and Y or X and 0) determine the sex of the individual. Through the process of crossing over, chromosomes introduce variations. Mutations are produced due to change in gene chemistry. The role of chromosomes in heredity was suggested independently by **Walter Sutton** and **Theodor Boveri** in 1902. This and various other functions of chromosomes may be summarized as under.

1. It is universally accepted that DNA is the genetic material, and that in eukaryotes almost all the DNA is present in chromosomes. Thus, the most important function of chromosomes is to provide the genetic information for various cellular functions essential for growth, survival, development, reproduction, etc., of organisms.
2. Another very important function of chromosomes is to protect the genetic material (DNA) from being damaged during cell division. Chromosomes are coated with histones and other proteins which protect it from both chemical (e.g., enzymes) and physical forces.
3. The chromosomes are capable of **self-duplication**. During duplication process the DNA strands unwind. As unwinding starts, each template of DNA forms its complementary strand in double-helix nature. The conversion of the old DNA molecule into two new molecules helps in duplicating the chromosomes.
4. The properties of chromosomes ensure a precise distribution of DNA (genetic material) to the daughter nuclei during cell division. Centromeres of chromosomes perform an important function in chromosome movements during cell division which is due to the contraction of spindle fibres attached to the centromeric regions of chromosomes.
5. Gene action in eukaryotes is believed to be regulated through histone and non-histone proteins associated with chromosomes. They help in expression of different characters in an organism by synthesizing proteins in cells. A definite protein is accumulated to produce a definite character
6. As carrier of genes they transmit characters from generation to generation, i.e. parents to offspring. Chromosomes form a link between the offspring and the parents.
7. The chromosomes control the physiological and biochemical processes in the body of the organism.

3.4 SUMMARY

1. Chromosomes are tiny thread-like structures found in the nucleus of a eukaryotic cell and as **nucleoid** (without nucleus) in prokaryotic cell.
2. Chromosomes store and transmit the coded information which is responsible for all the life processes of an organism. Hence, chromosomes are commonly described as carriers of heredity.
3. In bacteria, entire hereditary material is present as a single, irregularly packed, compact mass called nucleoid or **bacterial chromosome**. Bacterial chromosomes lack a nuclear membrane and are not associated with histones.
4. Prokaryotic genome is very compact; contain very little non-coding DNA sequences. However, eukaryotic chromosomes contain large amount of non-coding sequences. Eukaryotic chromosomes are visible only during metaphase stage of mitosis.
5. Chromosomes store and transmit genetic information from one generation to another in form of **genes**.
6. Chromosomes occur in pairs and the chromosome number of a particular species is a fixed diploid number (2n). **Haploid** number of chromosomes occurs in gametes and spores. Some adult organisms (like male honey bee) have haploid number of chromosomes. **Genome** is the term used to describe the sum total of all the genes present in a haploid set of chromosomes.
7. Chromosomes range in size from 0.1 to 30 mm in length and 0.2 to 2.0 mm in thickness. When the number is less, the chromosomes are larger in size. Each chromosome contains one DNA molecule
8. At metaphase stage of mitosis, chromosome consists of two identical components called **chromatids**, which are joined by centromere (**primary constriction**). Each chromatid has two arms out of which 'q' are longer arm and 'p' is shorter arm. A **secondary constriction** may occur sometimes. Such a chromosome is called sat-chromosome.
9. Chromatin exists in two physiological forms: euchromatin and heterochromatin.
10. Chemically, the chromosome is composed of nucleoproteins present in the form of a highly coiled chromonema. **Chromonema** is composed of a chain of bead-like structures called nucleosomes.
11. A nucleosome has a core particle formed by proteins called **histones** surrounded by a DNA strand. Nucleosome is composed of histone proteins H2A, H2B, H3, and H4. The part of DNA not associated with histones is called Linker DNA. Histones proteins allow packing of large size DNA molecule into a small sized nucleus.
12. Normally, a chromosome has only one centromere (**Monocentric**). It can be sometimes dicentric or polycentric. Very rarely it may lack a centromere (Acentric). Based on the position of centromere, Monocentric chromosome can be distinguished into Metacentric, Submetacentric, Acrocentric and Telocentric.

13. In unisexual organisms the chromosomes can be distinguished into **autosomes** (somatic chromosomes) and **allosomes** (sex chromosomes). Allosomes have a role in sex-determination and in the expression of sex-linked characters.
14. Chromosomes, which are extremely larger than the normal ones are called **giant chromosomes**. They occur in some animal cells.
15. Lampbrush chromosomes of amphibian oocytes and polytene chromosomes of *Drosophila* are common examples of giant chromosomes.
16. **Polytene** Chromosomes were discovered by **Balbiani** (1881) in the salivary glands of *Chironomus tentans* (Midge). They are also found in salivary glands of many insect.
17. Polytene chromosomes are formed by repeated DNA replication without cell division. All the polytene chromosomes remains attached to a common chromocenter. When stained with basic dyes, the chromosomes show dark bands and light interbands.
18. **Lampbrush** chromosomes are large sized diplotene chromosome bivalents with a length of 400–1000 nm each. A lampbrush chromosome is made of two homologous chromosomes joined to one another by chiasmata at several places.
19. Many of the chromomeres give out lateral loops of various sizes. Loops possess a number of copies of the same gene and are meant for rapid transcription and production of materials like yolk. Lampbrush chromosomes occur in oocytes.

3.5 GLOSSARY

Acrocentric chromosome: A chromosome with the centromere near one end so that one chromosomal arm is short and one is long

Allosomes: A sex chromosome that differs from an ordinary autosome in form, size, or behavior. The human sex chromosomes are a typical pair of allosomes.

Autosomes: Any chromosome other than a sex chromosome.

B-chromosome: Also known as supernumerary, are extra chromosomes to the standard complement that occur in many organisms. They can originate in a number of ways including derivation from autosomes and sex chromosomes in intra- and interspecies crosses.

Centromere: A specialized structure on the chromosome, appearing during cell division as the constricted central region where the two chromatids are held together and form an X shape.

Chromatid: One of two identical chromosomal strands into which a chromosome splits longitudinally preparatory to cell division.

Chromatin: Readily stainable substance of a cell nucleus, consisting of DNA, RNA, and various proteins, that forms chromosomes during cell division.

Chromonemata: A chromosome thread that is relatively uncoiled at early prophase but assumes a spiral form at metaphase.

Chromosome: A thread-like structure of nucleic acids and protein found in the nucleus of most living cells, carrying genetic information in the form of genes.

Constitutive: Forming a part or constituent of something.

Diploid: Having two similar complements of chromosomes.
Endoreduplication: Replication of the nuclear genome in the absence of cell division, which leads to elevated nuclear gene content and polyploidy.
Euchromatin: Chromosome material which does not stain strongly except during cell division. It represents the major genes and is involved in transcription
Facultative: Capable of but not restricted to a particular function or mode of life.
Gene: A unit of heredity which is transferred from a parent to offspring.
Genome: A full set of chromosomes; all the inheritable traits of an organism.
Haploid: An organism or cell having only one complete set of chromosomes, ordinarily half the normal diploid number.
Heredity: The passing on of physical or mental characteristics genetically from one generation to another
Heterochromatin: Chromosome material of different density from normal (usually greater), in which the activity of the genes is modified or suppressed.
Histone: Any of a group of five small basic proteins, occurring in the nucleus of eukaryotic cells, that organize DNA strands into nucleosomes by forming molecular complexes around which the DNA winds.
Karyotype: The chromosomes of a cell, usually displayed as a systematized arrangement of chromosome pairs in descending order of size.
Kinetochore: The place on either side of the centromere to which the spindle fibers are attached during cell division.
Lampbrush chromosome: A special form of chromosome found in the growing oocytes (immature eggs) of most animals, except mammals.
Linker DNA: Linker DNA is double-stranded DNA in between two nucleosome cores that, in association with histone H1, holds the cores together
Matrix: The intercellular substance of a tissue.
Meiosis: Part of the process of gamete formation, consisting of chromosome conjugation and two cell divisions, in the course of which the diploid chromosome number becomes reduced to the haploid.
Metacentric: Relating to any chromosome or chromatid whose centromere is centrally located, creating two apparently equal chromosome arms.
Microtubules: A hollow cylindrical structure in the cytoplasm of most cells, involved in intracellular shape and transport.
Mitosis: A type of cell division that results in two daughter cells each having the same number and kind of chromosomes as the parent nucleus, typical of ordinary tissue growth.
Morphogenesis: The development of structural features of an organism or part.
Nucleoid: The central region in a prokaryotic cell, as a bacterium, that contains the chromosomes and that has no surrounding membrane.
Nucleoprotein: Any of the class of conjugated proteins occurring in cells and consisting of a protein combined with a nucleic acid, essential for cell division and reproduction.

Nucleosome: A structural unit of a eukaryotic chromosome, consisting of a length of DNA coiled around a core of histones.

Polytene chromosome: A giant chromosome which is composed of many parallel copies of the genetic material, as found in Drosophila fruit flies where they are much used in genetic research.

Prophase: A stage of mitosis during which chromatin condenses into two rod-shaped structures called chromosomes.

Ribonucleoproteins: A nucleoprotein in which the nucleic acid is RNA.

Sat-chromosome: A chromosome segment that is separated from the main body of the chromosome by such a secondary constriction.

Sex chromosome: A chromosome, differing in shape or function from other chromosomes, that determines the sex of an individual.

Telocentric: Relating to any chromosome or chromatid whose centromere is positioned at its end, creating one chromosome arm.

Telomeres: The segment of DNA that occurs at the ends of chromosomes.

3.6 SELF ASSESSMENT QUESTIONS

3.6.1 Multiple choice questions:

1. Bacteria have single chromosome, called as?

(a) Chromatid (b) nucleus

(c) Nucleoid (d) plasmid

2. An estimate measurement of a chromosome in close circle of *Escherichia coli* is?

(a) 1400 µm (b) 1100 µm

(c) 1200 µm (d) 1000 µm

3. How many pair of autosomes is present in human beings?

(a) 22 (b) 23

(c) 01 (d) 04

4. An octamer of 4 histones complexes with DNA forms?

(a) Endosome (b) Nucleosome

(c) Mesosome (d) Centromere

5. Nucleosome core is made of?

(a) HI, H2A, H2B and H3 (b) HI, H2A, H2B, H4

(c) HI, H2A, H2B, H3 and H4 (d) H2A, H2B, H3 and H4

6. The point, at which polytene chromosome appear to be attached together, is called?

(a) Centromere (b) Chromomere

(c) Chromocenter (d) Centriole.

7. The polytene chromosomes were discovered for the first time in?
(a) *Drosophila* (b) *Musca domestica*
(c) *Chironomus* (d) *Musca nebula*

8. Lampbrush Chromosomes are seen in which typical stage?
(a) Mitotic metaphase (b) Meiotic prophase
(c) Mitotic anaphase (d) Mitotic prophase.

9. Extranuclear chromosomes occur in?
(a) Peroxisome and ribosome (b) Chloroplast and mitochondria
(c) Mitochondria and ribosome (d) Chloroplast and lysosome.

10. Extra chromosomal DNA is found in cell organelles like?
(a) Mitochondria (b) Chloroplast
(c) Both a and b (d) Ribosomes

11. The salivary gland chromosomes in the dipterans larvae, are useful in gene mapping because they are?
(a) Fused (b) Much longer in size
(c) Easy to stain (d) Endoreduplicated chromosomes

12. Which of the following is a V-shaped chromosome?
(a) Telocentric (b) Acrocentric
(c) Metacentric (d) Submetacentric

13. Diffused centromere is found in?
(a) Animals (b) Insects
(c) Fungi (d) Bacteria

14. Accessory chromosomes are also known as?
(a) Giant chromosomes (b) B-chromosome
(c) Polytene chromosome (d) Balbiani rings

15. Polytene chromosomes exhibit higher level of gene expression due to?
(a) Multiple copies of gene (b) Large length of polytene chromosome
(c) Presence of interbands (d) None of these

16. Prokaryotic DNA differ from eukaryotic in not having?
(a) Promoter (b) Transposons
(c) Genes (d) Histone

17. In polytene chromosomes bands /puffs are formed due to?
(a) Coiling of chromatin fibres (b) Uncoiling of chromatin fibres
(c) Both coiling and uncoiling of chromatin fibre (d) Enhanced RNA synthesis

18. Satellite chromosomes are?
(a) Chromosomes having secondary constriction
(b) Chromosomes having primary constriction only
(c) Chromosomes having no telomeric region
(d) Both b and c

19. *Drosophila melanogaster* contains how many pairs of autosomes?
(a) Four (b) Three
(c) Two (d) One

20. In Fishes:
(a) Males are homogametic and female heterogametic
(b) Males are heterogametic and females are homogametic
(c) Both are homogametic
(d) Both are heterogametic

3.6.2 State whether the following statements are true or false.

1. Histones are the proteins associated with prokaryotic and eukaryotic DNA.
2. Chromatin is made up of DNA and proteins.
3. The position of centromere is fixed for each chromosome.
4. In Acrocentric chromosome both p and & q arm are of equal length.
5. Secondary constriction is not found in chromosomes of human beings.
6. Bacterial chromosomes are not enclosed by nuclear membrane.
7. Extra chromosome DNA or extra nuclear DNA is always circular.
8. Protozoan's can have 300 or more chromosomes.
9. Formation of lateral loops is a feature of polytene chromosome.
10. Dark bands of polytene chromosomes are euchromatic in nature.
11. Multiple copies of gene in polytene chromosome results in higher level of gene expression.
12. Supernumerary chromosomes are also called as accessory chromosomes.
13. Presence of heterochromatin is a peculiar feature of eukaryotic DNA.
14. One of the functions of chromatin is to protect DNA from damage.
15. H1 histone protein is not a part of nucleosome.

3.6.3 Fill in the blanks:

1. Puffs present in polytene chromosomes are active sites of_____.

2. Polytene chromosome was first of all observed by _____ in salivary glands of larva of _____ *midges*.
3. The subunit designation of chromosome is_____.
4. Fundamental unit of chromatin is _____.
5. Chromosomes having more than two centromeres are called _____.
6. Chromosomes of bacteria are commonly known as _____.
7. _____ are also known as giant chromosomes.
8. _____ are formed due to unwinding of chromatin fibres in loops of polytene chromosome.
9. Prokaryotes contain _____ copy of each gene.
10. Presence of supernumerary chromosomes was discovered in _____.
11. Spindle fibres are attached to _____part of chromosome.
12. Nucleolar organizer is present in _____ region of chromosome.
13. Lampbrush chromosomes are seen at _____ stage of meiosis.
14. _____ has single stranded DNA polytene chromosome as genetic material.

3.6.4 Very short answer type questions

1. What are centromeres?
2. What are telomeres?
3. How many pair of chromosomes does human have?
4. Define nucleoid?
5. Name a plant possessing diffused centromere?
6. Which type of chromosomes is known as satellite chromosomes?
7. What is nucleolus organizer region?
8. What is endoreplication?
9. How does facultative heterochromatin differ from constitutive heterochromatin?
10. Mention the function of heterochromatin?

3.6.1 Answers Key: 1-(c), 2-(b), 3-(a), 4-(b), 5-(d), 6-(a), 7-(c), 8-(b), 9-(b), 10-(c), 11-(d), 12-(c), 13-(b), 14-(b), 15-(a), 16-(d), 17-(a), 18-(b), 19-(a), 20-(a)

3.6.2 Answers Key: 1-(F), 2-(T), 3-(T), 4-(F), 5-(F), 6-(T), 7-(T), 8-(T), 9-(F), 10-(T), 11-(T), 12-(T), 13-(T), 14-(T), 15-(T).

3.6.3 Answers Key: 1-RNA synthesis, 2-Balbiani & *Chironomus*, 3-Chromatin, 4-Nucleosome, 5-Polycentric, 6-Nucleoid, 7-Polythene chromosomes, 8-Puffs, 9-One, 10-Metapodius, 11-Kinetochore, 12-Secondary constriction, 13-Diplotene, 14-Tobacco mosaic virus

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3.9 TERMINAL QUESTIONS

3.9.1 Short answer type questions:

1. What is significance of telomeric region in chromosomes?
2. How do autosomes differ from sex chromosomes?
3. Briefly mention about function of polytene chromosomes?
4. Do all living organism have same type of chromosomes?
5. Write short note on supernumerary chromosome?
6. What are Balbiani rings? Where and how are they formed?
7. In what kind of organisms is polytene and lampbrush chromosomes are found?
8. What is a karyotype? Also mention about its utility?
9. Classify chromosomes based upon number of centromeres?
10. Briefly mention about genetic material found in viruses?

3.9.2 Long answer type questions:

1. Define chromosomes. With self explanatory diagram classify chromosomes based upon position and number of centromere?
2. Describe about different regions present in structure of chromosomes?
3. With the help of well labeled diagram explain DNA packing in eukaryotic chromosomes?
4. What are special chromosomes? Why are they called so? Explain in detail about some special chromosomes?
5. Differentiate between
   (a)Euchromatin and heterochromatin
   (b) Prokaryotic and eukaryotic chromosome
   (c)Autosomes and sex chromosomes

UNIT–4 CELL DIVISION

4.1 OBJECTIVES

After reading this unit students will be able:

- To understand preliminary idea of cell cycle
- To study how does mitosis occurs in plant and animal cells
- To study how the process of meiosis occurs in
- To learn about similarities and differences between mitosis and meiosis.
- To know significance of occurrence of mitosis and meiosis.

4.2 INTRODUCTION

Cells are structural and functional unit of life. All the living organisms whether unicellular or multicellular require the cells to divide for their growth, development and reproduction. There are several examples which can be cited to understand this, for instance a single celled zygote divides and re-divides to form large number of cells which ultimately leads to formation of multicellular organism. In plants, cell division in meristematic tissue is responsible for growth as well as formation of new shoots, leaves and roots throughout the life of a plant. Living organism can be classified as eukaryotic or prokaryotic. In eukaryotes, two types of cell division are known i.e., mitosis and meiosis, out of which mitosis occurs in somatic cells and meiosis occurs in gametes.

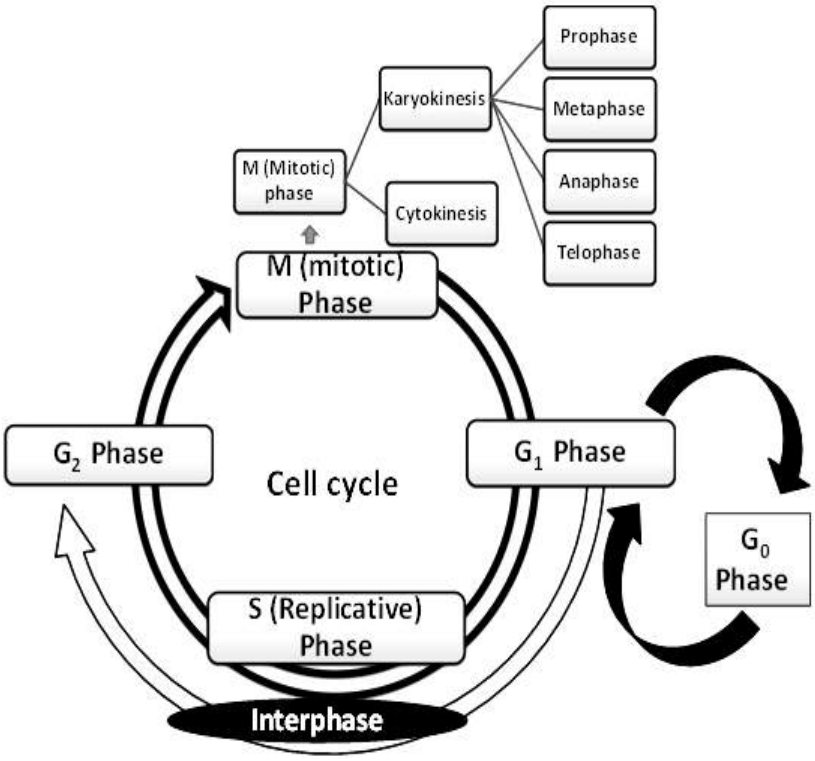

Fig. 4.2: Overview of cell cycle

For cell division to occur, cells undergo a process called as **Cell Cycle**. Cell cycle is a series of events that take place in a cell which involves duplication of its DNA (**DNA replication**) and its division to produce daughter cells. There are four phases of cell cycle; G_1, S, G_2 and M phase along with a G_0 phase. G_0 phase is also known as resting phase. Cell division is a strictly regulated and controlled process. It means that a cell will undergo the process of cell division only when formation of new cells is required; otherwise no unnecessary cell division occurs in living organism. Now, we can better understand why G_0 phase is called **resting phase**.

When a cell is not dividing, it is said to remain in resting phase and cell cycle is paused or halted, but when, formation of new cells required, cell cycle is resumed.

Except **M phase,** rest of cell cycle is known as **Interphase**. G_1 is the first phase of interphase, from the end of the M phase till the beginning of DNA synthesis, is called as **G_1 phase**. It is also called the growth phase where the cell increases its proteins, number of organelles and size. **S phase** is known as synthetic or replicative phase where replication of DNA occurs. **G_2 phase** is the period between S and M phase. In this phase of cell cycle, protein synthesis and rapid cell growth occurs which prepares cell for mitosis. M phase is **mitotic phase** where karyokinesis (division of nucleus) followed by cytokinesis (division of cytoplasm) occurs.

Table-1: Differences between Karyokinesis and Cytokinesis

S.No.	Karyokinesis	Cytokinesis
1	It is the process of splitting of the chromosomal material in the nucleus.	It is the process of splitting of the cytoplasm
2	It is followed by cytokinesis. It also occurs without being followed by the cytokinesis.	Cytokinesis occurs only when the karyokinesis take place.
3	It is a sequential process.	It is equal distribution of the cell organelles and the nuclei into the daughter cells formed
4	It is the first step in the M phase of the life cycle.	It is the second step of the M phase of the life cycle.

4.3 CELL DIVISION

There are two types of cell division known which is mitosis (or indirect cell division) and meiosis (or reductional cell division). Mitosis occurs in somatic cells where as meiosis occurs in reproductive cells or gametes. Beside this there is another type of cell division called as **Amitosis** (**or direct cell division)** which occurs in unicellular organism such as protozoa and also in yeast. Amitosis also occurs in foetal membrane of vertebrates. There is no condensation of chromosomes and spindle formation in this type of cell division.

Advantage of amitosis is that it occurs in very short period of time and disadvantage is that there is no possibility of genetic recombination. During amitosis, nucleus elongates and assumes dumbbell-shape followed by splitting of nucleus by constriction. Along with this, constriction in cytoplasm occur which results in division of cell into two.

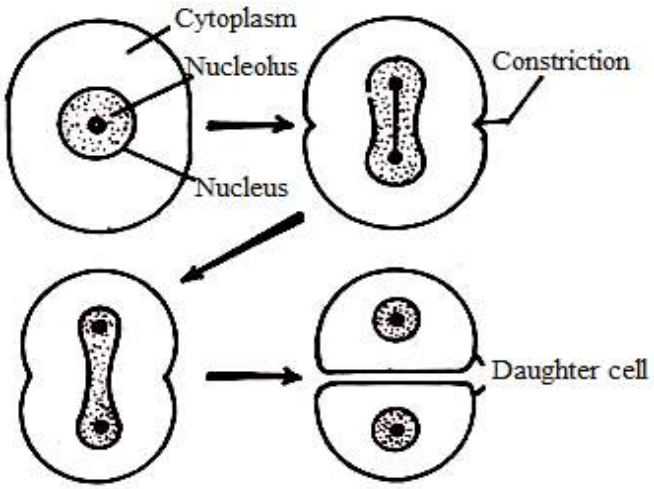

Fig.4.3: Diagrammatic representation of amitosis

Amitosis is characterized by:

1. Intact nuclear envelope is found throughout the division.
2. Chromatin does not condense into definite chromosomes.
3. A spindle is not formed.
4. Chromatin distribution occurs unequally which causes abnormalities in metabolism and reproduction.
5. Cytokinesis may or may not follow karyokinesis.

The primary concern of cell division is the maintenance of the original cell's genome. Before division can occur, the genomic information that is stored in chromosomes must be replicated, and the duplicated genome must be separated cleanly between cells. A great deal of cellular infrastructure is involved in keeping genomic information consistent between generations.

The importance of cell division can be appreciated by realizing the following facts:

1. Cell division is a prerequisite for the continuity of life and forms the basis of evolution to various life forms.
2. In unicellular organisms, cell division is the means of asexual reproduction, which produces two or more new individuals from the mother cell. The group of such identical individuals is known as **clone**.
3. In multicellular organisms, life starts from a single cell called zygote (fertilized egg). The zygote transforms into an adult that is composed of millions of cells formed by successive divisions.
4. Cell division is the basis of repair and regeneration of old and worn out tissues.

4.3.1 Mitosis

The mitosis is a part of somatic cell division which includes the division of the nucleus (called mitosis or karyokinesis) and the division of the cytoplasm (called cytokinesis). **Strasburger** (1875), a German botanist, was the first to work out the details of mitosis. Later on, **W. Flemming** (1879) discovered it in animal cells. The term mitosis was coined by **Flemming** (1882). Mitosis can be studied best in the root tip and shoot tip of several plants. But the most favourable material is the apices of onion roots.

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 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. The replicated chromosomes are attached to a 'mitotic apparatus' that aligns them and then separates the sister chromatids to produce an even partitioning of the genetic material. This separation of the genetic material in a mitotic nuclear division (or **karyokinesis**) is followed by a separation of the cell cytoplasm in a cellular division (or **cytokinesis**) to produce two daughter cells.

In some single-celled organisms mitosis forms the basis of asexual reproduction. In diploid multicellular organisms, sexual reproduction involves the fusion of two haploid gametes to produce a diploid zygote. Mitotic divisions of the zygote and daughter cells are responsible for the subsequent growth and development of the organism. In the adult organism, mitosis plays a role in cell replacement, wound healing and tumour formation. In mitosis, the metabolic nucleus passes through a complicated system of changes in the form of four different stages, viz., prophase, metaphase, anaphase and telophase.

Karyokinesis: Karyokinesis is the name of nuclear division. It has been divided into five phases; Prophase, Prometaphase, Metaphase, Anaphase and Telophase. Some important aspects of all these stages are discussed below.

1. Prophase

- It is the first phase of mitotic karyokinesis in which chromatin fibres condense to form chromosomes. Prophase has three sub phases i.e. early, middle and late.
- Nucleus becomes spherical and cytoplasm becomes more viscous.
- The chromatin slowly condenses into well-defined chromosomes.
- Each chromosome appears as two sister chromatids joined at the centromere.
- The spindle (microtubules) begins to form outside nucleus. In plants, the spindle apparatus or mitotic spindle is anastral. In animals and brown algae, the mitotic spindle is amphiastral which include two asters in opposite poles of the spindle. Each aster consists of two centrioles surrounded by astral rays.

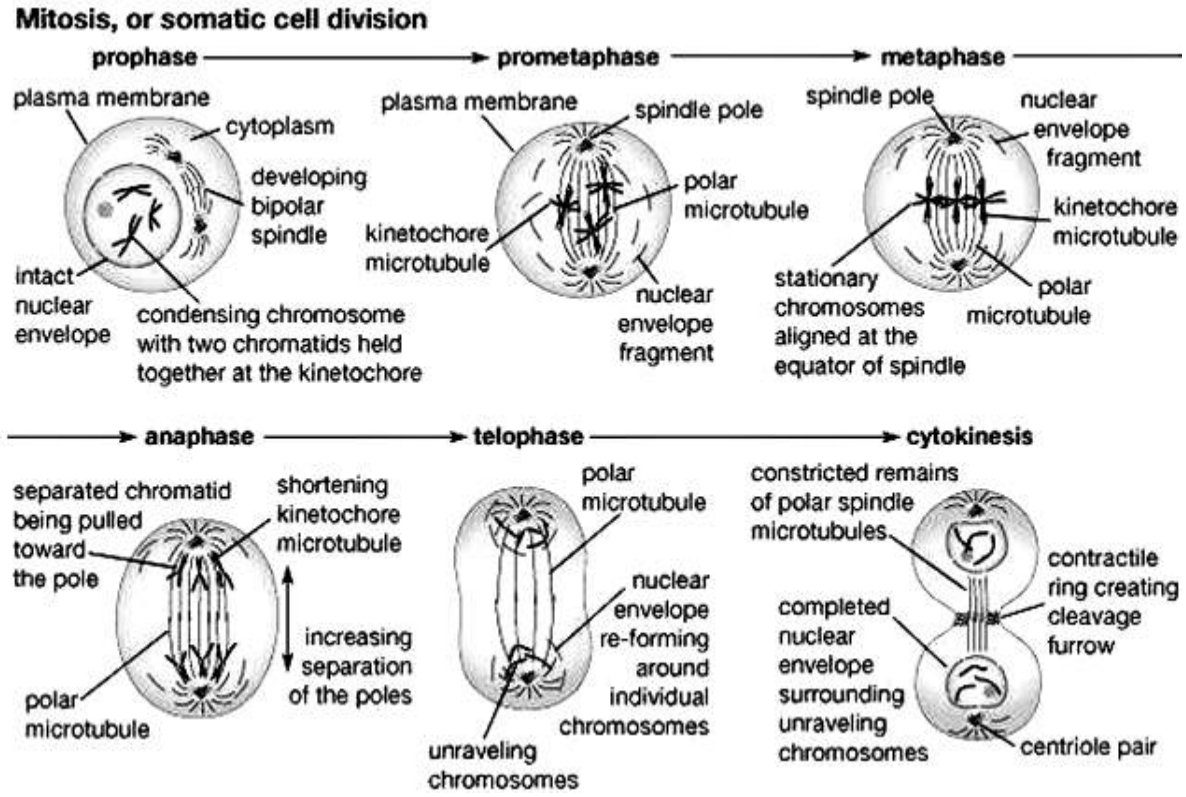

Fig. 4.4: Different stages of karyokinesis and cytokinesis

2. Prometaphase

- Nuclear envelop breaks down into membrane vesicles and the chromosomes set free into the cytoplasm.
- Chromosomes are attached to spindle microtubules through kinetochores. Specialized protein complexes that mature on each centromere are called **Kinetochores**.
- Nucleolus disappears.

3. Metaphase

- Kinetochore microtubules align the chromosomes in one plane to form metaphasic plate or equatorial plate. The process of formation of metaphasic plate is called **congression**.
- Centromeres lie on the equatorial plane while the chromosome arms are directed away from the equator called **auto orientation**.
- Smaller chromosomes remain towards the centre while larger ones occupy the periphery.

4. Anaphase

- Chromosomes split simultaneously at the centromeres so that the sister chromatids separate. They are now called daughter chromosomes. Where each one consists of single chromatid.
- The separated sister chromatids move towards opposite poles at the speed of 1µm per minute.

- Poleward movement of daughter chromosomes occurs due to shortening of kinetochore microtubules; appearance and elongation of **inter-zonal fibers**.
- Daughter chromosomes appear V-shaped (metacentric), L-shaped (Submetacentric), J-shaped (Acrocentric) and I-shaped (Telocentric).
- Chromosomal fibres shorten and disappear when chromosomes reach the poles.
- At the end of anaphase, two groups of single stranded chromosomes are formed.
- It is the shortest of all stages of mitosis.

5. Telophase

- In this stage of karyokinesis, reformation of nuclei occurs.
- Daughter chromosomes arrive at the poles.
- Kinetochore microtubules disappear.
- Nuclear envelope reforms around each chromosome cluster of each pole.
- Decondensation or unfolding of chromosomes occurs. Chromosomes uncoil into chromatin.
- Formation of nucleolus and nuclear membrane occur.
- Nucleolus reappears. Two daughter nuclei are formed at each pole.
- It is considered as the reverse of prophase.
- Golgi complex, ER etc. reforms.
- In animal cells, astral rays and spindle fibres completely disappear in telophase. In plant cells the spindle fibres disappear from near the poles but remain intact towards the equator.

6. Cytokinesis: or C-phase is the division of parent cell having undergone karyokinesis to produce two daughter cells each with a daughter nucleus. It is the cytoplasmic division that starts during mid-anaphase and completed by the end of telophase. Animal cell cytokinesis significantly differs from plant cell cytokinesis. It takes place by two bare different methods i.e. **cell plate** method and **cleavage** or cell furrowing method.

(a) Cell plate cytokinesis: It occurs in plant cells. The spindle fibres persist at equatorial plane. The Golgi vesicles fuse at the centre to form barrel-shaped **phragmoplast**. Further addition of vesicles causes the phragmoplast to grow centrifugally till it meets with plasma membrane of the mother cell. The contents of phragmoplast solidify to become cell plate or future middle **lamella** which separates the two daughter cells. The daughter protoplast secretes primary wall materials on both sides of the cell plate or middle lamella.

(b) Cleavage cytokinesis: It occurs in animal cells and pollen mother cells of some angiosperms. In this process, a cleavage furrow appears at the middle, which gradually deepens and breaks the parent cell into two daughter cells. A special structure called **midbody** is formed in the centre, and it is a centripetal process.

Duration of mitosis

The duration of mitosis varies from species to species. The time required for mitosis ranges from 6 minutes to several hours. Temperature and nutrition are also known to affect duration of mitosis. If we talk of different phases of mitosis, anaphase is shortest phase, metaphase is intermediate in duration and prophase and telophase are of longest duration.

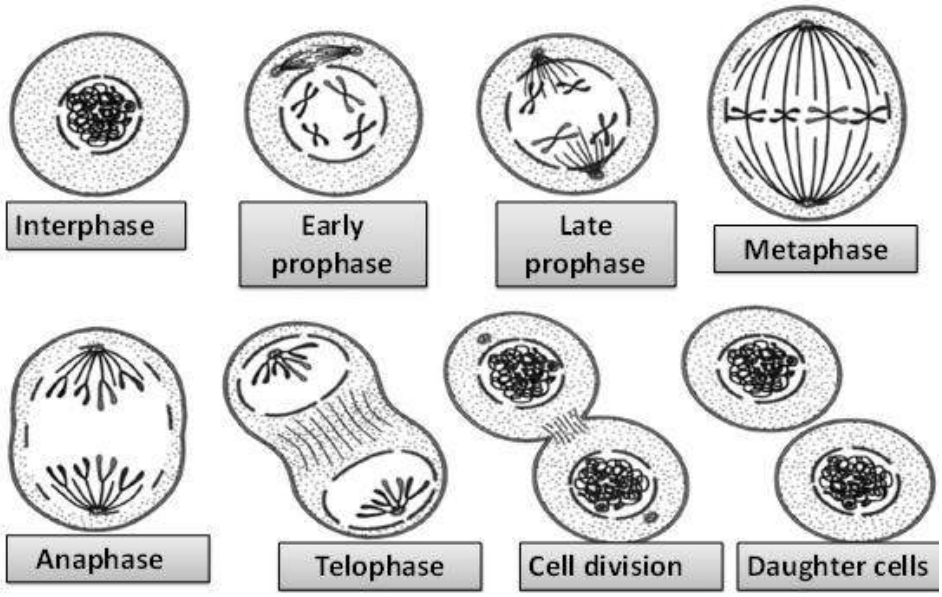

Fig. 4.5: Various stages of mitosis occurring in animal cell

Mitotic poison

There are several chemical substances which are known to inhibit the process of mitosis or prevent the entry of cell into the process of mitosis. Such substances are called as **mitotic poison**. One of the most commonly known mitotic poisons is **colchicines** which arrests the cell at metaphase stage. Colchicines is obtained from *Colchicum autumnale* and other species of Liliaceae family, it inhibits formation of **spindle fibres**. Another well known mitotic poison is **ribonuclease enzyme** which acts as prophase poison. Mitotic poison functions to inhibit or arrest the process of mitosis at a particular stage, but if cells are exposed to higher concentration of some of these poison, it may result in immediate cell death.

Table-2: Differences between mitosis in animal and plant cell

S.No.	Animal cell mitosis	Plant cell mitosis
1	It is found in bone marrow and epithelial cells	It occurs in meristematic cells
2	Animal cell become round before mitosis.	There is no change in shape of plant cell before the cell division.
3	Centrosome is required for mitosis	No centrosome is present in plant cells.

4	Formation of asters occurs during mitosis in animal cells.	No asters formation
5	Cytokinesis occurs through cleavage.	Cytokinesis occurs through cell plate formation.

4.3.1.1 Significance of mitosis

1. It is an equational division which maintains equal distribution of chromosomes after each cell cycle, through which identical daughter cells are produced having the same amount and type of genetic constitution as that of the parent cell.
2. Mitosis results in the formation of two daughter cells which inherit identical chromosomal material (hereditary material) with that of the parental cell. Both the daughter cells formed after mitosis have the same genetic constitution, qualitatively (i.e. genetic makeup or characters) as well as quantitatively (i.e. numbers), as the parent cell.
3. By this process, DNA, the main component of chromosomes, is distributed equally among the two newly formed nuclei. Mitosis maintains constant number of chromosomes in all body cells of an organism. The number of chromosomes remains the same from one generation to another generation.
4. It helps to maintain the equilibrium in the amount of DNA and RNA contents of a cell as well as the nuclear and cytoplasmic balance in the cell. It also helps the cell in maintaining proper size.
5. It is responsible for growth and development of multi-cellular organisms from a single-celled zygote.
6. Mitosis is also required for vegetative reproduction in plants. The characters of the plants grown by vegetative reproduction may be preserved for a long period. Even in sexual reproduction, mitosis occurs in first stage of gametogenesis to increase the number of cells which will undergo meiosis.
7. The number of chromosomes remains the same in all the cells produced by this division. Thus, the daughter cells retain the same characters as those of the parent cell.
8. It is a method of multiplication in unicellular organisms. It helps asexual reproduction, growth and development of organisms.
9. Dead cells are replaced by newly formed cells through mitosis. Mitosis helps in restoring wear and tear in body tissues, replacement of damaged or lost part, healing of wounds and regeneration of detached parts (as in tail of lizards). It thus helps in the repair of the body.
10. There exists a particular ratio between nucleus and cytoplasm. When cell increases in size the ratio between of size between nucleus and cytoplasm reduces. Mitosis is required to restore the ratio.
11. Cell division keeps cell small, this is important because smaller cells have higher surface volume ratio (as compared to large cells) which makes cells more efficient in exchange of material.

12. If mitosis remains unchecked, it may result in uncontrolled growth of cells leading to cancer or tumour.

4.3.2 Meiosis

Meiosis (from Greek, meiosis, which means lessening) is a specialized type of cell division that reduces the chromosome number by half, creating four haploid cells, each genetically distinct from the parent cell that gave rise to them. This process occurs in all sexually reproducing single-celled and multicellular eukaryotes, including animals, plants, and fungi. It is the form of eukaryotic cell division (reductional division) which occurs during the process of formation of gametes in sexually reproducing organisms and produces haploid sex cells or gametes from diploid cells. The process also occurs during formation of spores in some organisms. In meiosis a diploid cell (2N) undergoes two successive divisions to form four haploid (N) daughter cells. The haploid cells give rise to gametes.

Meiosis was discovered and described for the first time in sea urchin eggs in 1876 by the German biologist **Oscar Hertwig**. It was described again in 1883, at the level of chromosomes, by the Belgian embryologist **Edouard Van Beneden**, in Ascaris roundworm eggs. While working on the horse threadworm (*Parascaris equorum*), he observed that there were twice as many chromosomes visible during mitosis in the fertilized egg as there had been in the sperm and egg nuclei before the mitosis. By this observation, he concluded that the contribution of each of the female and male gametes was half the chromosome number to the zygote. **A. Weismann** (German biologist) suggested in 1887 that in each generation there must occur reduction division at some stage in which the chromosome number is reduced to half. **Flemming** (1887) and **Strasburger** (1888) observed that two nuclear divisions take place in rapid succession just prior to the formation of mature eggs and sperms in animals and formation of pollen grains in angiosperms. The entire process of reduction division leading to the formation of gametes was termed as “meiosis” in 1905.

In all organisms the chromosomes remain in pairs. The organisms reproducing asexually multiply by mitosis. Thus, there exists no chance of alteration of chromosome number. On the contrary, sexual reproduction demands contribution from two individuals. Thus there lies a risk of chromosomal imbalance. The process of meiosis helps to avert this probability by reducing the number of chromosomes to half. It may happen after gametic union (as in sporozoa) or before fertilization (in all higher organisms). In higher organisms, therefore, mitosis occurs in both somatic and germ cells but meiosis takes place in the germ cells alone and only during the formation of gametes. 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.

Meiosis is divided into meiosis I and meiosis II which are further divided into Karyokinesis I and Cytokinesis I and Karyokinesis II and Cytokinesis II respectively. The

preparatory steps that lead up to meiosis are identical in pattern and name to interphase of the mitotic cell cycle. Interphase is divided into three phases: Growth 1 phase, synthesis phase, and growth 2 phase.

(1) Growth 1 (G1) phase: In this very active phase, the cell synthesizes its vast array of proteins, including the enzymes and structural proteins it will need for growth. In G1, each of the chromosomes consists of a single linear molecule of DNA.

(2) Synthesis (S) phase: The genetic material is replicated; each of the cell's chromosomes duplicates to become two identical sister chromatids attached at a centromere. This replication does not change the ploidy of the cell since the centromere number remains the same. The identical sister chromatids have not yet condensed into the densely packaged chromosomes visible with the light microscope. This will take place during prophase-I in meiosis.

(3) Growth 2 (G2) phase: G2 phase as seen before mitosis is not present in meiosis. Meiotic prophase corresponds most closely to the G2 phase of the mitotic cell cycle.

Interphase is followed by meiosis I and then meiosis II. Meiosis I separates homologous chromosomes, each still made up of two sister chromatids, into two daughter cells, thus reducing the chromosome number by half. During meiosis II, sister chromatids decouple and the resultant daughter chromosomes are segregated into four daughter cells. For diploid organisms, the daughter cells resulting from meiosis are haploid and contain only one copy of each chromosome.

Meiosis I: It is the first division of meiosis where a diploid cell divides into two daughters each having half the number of replicated chromosomes. Meiosis I segregates homologous chromosomes, which are joined as tetrads (2n, 4c), producing two haploid cells (n chromosomes, 23 in humans) each contain chromatid pairs (1n, 2c). Because the ploidy is reduced from diploid to haploid, meiosis-I is referred to as a **reductional division**. The first meiotic division consists of four stages; Prophase I, Metaphase I, Anaphase I and Telophase I.

1. Prophase I: It is typically the longest phase of meiosis. During prophase I, homologous chromosomes pair and exchange DNA (homologous recombination). This often results in chromosomal crossover. This process is critical for pairing between homologous chromosomes and hence for accurate segregation of the chromosomes at the first meiosis division. The paired and replicated chromosomes are called bivalents or tetrads, which have two chromosomes and four chromatids, with one chromosome coming from each parent. The process of pairing the homologous chromosomes is called synapsis. At this stage, non-sister chromatids may cross-over at points called chiasmata. Prophase I has been divided into a series of sub-stages which are named according to the appearance of chromosomes.

(a) Leptotene:

1) Formation of homologous chromosomes occurs. Homologous chromosomes resemble each other in their length, position of centromeres, chromomeres and alleles.

2) In each homologous pair of chromosomes one chromosome is paternal and other is maternal.
3) In animal cell the chromosomes are found to coverage towards the side of centrosomes like a bouquet. Hence this stage is also called bouquet stage.
4) Each chromosome is attached to nuclear envelope by its both ends.
5) Centrosome or centrioles pairs, separate and development astral rays occurs.

(b) Zygotene:

1) Pairing of homologous chromosomes.
2) The process of attachment or pairing of homologous chromosomes is called synapsis. Synapsis produces pairs of chromosomes called bivalents.
3) The nucleoprotein complex that helps in adherence of sister chromatids and then homologous chromosomes is called **synaptonemal complex**.
4) Asters continue to move away from each other.

(c) Pachytene:

1) It is of longer duration as compared to Leptotene and Zygotene.
2) It is one of the most important stages/ sub-stage of meiosis as chromosome thickening and crossing over takes place in this sub-stage.
3) Each bivalent at the end of Zygotene is made up of two homologous chromosomes and each chromosome comprises of two chromatids.
4) The two chromatids of the same chromosome are called sister chromatids while chromatids belonging to different chromosomes of the homologous pair are known as non-sister chromatids.
5) Crossing over (exchange of chromatid segments or genetic material between the homologous chromosomes) always occurs between non-sister chromatids.
6) With the end of Pachytene disintegration of synaptonemal complex begins.
7) Asters move further away from each other.

(d) Diplotene:

1) Separation of homologous chromosomes occurs except in the region of chiasmata.
2) Dissolution of synaptonemal complex begins to dissolve bivalent.
3) Diplotene is prolonged in many animal oocytes. For example, all the oocytes of human female reach the diplotene stage in the fifth month of foetus and remain so for many years till ovulation is to occur.
4) In oocytes of many fishes, amphibians, reptiles and birds the bivalents elongate and become converted into lampbrush chromosomes.

(e) Diakinesis:

1) Chromosomes condense further during the Diakinesis stage, from Greek words meaning "moving through".

2) This is the first point in meiosis where the four parts of the tetrads are actually visible. Reduction in number of chiasmata is observed.
3) In this last stage of the first meiotic prophase the chromosomes are shortest and thickest.
4) In each pair the chromatids of a chromosome remain attached in the region of centromere. There is also an attachment between non-sister chromatids of the two homologous chromosomes in the region of chiasmata.
5) The chromosomes bivalents move towards the periphery of the nucleus and remain connected only at the points of chiasmata.
6) Chiasmata slip from their original position and pass outwardly. The phenomenon is called terminalization.
7) In case of animal cells, asters reach the opposite sides in position of poles.
8) Sites of crossing over entangle together, effectively overlapping, making chiasmata clearly visible. Other than this observation, the rest of the stage closely resembles Prometaphase of mitosis; the nucleoli disappear, the nuclear membrane disintegrates into vesicles, and the meiotic spindle begins to form.
9) The chromosomes are finally released into the cytoplasm.

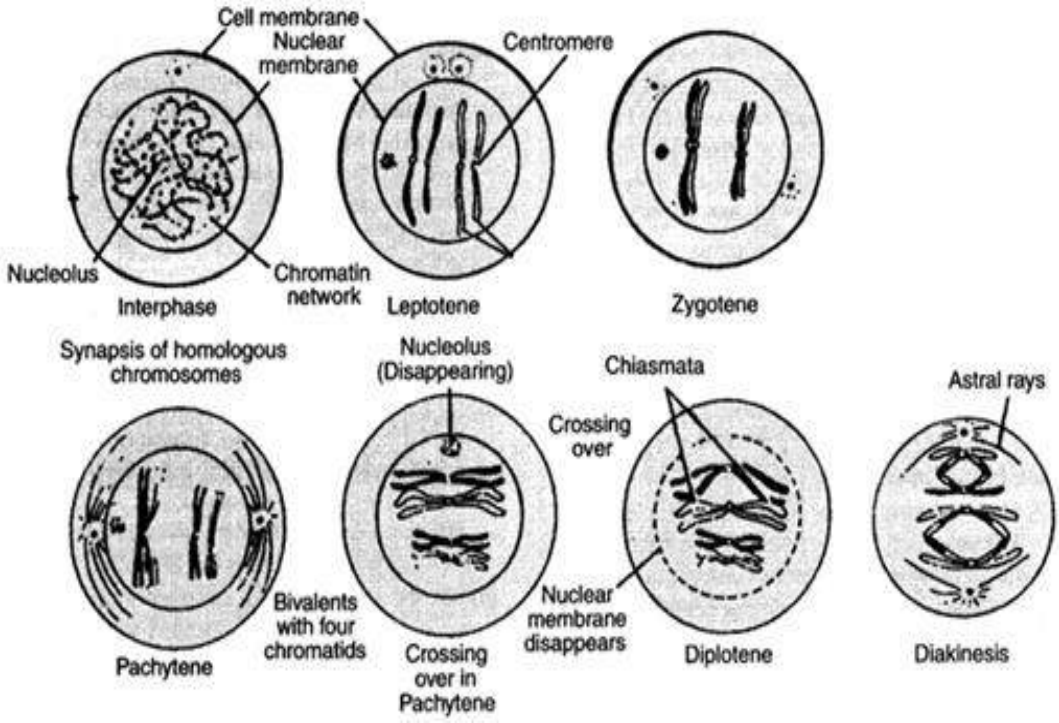

Fig. 4.6: Different stages of Prophase I of meiosis I

2. Metaphase I: Two major events of metaphase I include complete disintegration of nuclear membrane and the formation of spindle. All the chromosomes, each along with their two chromatids, move to the equatorial region of the newly formed spindle.

Differing from the metaphase stage of mitosis, the centromeres of chromosome pairs in metaphase stage of meiosis I become attached with the spindle fibres near the equatorial region. The centromeres remain clearly apart from each other and face the opposite poles while the arms of the chromosome pairs lie towards the equator.

1) Nuclear envelop degenerate and spindle fibres appear.
2) At this stage, bivalents get arranged on the equator.
3) Chromosomes occur in bivalents having chiasmata.
4) Chromosomes of a bivalent get attached to spindle fibre belonging to different poles.

3. Anaphase I: There is first a repulsion and then movement of the two centromeres of the homologous chromosomes towards the opposite poles of the spindle in anaphase-I. A centromere carries either a paternal or a maternal chromosome to one pole but not both the chromosomes. This actually reduces the chromosome number from diploid (2n) to haploid (n), which is the main feature of meiosis of reduction division.

1) Homologous chromosomes separate and move to opposite poles due to disintegration of chiasmata.
2) This results in formation of two haploid sets of chromosomes.
3) Separation of homologous chromosomes is known as disjunction, separated chromosomes are called univalent.
4) At the end of anaphase-I, two groups of chromosomes are formed, with half number of chromosome present in the mother cell.

4. Telophase I: A nuclear membrane develops around each group of homologous chromosomes present on the two opposite poles in the form of a compact group in Telophase-I. The nucleolus reappears. Both the so formed daughter nuclei contain haploid number (n) of chromosomes, and each chromosome contains a pair of chromatids.

Both the daughter nuclei may or may not be separated by a plasma membrane and soon pass on to the next division, i.e., meiosis division-II.

Meiosis II: It is the second meiotic division, and usually involves equational segregation, or separation of sister chromatids. Mechanically, the process is similar to mitosis, though its genetic results are fundamentally different. The end result is production of four haploid cells (n chromosomes, 23 in humans) from the two haploid cells (with n chromosomes, each consisting of two sister chromatids) produced in meiosis I. The four main steps of meiosis II are: Prophase II, Metaphase II, Anaphase II, and Telophase II.

1. Prophase II: The chromosomes separate into chromatids in both the haploid nuclei and cells formed after meiosis division-I. The separated chromatids remain connected only at the centromeres. The chromosomes start coiling and become shorter and thicker. The nuclear membrane and nucleolus start disintegrating and some spindle fibres also start appearing.

1) It is simple and of short duration.

2) Shortening of chromosomes occur.
3) Nucleolus disappears and nuclear envelope degenerate.
4) In animal cells, centrosomes develop astral rays and move to the opposite sides.

2. Metaphase II:

The chromosomes get arranged in an equatorial position in the newly-formed spindle. Very soon, the chromosome pair separates, of which each contains its own centromere. This is a very short phase of meiosis division-II.

1) Chromosome gets attached to spindle fibres, one from each pole.
2) Area of chromosome to which spindle fibres attach is called kinetochore.
3) Chromosomal fibres contract and bring the chromosomes get arranged on the equator.
4) The limbs of the two chromatids are divergent.

3. Anaphase II:

In this phase, the two sister chromosomes of each pair start to move towards the opposite poles of the spindle. They are being drawn towards the opposite poles by their centromeres.

1) Separation of centromere of two chromatids occurs.
2) The separated chromatids become daughter chromosomes.
3) Chromosomes move to opposite poles.
4) At the end of anaphase II, each pole has haploid number of chromosomes.

4. Telophase II:

Each polar group of chromosomes gets enveloped by a nuclear membrane, and there is the reappearance of nucleolus. Four cells are formed by cytokinesis, and the nucleuses in all these so formed four young cells contain haploid number (n) of chromosomes. In this way, four haploid cells are resulted from a single diploid cell in the process of meiosis.

1) De-condensation of chromosomes occurs.
2) Chromosomes organize to reform a nucleus, along with formation of nucleolus and nuclear envelope.
3) Astral rays and spindle fibres disappear.

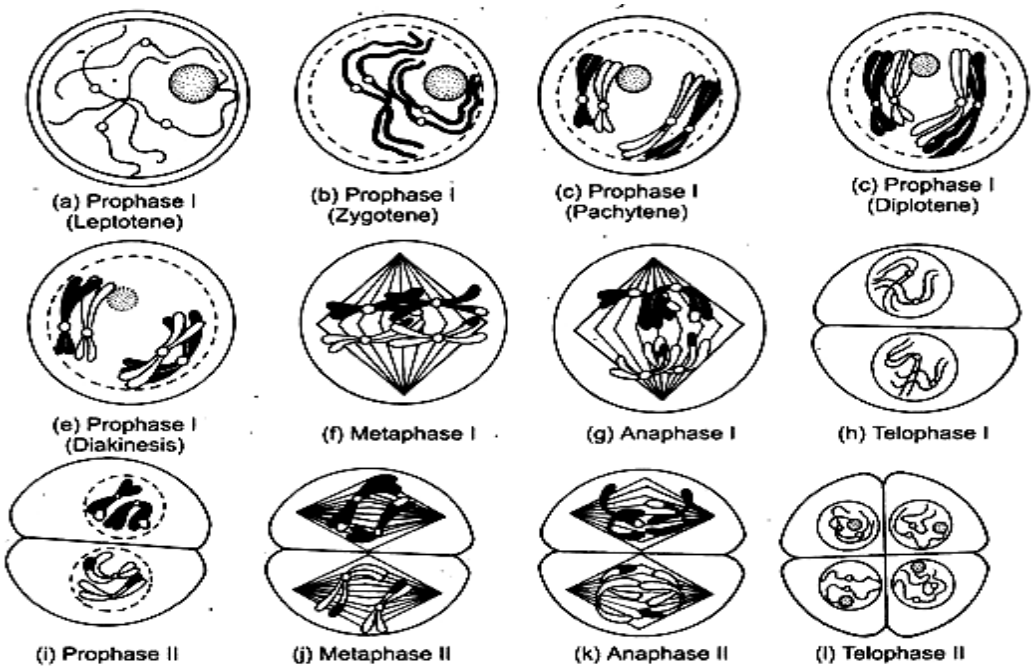

Fig. 4.7: Different stages of meiosis

Table-3: Differences between meiosis I and meiosis II

S.No.	Meiosis I	Meiosis II
1	It is heterotypic or reductional division.	It is homotypic or equational division.
2	It is a complex process and takes long duration of time.	It is a simple process and takes short duration of time.
3	There is present both growth phase and synthetic phase before interphase.	Only growth phase is present before interphase (called interkinesis). Synthetic phase is absent.
4	Sister chromatids have convergent arms in prophase-I	Sister chromatids have divergent arms in prophase-II
5	Crossing over occurs in prophase-I	No crossing over occurs in meiosis-II

Mitosis and meiosis are similar processes in that they both result in the separation of existing cells into new ones. They differ, however, in their specific processes as well as in their products. The reason for these differences lies in the difference in the class of cells that each process creates. Mitosis is responsible for reproducing somatic cells and meiosis is responsible for reproducing germ cells. In single-cell organisms, mitosis is the only form of cellular reproduction. One round of mitosis yields two genetically identical cells. In bacteria, this process results in an entirely new, independent organism. This is classified as asexual reproduction because it does not require sex for the creation of new organisms.

In multicellular organisms, like us, mitosis only occurs in somatic cells, which comprise all cells in an organism excluding germ cells. Cells that undergo mitosis duplicate their chromosomes, resulting in cells with two times their normal haploid or diploid numbers (4N chromosomes). Newly synthesized chromosomes remain closely associated with their like-chromosome. These two identical chromosomes are called sister chromatids. Once, the duplicated sisters' chromatids are separate such that one copy of each chromosome lines up on opposite ends of the cell. The cell then pinches in the center until it breaks into two different cells. A nucleus then forms around the chromosomes in each cell to yield two cells with the same original number of chromosomes as the preexisting cell.

There are two major differences between mitosis and meiosis. First, meiosis involves not one, but **two** cell divisions. Second, meiosis leads to the production of germ cells, which give rise to gametes. In meiosis, as in mitosis, the maternal and paternal homologues are replicated during DNA replication yielding two pairs of sister chromatids. After the first cell division, each of the resulting cells contains a pair of sister chromatids; one maternal pair and the other paternal. Unlike mitosis, meiosis does not end after one division; it continues with a second cell division. In this division, the sister chromatids are separated yielding four total haploid cells.

Table-4: Differences between mitosis and meiosis

S.No.	Mitosis	Meiosis
1	The division occurs in somatic cells and it is a single division.	It occurs in reproductive cells and it is a double division.
2	Mitosis takes place throughout the life of a multicellular organism.	Meiosis takes place only at the time of sexual reproduction.
3	The daughter cells resemble each other as well as their mother cell.	The daughter cells neither resemble one another nor their mother cell.
4	Chromosome number remains the same.	Chromosome number is halved.
5	Mitosis is required for growth, repair and healing. It does not introduce variations.	Meiosis has no such function. It introduces variations.
6	It occurs in both sexually and asexually reproducing organism.	Meiosis is found in only sexually reproducing organism.
7	Prophase: is shorter duration, generally of a few hours. Crossing over does not occur.	Prophase-I is longer duration, which may be of several days. Crossing over occurs.
8	Metaphase: Chromosomes are replicated but unpaired.	Chromosomes are replicated as well as paired to form bivalents.
9	Anaphase: Chromosomes are single stranded.	Chromosomes are double stranded in anaphase I and single stranded in anaphase-II.

10	Telophase: It is an essential component of mitosis	Telophase I may be absent. Telophase II always occurs.

4.3.2.1 Significance of Meiosis

In the process of sexual reproduction, the male and female gametes fuse to form a zygote which gives rise to the new off-springs. If the gametes contained the same number of chromosomes as that of their parents, the off-springs would have an ever-increasing chromosomes number in all future generations to come, and this might have resulted always in the formation of new and peculiar types of off-springs, much different from that of their parents. To solve this problem, nature has provided the phenomenon of meiosis to all sexually reproducing plants and animals. Meiosis maintains the haploid nature of gametes.

Another fascinating aspect of meiosis is that, it begins at the very early life in the individual but remains arrested for a considerably long time in the prophase state. In males the completion depends upon the attainment of sexual maturity. In the female, the completion of the division comes only shortly before or after fertilization. The process of meiosis not only reduces the chromosome number to half for the purpose of reproduction but also by random distribution of paternal and maternal chromosomes and by crossing-over through chiasma, it produces gametes, none of which are exactly alike. Thus, a large number of variations result, which have got great significance in evolution.

1. DNA, the sole hereditary material, is distributed equally among the gametes by the process of meiosis.
2. It provides stimulus for the formation of gemmates and spores.
3. Meiosis causes conversion from sporophytic generation to gametophytic generation in plants. It forms spores (n) from the spore mother cells (2n) and thus maintains the alternation of generations in organisms.
4. Meiosis-I reduce the number of chromosomes to one half or single genome where each chromosome is without its homologue.
5. The products of meiosis-I posses replicated or dyad chromosomes. Occurrence of dyad chromosome acts as a stimulus for meiosis-II to occur.
6. Meiosis causes segregation and random assortment of genes. Random assortment of paternal and maternal chromosomes produces genetic variations.
7. Crossing over brings about gene recombination or new combination of genes. It also produces genetic variation within the species. The variations are important raw materials for evolution and also help in improvement of races.
8. Non-disjunction and breakage of chromosomes may occur during anaphase-I due to non - dissolution of chiasmata. It produces chromosomal aberrations, aneuploidy and polyploidy.
9. Meiosis essentially maintains constancy in chromosomes from generation to generation.
10. It leads to the formation of haploid gametes (n) which is an essential process in sexually reproducing organisms. Fertilization restores the normal somatic (2n) chromosome number.

4.4 SUMMARY

1. **Amitosis** is a process of division found in prokaryotic cell. It occurs in lower group of organisms like protozoa, endosperm tissue of flowering plants and in cell of foetal membrane of vertebrates.
2. Every growing cell undergoes a cell cycle that consists of two phase an **interphase** and **mitotic** phase.
3. **Karyokinesis** is nuclear change taking place during mitosis and is divided into four phases namely prophase, metaphase, anaphase and telophase.
4. **Prophase** is the first phase and is the longest phase. In prophase chromatin becomes condensed resulting in formation of chromosomes. Nuclear membrane along with nucleolus disappears during late prophase. Single centriole divides into two and for each centriole astral rays are formed. Centrioles are absent in plant cells.
5. During metaphase chromosomes get arranged at equator. Centromere of chromosomes is attached to spindle fibres. **Metaphase** stage is the best stage to observe and count chromosomes.
6. Separation of chromosomes occurs in anaphase stage. During anaphase movement of chromatids occurs towards opposite poles. **Anaphase** is the shortest phase of mitosis.
7. Events of prophase are reversed in **telophase**. In telophase nuclear membrane and nucleolus reappear along with formation of chromatin network from chromosomes.
8. As a result after telophase, cell contains two separate nuclei (one at each pole). After four phases of karyokinesis, cytokinesis occurs. **Cytokinesis** is simply division of cytoplasm.
9. There is different in how the process of cytokinesis occurs in plant cell and in animal cells. A **cell furrow** is formed in animal cells which divides the cell into two cells. In plant cells, cytokinesis occurs by formation of **cell plate**.
10. Mitosis is essential for growth and development of organism and also for repair of damaged tissues. Mitosis is the basis of vegetative reproduction. **Colchicines** are an alkaloid used to arrest mitosis at metaphase stage.
11. Mitosis is known as **equational division** as daughter cells formed have same number of chromosomes as the mother cell.
12. In gonads, occurs reductional division called **meiosis**. After meiosis four daughter cells are formed with half the number of chromosomes present in mother cell. Cells which undergo meiosis are called **meiocytes**. Meiosis consists of two successive divisions called as **meiosis-I** and **meiosis-II**. Meiosis I is also known as **heterotypic** division and meiosis II is known as **homotypic** division.
13. Meiosis-I is divided into four phases prophase I, metaphase I, anaphase I and telophase I, whereas, meiosis II is divided into prophase II, metaphase II, anaphase II and telophase II.
14. **Prophase-I** is the longest phase and is further divided into sub-stages; Leptotene, Zygotene, Pachytene, diplotene and Diakinesis.
15. In **Leptotene** chromosomes are seen in the form of long thin threads.

16. In **Zygotene** stage, pairing between homologous chromosomes occurs. This is also called as **synapsis** formation. As a result of synapsis formation, bivalents are formed. During Zygotene formation of **synaptonemal complex** also occurs which facilitates genetic recombination.
17. In **Pachytene** stage of prophase-I shortening and thickening of bivalents occur. Each of the bivalent is made of four chromatids known as tetrad. **Crossing-over** between non-sisters chromatids occur in Pachytene.
18. In **Diplotene** separation of paired chromosomes occur but chromosomes remains attached at chiasmata.
19. In **Diakinesis** contraction of chromosomes increases. Number of chiasmata gets reduced in Diakinesis, known as **terminalization**.
20. In **Metaphase-I** stage, bivalents get arranged on equatorial plate.
21. Separation of homologous chromosomes occurs at **Anaphase I**. Separation of tetrad into dyads is known as **disjunction**, which results in reduction of chromosome number to half.
22. In **Telophase** chromosomes gets grouped at poles and nuclear membrane reappear.
23. **Meiosis II** is equational division, which is similar to mitosis.
24. In **Prophase II** nuclear membrane disappear, chromosomes become visible along with formation of spindle fibres.
25. Chromosomes get arranged on equator at **metaphase II**.
26. Division of centromere and separation of chromatids occur during **anaphase II**.
27. Reformation of nucleoli and formation of nuclear membrane occurs during **telophase II**.

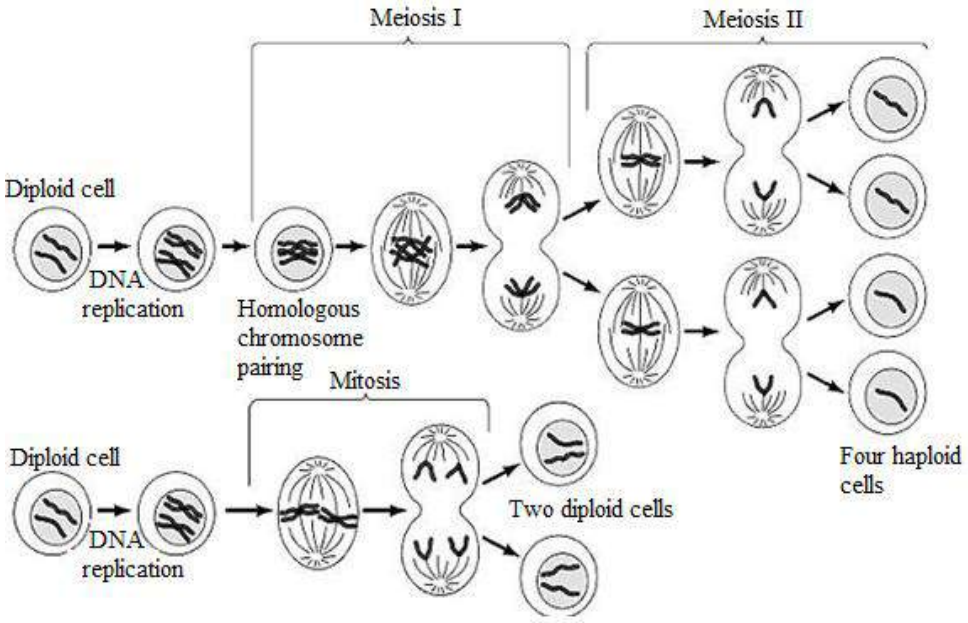

Fig: 4.8 Summary of events occurring during mitosis and meiosis

4.5 GLOSSARY

Acrocentric: A chromosome in which the centromere is located quite near one end of the chromosome.

Amitosis: Direct method of cell division, characterized by simple cleavage of the nucleus without the formation of chromosomes.

Amphiastral: It is found in animals in which spindle has two asters, one at each pole of the spindle.

Anaphase: The stage in mitosis or meiosis following metaphase in which the daughter chromosomes move away from each other to opposite ends of the cell.

Anaphase I: The third stage of meiosis I, in which the homologous chromosomes separate and move to opposite ends of the cell.

Anaphase II: The third stage of meiosis II, in which the sister chromatids of double-stranded chromosomes separate and move to opposite ends of the cell.

Aneuploidy: Presence of an abnormal numbers of chromosomes in a cell, for example a human cell having 45 or 47 chromosomes instead of the usual 46.

Asters: A structure formed in a cell during mitosis, composed of astral rays radiating about the centrosome.

Astral ray: An aster is a cellular structure shaped like a star, formed around each centrosome during mitosis in an animal cell. Asters do not form during mitosis in plants. Astral rays, composed of microtubules, radiate from the centrospheres and look like a cloud.

Cell Cycle: The cell cycle or cell-division cycle is the series of events that take place in a cell leading to its division and duplication of its DNA to produce two daughter cells.

Cell Plate: A cellular structure formed during a cytokinesis in a plant cell, which divides the cytoplasm of the cell into two equal portions and gives rise to the cell walls of the two newly formed cells.

Centriole: A cellular organelle found in the center of animal cells, help in organizing microtubule assembly so that a spindle is formed during cell division.

Centromere: A region on a chromosome that joins two sister chromatids.

Centrosome: A small region near the nucleus in the cell cytoplasm, containing the centrioles.

Chiasmata: A point of overlap of paired chromatids at which fusion and exchange of genetic material take place during prophase of meiosis.

Chromatid: One of two identical chromosomal strands into which a chromosome splits longitudinally preparatory to cell division.

Chromatid: One of two identical copies of a replicated chromosome.

Chromatin: The readily stainable substance of a cell nucleus, consisting of DNA, RNA, and various proteins, that forms chromosomes during cell division.

Chromosome: Any of several threadlike bodies, consisting of chromatin, that carry the genes in a linear order: the human species has 23 pairs, designated 1 to 22 in order of decreasing size and X and Y for the female and male sex chromosomes respectively.

Colchicines: A pale yellow, crystalline alkaloid, $C_{22}H_{25}NO_6$, the active principle of colchicum. It also acts as mitotic poison.

Cytokinesis: The division of the cell cytoplasm that usually follows mitotic or meiotic division of the nucleus.

Daughter Cell: A cell resulting from the replication and division of a single parent cell.

Diakinesis: The last stage in prophase, in which the nucleolus and nuclear envelope disappear, spindle fibers form, and the chromosomes shorten in preparation for anaphase.

Diploid: Having two similar complements of chromosomes.

Diplotene: A late stage of prophase during meiosis, in which the chromatid pairs of the tetrads begin to separate and chiasmata can be seen.

Germ cell: The sexual reproductive cell at any stage from the primordial cell to the mature gamete.

Haploid Cell: a cell that contains one complete set of chromosomes.

Interphase: The phase of the cell cycle in which a typical cell spends most of its life. During this phase, the cell copies its DNA in preparation for mitosis.

Karyokinesis: The series of active changes that take place in the nucleus of a living cell in the process of division.

Kinetochore: A specialized region on the centromere of chromosome where spindle fibers attach to the chromosome.

Leptotene: A stage of cell division during the prophase of meiosis, in which the chromosomes are not distinct but appear as a mass of entangled threads.

Meiocytes: A type of cell that differentiates into a gamete through the process of meiosis. Through meiosis, the diploid meiocytes divides into four genetically different haploid gametes.

Meiosis: Part of the process of gamete formation, consisting of chromosome conjugation and two cell divisions, in the course of which the diploid chromosome number becomes reduced to the haploid.

Meiosis I: The first meiotic division during which the parent cell divides into 2 cells, each with a reduced of chromosomes.

Meiosis II: The second meiotic division during which the chromatids within the 2 cells formed from meiosis I separate. The cells then divide to form 4 haploid cells.

Meristematic: Consisting of or having the properties of meristem.

Metacentric: Of or relating to any chromosome or chromatid whose centromere is centrally located, creating two apparently equal chromosome arms.

Metaphase: The stage in mitosis or meiosis in which the duplicated chromosomes line up along the equatorial plate of the spindle.

Microtubule: A hollow cylindrical structure in the cytoplasm of most cells, involved in intracellular shape and transport.

Mitosis: The usual method of cell division, characterized typically by the resolving of the chromatin of the nucleus into a threadlike form, which condenses into chromosomes, each of

which separates longitudinally into two parts, one part of each chromosome being retained in each of two new cells resulting from the original cell.

Mitotic poison: Substance which inhibits mitosis, e.g. ribonuclease enzyme act as poison at prophase, colchicines inhibits the formation of mitotic spindle.

Nucleolus: A conspicuous, rounded body within the nucleus of a cell.

Pachytene: The third stage of prophase in meiosis, during which each chromosome pair separates into sister chromatids with some breakage and crossing over of genes.

Periphery: The external boundary of any surface or area.

Polyploidy: Having a chromosome number that is more than double the basic or haploid number.

Prophase: The first stage of mitosis or meiosis in eukaryotic cell division, during which the nuclear envelope breaks down and strands of chromatin form into chromosomes.

Regeneration: The restoration or new growth by an organism of organs, tissues, etc., that have been lost, removed, or injured.

S phase: The synthesis phase of the cell cycle in which the DNA is replicated.

Somatic cell: One of the cells that take part in the formation of the body, becoming differentiated into the various tissues, organs, etc.

Spindle fiber: One of a network of achromatic filaments that extend inward from the poles of a dividing cell, forming a spindle-shaped figure. They are aggregates of microtubules that move chromosomes during cell division.

Submetacentric: Having the centromere near the center but not in the middle, so that one arm is shorter than the other. Used of a chromosome.

Synapsis: The pairing of homologous chromosomes, one from each parent, during early meiosis.

Synaptonemal complex: A ladder-like series of parallel threads visible in electron microscopy adjacent to and coaxial with pairing chromosomes in meiosis.

Telocentric: Relating to any chromosome or chromatid whose centromere is positioned at its end, creating one chromosome arm

Telophase: The final stage of meiosis or mitosis, in which the separated chromosomes reach the opposite poles of the dividing cell and the nuclei of the daughter cells forms around the two sets of chromosomes.

Tetrad: A group of four chromatids formed by synapsis at the beginning of meiosis.

Zygote: The cell produced by the union of two gametes, before it undergoes cleavage.

Zygotene: The second stage of prophase in meiosis, during which strands of homologous chromosomes line up and become pairs.

4.6 SELF ASSESSMENT QUESTIONS

4.6.1 Objective type questions:

1. In which stage of meiosis does the genetic constitution of gametes is finally decided

(a) Metaphase I (b) Anaphase I
(c) Metaphase II (d) Anaphase II

2. Meiosis occurs in organisms during
(a) Sexual reproduction (b) Vegetative reproduction
(c) Both Sexual and vegetative (d) None of the above

3. During anaphase-I of meiosis
(a) Homologous chromosomes separate (b) Non- homologous autosomes separate
(c) Sister chromatids separate (d) Non- sister chromatids separate

4. A bivalent of meiosis-I consists of?
(a) 2 chromatids and 1 centromere (b) 2 chromatids and 2 centromeres
(c) 4 chromatids and 2 centromeres (d) 4 chromatids and 4 centromeres

5. Select the correct statement about G_1 phase
(a) Cell is metabolically inactive
(b) DNA in the cell does not replicate
(c) It is not a phase of synthesis of macromolecules
(d) Cell stops growing

6. G_1, S and G_2 phases are observed in
(a) Prophase (b) Anaphase
(c) Interphase (d) Metaphase

7. The stage when chiasmata are observed is
(a) Leptotene (b) Diplotene
(c) Zygotene (d) Pachytene

8. Synapsis occurs between
(a) Spindle fibres and centromeres (b) mRNA and ribosomes
(c) A male and a female gamete (d) Two homologous chromosomes

9. Name the phase of prophase-I when synaptonemal complex dissolves, chromatids become clear and bivalents are called tetrads?
(a) Pachytene (b) Diakinesis
(c) Diplotene (d) Zygotene

10. Correct sequence of different phase of cell cycle is
(a) S- G_1 - G_2 →M (b) G_1- S - G_2 →M

(c) G_1 - G_2- S →M (d) G_2 - S- G_1→M

11. DNA molecules present in a bivalent is
(a) 4 (b) 3
(c) 2 (d) 6

12. In mitosis the process of Karyokinesis occurs in?
(a) Telophase (b) Interphase I
(c) Prophase (d) Interphase

13. Single celled organisms are reproduced by?
(a) Binary division (b) Meiosis
(c) Mitosis (d) Intimation

14. Meiosis was first discovered by?
(a) Oscar Hertwig (b) Walther Flemming
(c) Charles Darwin (d) Robert H. Brown

15. Which of the following cellular structures always disappears during mitosis and meiosis?
(a) Plasma membrane (b) Nucleolus and nuclear envelope
(c) Plastids (d) None of these

16. The stage in which daughter chromosomes move towards the poles of the spindle
(a) Anaphase (b) Metaphase
(c) Prophase (d) Telophase

17. Chromosomes can be counted best at the stage of?
(a) Late prophase (b) Late anaphase
(c) Telophase (d) Metaphase

18. During which stage of the cell cycle do chromosomes duplicate?
(a) G1 (b) G2
(c) Mitosis (d) S phase

19. The process of cell division that results in two genetically identical daughter cells developing from a single parent cell is?
(a) Cell division (b) Mitosis
(c) Meiosis (d) Binary division

20. A bivalent consists of?

(a) Two chromatids and one centromere (b) Four chromatids and two centromeres
(c) Two chromatids and two centromeres (d) Four chromatids and four centromeres

21. At what stage of meiosis does crossover occur?
(a) Anaphase I (b) Metaphase I
(c) Prophase I (d) Telophase I

22. The daughter cells produced by telophase-I of mitosis are:
(a) Diploid (b) Gametes
(c) Tetrad (d) Haploid

23. Centrosomes are duplicated during which phase of the cell cycle?
(a) Anaphase (b) Interphase
(c) Metaphase (d) Telophase

24. At metaphase-I, the kinetochores of sister chromatids face _____.
(a) Opposite pairs (b) Opposite poles
(c) The same pole (d) The same tetrad

25. During cell division in apical meristem, the nuclear membrane appears in?
(a) Metaphase (b) Cytokinesis
(c) Anaphase (d) Telophase

26. Number of chromatids at metaphase is?
(a) Two each in mitosis and meiosis (b) Two in mitosis and one in meiosis
(c) Two in mitosis and four inn meiosis (d) One in mitosis and two in meiosis

27. How many mitotic divisions are needed for a single cell to make 128 cells?
(a) 07 (b) 14
(c) 28 (d) 32

28. Which aspect of mitosis is affected by colchicines in inducing polyploidy?
(a) DNA replication (b) Chromosome doubling
(c) Formation of cell plate (d) Spindle formation

29. Which of the following represents the best stage to view the shape, size and number of chromosomes?
(a) Interphase (b) Prophase
(c) Metaphase (d) Telophase

30. During mitosis, ER and nucleolus begin to disappear at
(a) Late prophase (b) Early prophase
(c) Late metaphase (d) Early metaphase

4.6.2 Fill in the blanks:

1. G_0 phase of cell cycle is also known as _____.
2. Crossing over occurs at _____ stage of prophase-I.
3. Prokaryotic cells divide by a process of _____.
4. Chromosomes are arranged at equator in _____stage.
5. Somatic cells multiply by _____.
6. _____ is called as reductional division.
7. Centromere split and chromatids separate during _____.
8. Pairing of homologous chromosomes take place during _____.
9. _____ can be used to arrest mitosis at metaphase stage.
10. _____ is a brief interphase between meiosis I and meiosis II.

4.6.3 State whether following statements are true or false:

1. Meiosis-II is very similar to mitosis.
2. Meiosis occurs in somatic cells.
3. Animal's cells divides by cell plate formation.
4. Meiosis is also known as reductional division.
5. DNA synthesis occurs in cell cycle during G_2 phase of interphase.
6. Bivalents are pair of homologous chromosomes synapse together during Zygotene.
7. Chiasmata are point of attachment present between homologous chromosomes after crossing over.
8. During prophase stage of mitosis chromosomes move to poles through spindle fibres.
9. Cytokinesis is followed by karyokinesis during mitosis.

4.6.4 Very short answer questions:

1. What are mitogens? Name one.
2. Name the scientist who coined the term mitosis.
3. What is interphase?
4. What is amitosis?
5. What is heterotypic division?
6. Name the various sub-stages of Prophase-I?
7. What is bouquet stage? Where do we find it?
8. Why mitosis is called equational division?
9. Which tissue of animals and plants exhibits meiosis?
10. Why G_0 phase of cell cycle is called a resting phase.
11. Between a prokaryote and a eukaryote, which cell has a shorter cell division time?

12. Which of the phases of cell cycle is of longest duration?
13. Which tissue of animals and plants exhibits meiosis?
14. Given that the average duplication time of E. coli is 20 minutes, how much time will two E. coli cells take to become 32 cells?
15. Which part of the human body should one use to demonstrate stages in mitosis?
16. What attributes does a chromatid require to be classified as a chromosome?
17. At what stage of cell cycle does DNA synthesis take place?

4.6.1 Answers Key: 1-(b), 2-(a), 3-(a), 4-(c), 5-(b), 6-(c), 7-(b), 8-(d), 9-(c), 10-(b), 11-(a), 12-(d), 13-(c), 14-(a), 15-(b), 16-(a), 17-(d), 18-(d), 19-(b), 20-(b), 21-(c), 22-(d), 23-(b), 24-(c), 25-(d), 26-(a), 27-(a), 28-(d), 29-(c), 30-(b)

4.6.2 Answers Key: 1-Resting phase, 2-Pachytene, 3-Amitosis, 4-Metaphase, 5-Mitosis, 6-Meiosis, 7-Anaphase, 8-Zygotene, 9-Colchicine, 10-Interkinesis

4.6.3 Answers Key: 1-True, 2-False, 3-False, 4-True, 5-False, 6-True, 7-True, 8-False, 9-False

4.7 REFERENCES

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4.9 TERMINAL QUESTIONS

4.9.1 Short answer type question:

1. How does cell division occur in prokaryotes?
2. Describe the events taking place during interphase?
3. Define crossing over? In which stage of meiosis does it take place?
4. State the role of centriole other than spindle formation?
5. Both unicellular and multicellular organisms undergo mitosis. What are the differences, if any observed in the process between the two?
6. How does cytokinesis in plant cells differ from that in animal cells?
7. Describe the following:
   (a) Synapsis
   (b) Bivalent
   (c) Chiasmata.
8. Name a cell that is found arrested in diplotene stage for months and years. Comment in 2-3 lines how it completes cell cycle?
9. How does cytokinesis in plant cells differ from that in animal cells?
10. A cell has 32 chromosomes. It undergoes mitotic division. What will be the chromosome number (N) during metaphase? What would be the DNA content (C) during anaphase?

4.9.2 Long answer type question:

1. Describe different phases of cell cycle? Elaborate about M phase of cell cycle?
2. Explain about different phases of mitosis? How does mitosis differ in plant and animal cells?

3. Summarize different events occurring in a cell during the process of meiosis?
4. With help of suitable diagram explain different stages of prophase-I of meiosis?
5. Differentiate between Meiosis I and meiosis II.
6. Comment on the statement – Telophase is reverse of prophase.
7. What are the various stages of meiotic prophase-I? Enumerate the chromosomal events during each stage?
8. Differentiate between the events of mitosis and meiosis
9. Write brief note on the following
    (a) Synaptonemal complex
    (b) Metaphase plate
10. Write briefly the significance of mitosis and meiosis in multicellular organism.

BLOCK-2 MOLECULAR BIOLOGY

UNIT-5 STRUCTURE AND COMPOSITION OF DNA

5.1 OBJECTIVES

After reading this unit student will be able-

- To study about components that make up nucleic acids
- To know about structure and function of DNA
- To have elementary idea of different morphological forms of DNA
- To understand how does DNA replication occurs in prokaryotes and eukaryotes
- To study about DNA-protein interaction

5.2 INTRODUCTION

Cells are known to contain many biomolecules such as proteins, lipids, carbohydrates and nucleic acids. Among these, nucleic acids are large and heavy biomolecules which have molecular weight from 30,000 to several millions. The large size of nucleic acids is because they are made up of millions of monomeric units. **Friedrich Miescher** (1869, Swiss physician and biologist) first of all isolated nucleic acid from pus cells. They were then named as ***nuclein***. Oscar Hertwig (1884, German zoologist and professor) proposed this nuclein to be the carrier of hereditary traits. Because of their acidic nature they were initially called as **nucleonic acid** which were later named as nucleic acid. Nucleic acids are found to be present in all living organisms' plants, animals, and bacteria and even in viruses.

There are two types of nucleic acid found to be present in living cells **DNA** (deoxyribonucleic acid) and **RNA** (ribonucleic acid). In eukaryotes DNA is present in membrane bound cell organelle called as nucleus, whereas, in prokaryotes DNA is present (single chromosome) free in cytoplasm. Such DNA found in bacterial cell is known as **nucleoid**. DNA is the genetic material of almost all living organisms except some viruses, which have RNA as their genetic material. DNA is basically made up of three components phosphoric acid, deoxyribose sugar and nitrogenous bases (purines and pyrimidines). Presence of purine and pyrimidines in nucleic acid was reported by Fisher (1880s) and Levene (1910) reported phosphoric acid and deoxyribose sugar to be present in DNA.

In eukaryotes, along with nucleus DNA is also present in cell organelles such as, mitochondria and chloroplast. Such DNA is known as **extra chromosomal** or **extra nuclear DNA**. In nucleus, DNA is present in form of chromosomes i.e. it is linear however; extra chromosomal DNA (present in mitochondria or chloroplast) is always circular. Beside nucleoid, prokaryotes also contain extra chromosomal DNA present in form of circular plasmids. Although, DNA present in prokaryotes and eukaryotes are similar in chemical components by which they are made and the function they perform (both serve as genetic material), there exists several differences between prokaryotic and eukaryotic DNA which are as follows:

Table 5.1 Differences between prokaryotic and eukaryotic DNA

S.No.	Prokaryotic DNA	Eukaryotic DNA
1	Prokaryotic genomic DNA is present free in cytoplasm and occurs as covalently closed circular DNA.	Eukaryotic genomic DNA is present in well defined nucleus and occurs as linear DNA with two ends.
2	The quantity of DNA is comparatively less.	More in amount than in prokaryotes.
3	It is made up of single chromosome.	Eukaryotic genomic DNA contains many chromosome pairs (number varies from species to species).
4	Extra chromosomal DNA is present in form of plasmids.	Circular extra chromosomal DNA is present in cellular organelles like mitochondria and chloroplast.
5	Only very few proteins interacts with prokaryotic DNA. Usually codes for 300 to 500 proteins.	Large number of proteins interacts with eukaryotic DNA. Codes for about thousands of proteins. Human genome code for about 40000 proteins.
6	Majority of DNA is coding, only very little non-coding region occurs in prokaryotic DNA.	Majority of DNA is non-coding; the size of coding region is less as compare to non-coding regions.
7	Introns absent in the coding regions of DNA. Prokaryotic DNA contains only exons no introns.	Introns occur inside the coding region. Eukaryotic DNA contains both introns and exons.

5.3 DNA

Deoxyribonucleic acid (DNA) is a molecule composed of two chains (made of nucleotides) which coil around each other to form a double helix carrying the genetic instructions used in the growth, development, functioning and reproduction of all known living organisms and many viruses. DNA and ribonucleic acid (RNA) are nucleic acids; alongside proteins, lipids and complex carbohydrates (polysaccharides), nucleic acids are one of the four major types of macromolecules that are essential for all known forms of life.

DNA is well-suited for biological information storage, since the DNA backbone is resistant to cleavage and the double stranded structure provides the molecule with a built-in duplicate of the encoded information. This information is replicated as when the two strands separate. A large part of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences.

The two strands of DNA run in opposite directions to each other and are thus anti-parallel. Attached to each sugar is one of four types of nucleobases (informally, bases). It is the sequence of these four nucleobases along the backbone that encodes genetic information. RNA strands are created using DNA strands as a template in a process called transcription. Under the genetic code, these RNA strands are translated to specify the sequence of amino acids within proteins in a process called translation.

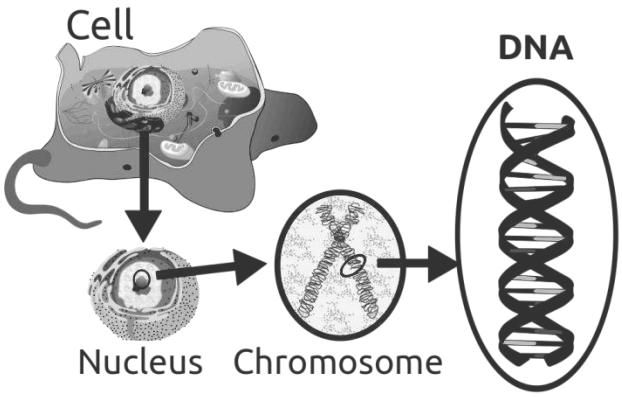

Fig. 5.1 – DNA in Eukaryotic cell

Within eukaryotic cells, DNA is organized into long structures called chromosomes. Before typical cell division these chromosomes are duplicated in the process of DNA replication, providing a complete set of chromosomes for each daughter cell. Eukaryotic organisms (animals, plants, fungi and protists) store most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts. In contrast, prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm. Within eukaryotic chromosomes, chromatin proteins such as histones compact and organize DNA. These compact structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed.

5.3.1 Components of DNA

There are three components, a pentose sugar, phosphoric acid and nitrogenous bases which combine to form monomer unit called as nucleotide. Large number of nucleotides joins to form polynucleotide chain. Each nucleotide is composed of one of four nitrogen-containing nucleobases i.e., cytosine (C), guanine (G), adenine (A) or thymine (T), a sugar called deoxyribose, and a phosphate group. The nucleotides are joined to one another in a chain by covalent bonds between the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. The nitrogenous bases of the two separate polynucleotide strands are bound together, according to base pairing rules (A with T and C with G), with hydrogen bonds to make double-stranded DNA.

The three components of DNA are:

(1) Sugar:

The sugar present in DNA is a pentose sugar (Fig. 5.2) called as deoxyribose sugar. Its name indicates that it is derived from ribose sugar by loss of an oxygen atom. Deoxyribose sugar joins with a nitrogenous base to form nucleoside.

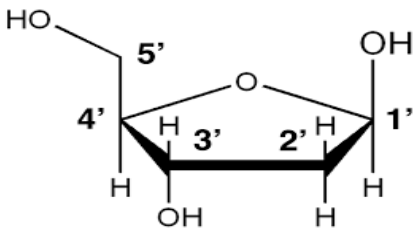

Fig.5.2.Structure of deoxyribose sugar

(2) Phosphoric acid

Phosphoric acid along with sugar molecule forms the backbone of polynucleotide chain. The bond formed by a phosphate between the sugar molecules of two different nucleotides is called phosphodiester bond. In one strand of DNA helix the phosphodiester bond is formed in the direction 3'-5' direction and in the other strand of the helix phosphodiester bonds are formed in 5'-3' direction.

(3) Nitrogenous bases

There are four nitrogenous bases present in structure of DNA which are grouped into two classes called as purines and pyrimidines. The complementary nitrogenous bases are divided into two groups, pyrimidines and purines. In DNA, the pyrimidines are thymine and cytosine; the purines are adenine and guanine.

Pyrimidines

Pyrimidines are simple aromatic compounds composed of carbon and nitrogen atoms in a six-membered, heterocyclic ring system. The name also refers to a specific compound (composition $C_4H_4N_2$), not found in nature that can be regarded as the parental structure of a wide range of naturally occurring chemical species. The most abundant naturally occurring pyrimidines are uracil (2, 4-dihydroxypyrimidine), cytosine (2-hydroxy-4-aminopyrimidine), and thymine (2, 4-dihydroxy-5-methyl pyrimidine). The first two are found predominantly in RNA, while the latter two are found predominantly in DNA. Small amounts of thymine are found in transfer RNA. The two pyrimidines found in DNA are usually base-paired with a purine residue on the complementary strand, so the purine to pyrimidine ratio in DNA is unity. In RNA, which is single-stranded, this ratio varies widely.

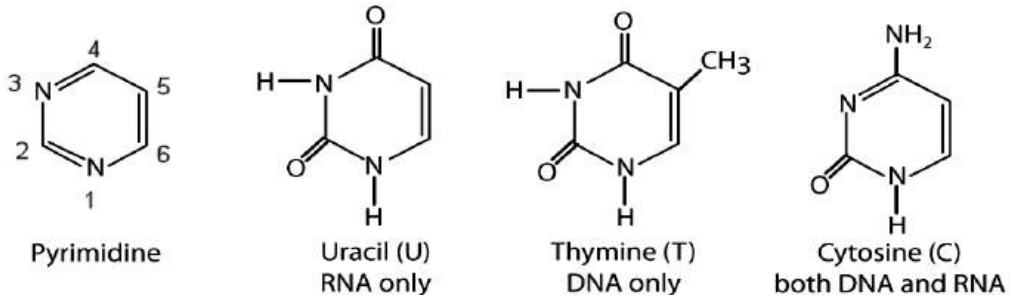

Fig. 5.3 - Structure of Nitrogenous base (Pyrimidines)

The term pyrimidine is also used to refer to pyrimidine derivatives, most notably the three nitrogenous bases that, along with the two purines, are the building blocks of both deoxyribonucleic acids (DNA) and ribonucleic acid (RNA). The pyrimidine nitrogenous bases are derived from the organic compound pyrimidine through the addition of various functional groups. Each pyrimidine ring has 2 nitrogen atoms present at first and third position and 4 carbon

atoms present at 2nd, 4th, 5th and 6th position of pyrimidine ring. In all three pyrimidines C-2 is linked to oxygen by double bond.

Purines

A purine is a heterocyclic aromatic organic compound that consists of a pyrimidine ring fused to an **imidazole ring**. Purine gives its name to the wider class of molecules, *purines*, which include substituted purines and their tautomers, are the most widely occurring nitrogen-containing heterocycle in nature. Purine is water soluble, are found in high concentration in meat and meat products, especially internal organs such as liver and kidney.

All purines contain a double-ringed structure that consists of a six-membered ring fused to a five-membered ring; think of a honeycomb cell attached to a pentagon. The purine ring is considered a heterocyclic molecule, meaning it is a closed ring containing at least two different kinds of atoms. Each of purines rings contains two nitrogen atoms, for a total of four within the double-ringed structure. These nitrogen atoms are located in the same positions in all purines. The remaining five positions within the rings are occupied by carbon atoms. The purine ring is encircled by hydrogen atoms, which can be replaced by other atoms or groups of atoms to form different purines.

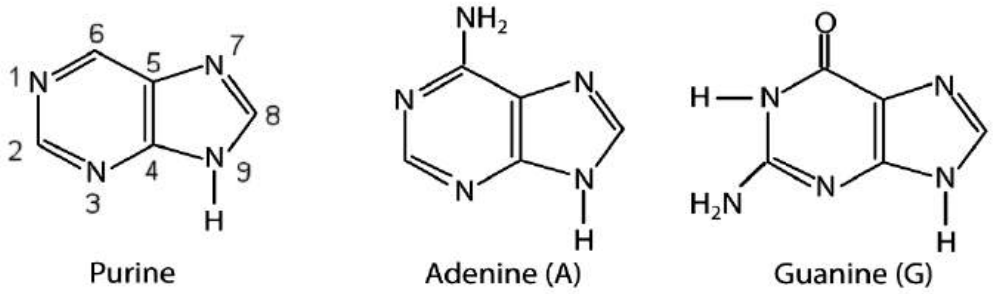

Fig. 5.4- Structure of Nitrogenous base (Purines)

Purines are double ringed nitrogenous bases found to be present in nucleic acids. Both DNA and RNA contain two types of purines which are adenine and guanine. Carbon atoms in purine ring are numbered in anti-clock wise direction and carbon atoms in imidazole ring are numbered in clockwise direction. C-4 and C-5 are common to both the rings. The four nitrogen atoms are present at 1st, 3rd, 7th and 9th position.

Nucleoside

Nucleoside is a molecule formed by association of nitrogenous base and pentose sugar. Nitrogenous bases get attached to C-1 of pentose sugar. When a glycosidic bond is formed between sugar and nitrogenous base, C-1 of sugar is always involved. If the nitrogenous base is a purine, the nitrogenous base is linked to the sugar via its N-9 atom, while if it's a pyrimidine, it is linked via its N-1 atom. Depending upon the type of sugar present, nucleosides are of two types;

Ribonucleosides and Deoxyribonucleosides. Because the pentose sugar present in DNA is deoxyribose, hence, nucleoside present in DNA called **Deoxyribonucleosides**.

There are four types of deoxyribose nucleosides present in DNA molecule:

1. Deoxyadenosine = Adenine + Deoxyribose
2. Deoxyguanosine = Guanine + Deoxyribose
3. Deoxycytidine = Cytosine + Deoxyribose
4. Deoxythymidine = Thymine + Deoxyribose

Nucleotide

Nucleotides are monomers which get linked to one another to form a polynucleotide chain present in DNA molecule. Nucleotides are the building blocks of nucleic acids; they are composed of three subunit molecules: a nitrogenous base, a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group. Nucleotide is formed by addition of phosphoric acid to nucleoside. Hence it can be said that a nucleotide is **nucleoside monophosphate**.

The phosphate group gets attached to C-5 of deoxyribose sugar. Because there are four types of nitrogenous bases, hence, there exist four types of nucleotides which are: Deoxyadenosine monophosphate (dAMP), Deoxyguanosine monophosphate (dGMP), Deoxycytidine monophosphate (dCMP) and Deoxythymidine monophosphate (dTMP)

1. Deoxyadenylic acid or (dAMP) = Adenine + Deoxyribose + Phosphoric acid
2. Deoxyguanylic acid or (dGMP) = Guanine + Deoxyribose + Phosphoric acid
3. Deoxycytidylic acid or (dCMP) = Cytosine + Deoxyribose + Phosphoric acid
4. Deoxythymidylic acid or (dTMP) = Thymine + Deoxyribose + Phosphoric acid

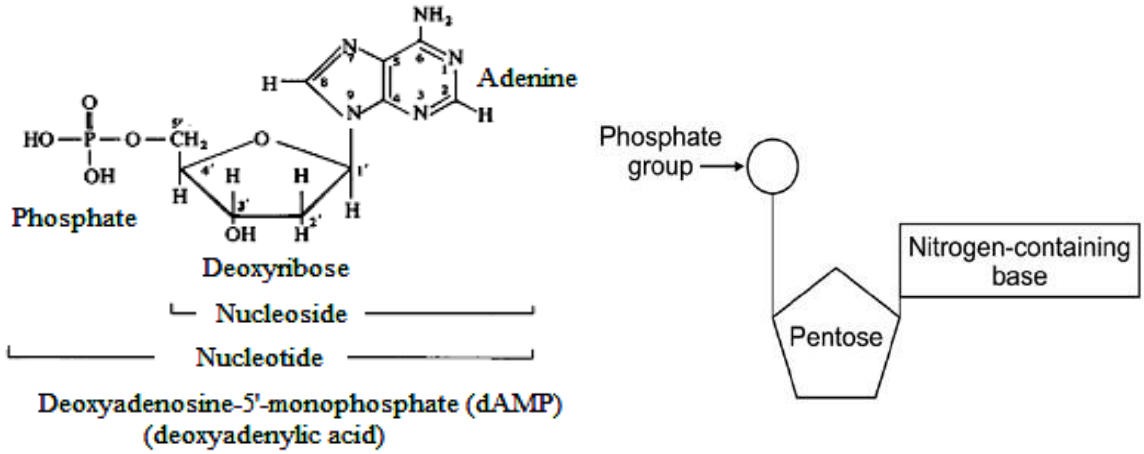

Fig. 5.5 - Structure of nucleotide

Chargaff's Rule

Erwin Chargaff (1950), an Austro-Hungarian biochemist, made observations on the bases and other components of DNA. These observations or generalizations are **called as Chargaff's base equivalence rule**.

1. Purine and pyrimidine base pairs are in equal amount, i.e., adenine + guanine = thymine + cytosine. [A + G] = [T + C], also, [A+G] / [T+C] = 1
2. Molar amount of adenine is always equal to the molar amount of thymine. Similarly, molar concentration of guanine is equaled by molar concentration of cytosine.
   [A] = [T], i.e., [A] / [T] = 1; and [G] = [C], i.e., [G] / [C] = 1
3. Sugar deoxyribose and phosphate occur in equimolar proportions.
4. A-T base pairs are rarely equal to C-G base pairs.
5. The ratio of [A+T] / [G+C] is variable but constant for a species (**Table-5.2)**. It can be used to identify the source of DNA. The ratio is low in primitive organisms and higher in advanced ones.

Table 5.2 Base Composition of DNA from Various Sources

Species	A	G	C	T	A+T/C+G
Man	30.4	19.0	19.9	30.1	1.55
Calf	29.0	21.2	21.2	28.5	1.35
Wheat germ	28.1	21.8	22.7	27.4	1.25
Pea	30.8	19.2	18.5	30.5	1.62
Euglena	22.6	27.7	25.8	24.4	0.88
Escherichia coli	24.7	26.0	25.7	23.6	0.93

5.3.2 Structure of DNA

Watson and crick model

In 1953, **James D. Watson** (American molecular biologist, geneticist and zoologist) and **Francis H.C. Crick** (British molecular biologist, biophysicist, and neuroscientist), studied physical and chemical properties of DNA and proposed a three dimensional helix model of physiological DNA (i.e. B-DNA), which is made up of two strands that are twisted around each other to form a right-handed helix. The model is commonly called as "Watson and crick model". The model is based upon X-ray Crystallographic analysis, obtained by two scientists, **Rosalind Franklin** and **Maurice Wilkins**.

The two DNA strands are anti-parallel, such that the 3′ end of one strand faces the 5′ end of the other (5.3.2). The 3′ end of each strand has a free hydroxyl group, while the 5′ end of each strand has a free phosphate group. The sugar and phosphate of the polymerized nucleotides form the backbone of the structure, whereas the nitrogenous bases are stacked inside. These nitrogenous bases on the interior of the molecule interact with each other, base pairing.

Analysis of the diffraction patterns of DNA has determined that there are approximately 10 bases per turn in DNA. The asymmetrical spacing of the sugar-phosphate backbones generates major grooves (where the backbone is far apart) and minor grooves (where the backbone is close together). These grooves are locations where proteins can bind to DNA. The binding of these proteins can alter the structure of DNA, regulate replication, or regulate transcription of DNA into RNA.

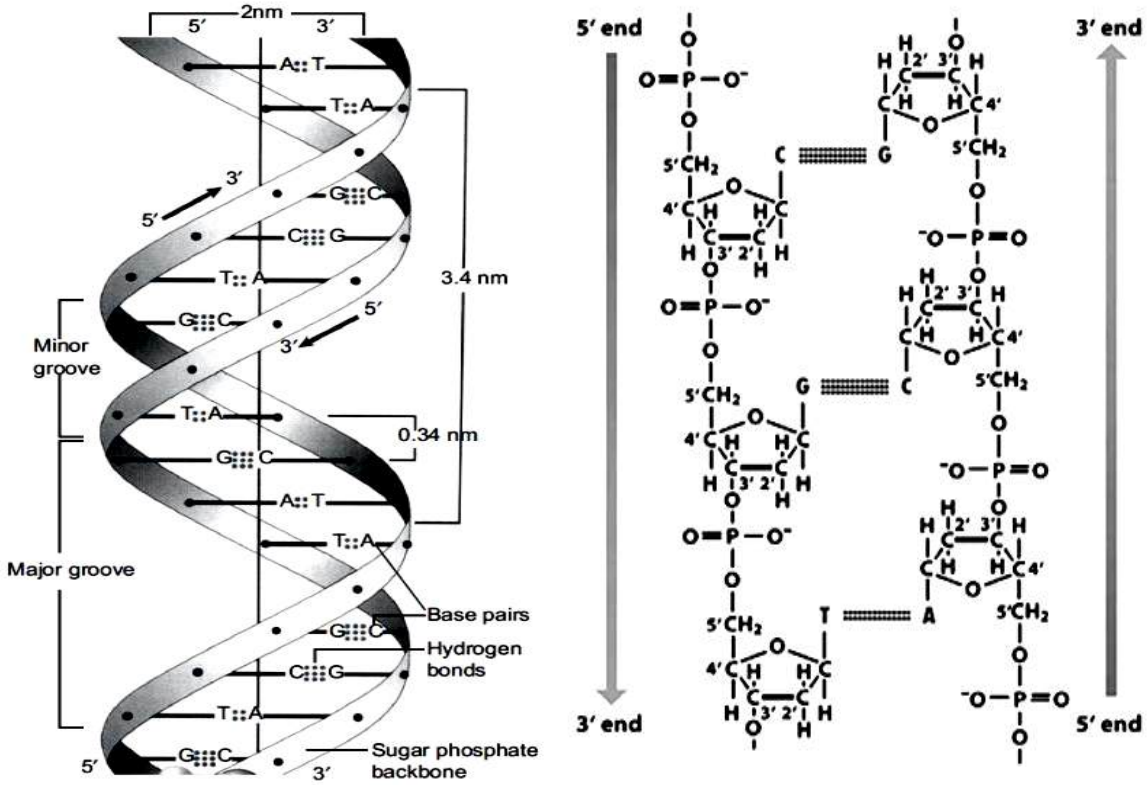

Fig. 5.6- Watson Crick's double helical structure of DNA molecule

Salient features of Watson and Crick model of DNA

1. The DNA molecule consists of two polynucleotide chains or strands that spirally twisted around each other and coiled around a common central axis to form a right-handed double-helix.
2. Each polynucleotide chain consists of several nucleotides. One nucleotide is linked to another by a phosphodiester bond.
3. The two polynucleotide chains in DNA molecules are antiparallel i.e. they ran in opposite directions so that the 3′ end of one chain facing the 5′ end of the other. The direction of one chain is 3'-5' and another is 5'-3'.
4. The two strands are held together by hydrogen bonds between the purine and pyrimidine bases of the opposite strands. Hydrogen bonds present between complement base pairs are main force which functions to hold the two strands in a helix together.

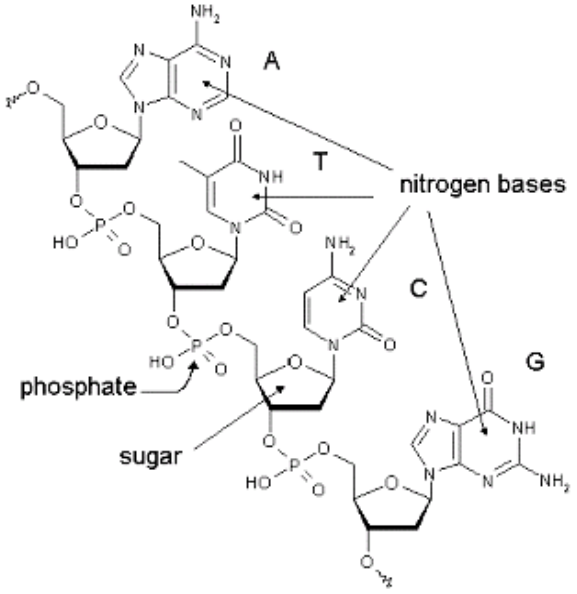

Fig. 5.7-Single polynucleotide chain of DNA helix

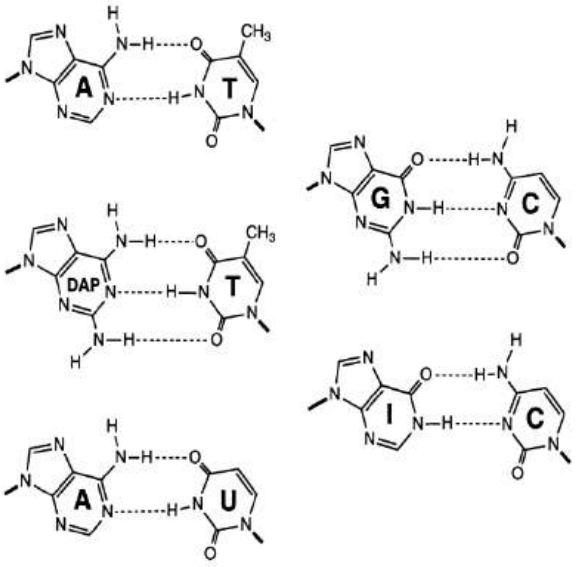

Fig. 5.8- Formation of hydrogen bonds between purines and pyrimidines

5. Every polynucleotide chain on outer side of helix has a backbone made up of sugar-phosphate, while the core of the helix contains the nitrogenous bases i.e., purine and pyrimidine.

6. Sugar molecules in two strands of a DNA helix are oriented in opposite direction. In one strand, all the sugar molecules are placed with their O atom facing upward and in other strand sugar molecules are upside down with their O atom facing downwards. Hence, the two strands are said to have opposite polarity and are antiparallel to each other.

7. Adenine (A) always pairs with thymine (T) by two hydrogen bonds and guanine (G) always pairs with cytosine (C) by three hydrogen bonds. This complimentarily is known as the base pairing rule. Thus, the two stands are complementary to one another (Fig 5.3.2-c). Also, by knowing the sequences of bases in one strand, the sequence of another strand can be easily determined.

8. The base compositions of DNA obey Chargaff s rules (E. Chargaff, 1950) according to which A=T and G=C. It also states that ratio of (A+T) and (G+C) is constant for a species.

9. The base sequence along a polynucleotide chain is variable and a specific sequence of bases carries the genetic information.

10. DNA double helix has two external grooves. One major groove, which is wide and deep and another minor groove which is narrow and shallow.

11. Each turn of Helix has about 10 base pairs and spacing between two adjacent base pairs is 0.34 nm or 3.4 Å in length. The length of a complete turn of helix is 3.4 nm or 34 Å i.e. there are 10 bp per turn.

12. The distance from phosphorous atom of backbone to central axis is 10 Å. Hence diameter of helix is 20 Å.

Different forms of DNA

There are six known different morphological forms of DNA double helix. These have been named as A, B, C, D, E and Z DNA. Out of these six forms only B-DNA and Z-DNA are found to occur as cellular DNA. While other forms, occur rigidly in controlled and experimental conditions. These six types of DNA differ from each other in following parameters:

- In the number of base pairs present per turn in them
- Angle between the base pairs
- Diameter of DNA molecule
- Hardness of coiling of double helix

(1) A-DNA

A-DNA is a rare type of structural conformation that a DNA can adopt under dehydrating conditions. A-DNA is a double stranded helical structure almost similar to B-DNA but with a shorter and more compact structural organization. A-DNA was discovered by **Rosalind Franklin** and the credit for the naming of A-DNA and B-DNA was also accounted to her. Important structural features of A-DNA are given below:

- A-DNA is formed from B-DNA under dehydrating condition.
- A-DNA is much wider and flatter than B-DNA.
- Similar to B-DNA, the A-DNA is also a right handed helix.
- A DNA is 20 to 25% shorter than B-DNA due to the smaller rise per turn. It contains 11.6 base pairs per turn. The distance between the adjacent base pairs is 2.9 Å.
- The helix diameter of A-DNA is 26 Å. The helix pitch (height of a turn) of A-DNA is 28.6 Å. The helical twist per base pair in A-DNA is 31^0 .
- A-DNA has an axial hole at the centre (hollow central core) and the base pairs are inclined to the helical axis.
- Individual base pairs in A-DNA are 20^0 tilted with respect to the helical axis.
- A-DNA has narrow and deep major groves. The minor groves of A-DNA are wide and shallow.
- The deoxyribose sugar pucker in A-DNA is C3-endo form.
- The conformation of glycosidic bond in A-DNA is in Anti-form.

(2) B-DNA

The B-DNA is the most common and predominate type of structural conformation of DNA in the cells. The DNA prefers to occur in B form under the natural physiological conditions (pH and salt concentration) in the cell. The B-DNA is better described as the Watson-Crick Model of DNA described for the first time by **James Watson** and **Francis Crick**. Important structural features of B-DNA are given below:

- Majority of the DNA in a cell is in B-DNA conformation.
- B-DNA is a right handed helix.

- In B-DNA, the bases occupy at the core whereas the sugar phosphate backbone occurs at the peripheral portion of the helix.
- In B-DNA only the edges of the base pairs are exposed to the solvent.
- Each base pair in B-DNA has the same width. The width of A–T and G–C in B-DNA is 10.85 Å.
- The helical diameter of B-DNA is 20 Å.
- Each turn on helix in B-DNA possess a helical height of 34 Å.
- Each turn in the B-DNA consists of 10 base pairs.
- The distance between adjacent base pairs in B-DNA is 3.4 Å. Each base pair will have a helical twist of 36^0 (360/10).
- The plain of inter-strand hydrogen bonds are perpendicular to the helical axis.
- B-DNA has a solid central core.
- The major grove of B-DNA is wide and deep. The minor grove of B-DNA is narrow and deep.
- The sugar pucker in B-DNA is C2'-endo form. The glycosidic bond conformation in B-DNA is in anti- form.

(3) Z-DNA

It is a left-handed double helical conformation of DNA in which the double helix winds to the left in a zig-zag pattern. The DNA strand with complementary nucleotides with alternating purines and pyrimidines can form Z-DNA conformation at high salt concentration. The existence of Z DNA was discovered by **Andres Wang** and **Alexander Rich**. It is one of the biologically active forms of DNA found in vivo in the cells. The exact biological function of Z-DNA is not clear. It is usually located upstream of the start site of a gene, and thus it may have some role in the regulation of gene expression. Important structural features of B-DNA are given below:

- The Z-DNA is a left-handed helical structure.
- The double helix winds in a zig-zag pattern.
- The helical diameter of Z-DNA is 18 Å. The total height of a helix turn is 44 Å.
- The nucleotide pairs in Z-DNA occur as nucleotide dimers.
- Each helical turn of Z-DNA contains 12 nucleotides (6 dimers).
- The distance between each nucleotide is 7.4 Å.
- The helical turn per base pair in Z-DNA is 9^0 for pyrimidine–purine step and 51^0 for purine–pyrimidine step.
- Z-DNA possesses a more or less flat major grove. The minor grove in Z-DNA is narrow and deep.
- Z-DNA has a solid core at the centre.
- The sugar pucker is C2'-endo for pyrimidine and C3'endo for purines.
- The glycosidic bond conformation is anti- for pyrimidines and syn- for purines.

(4) C-DNA

- This DNA is not found under normal conditions because it occurs only under condition of extreme dehydration.
- Number of bases per turn are 9.3.
- Diameter of helix is 19 Å.
- C-DNA is narrow and less compact than A-DNA.
- In both A-DNA and C-DNA the base pairs are tilled relative to the helix.

(5) D-DNA and E-DNA

- Both D-DNA and E-DNA are also Right-handed DNA.
- They are found where guanine is lack in a DNA structure (i.e. which have alternating A=T base pairs).
- D-DNA and E-DNA have 8 and 7 nucleotide per turn of helix respectively.
- They do not occur under normal / natural condition.
- D-DNA in nature has been found only in bacteriophage T_2.

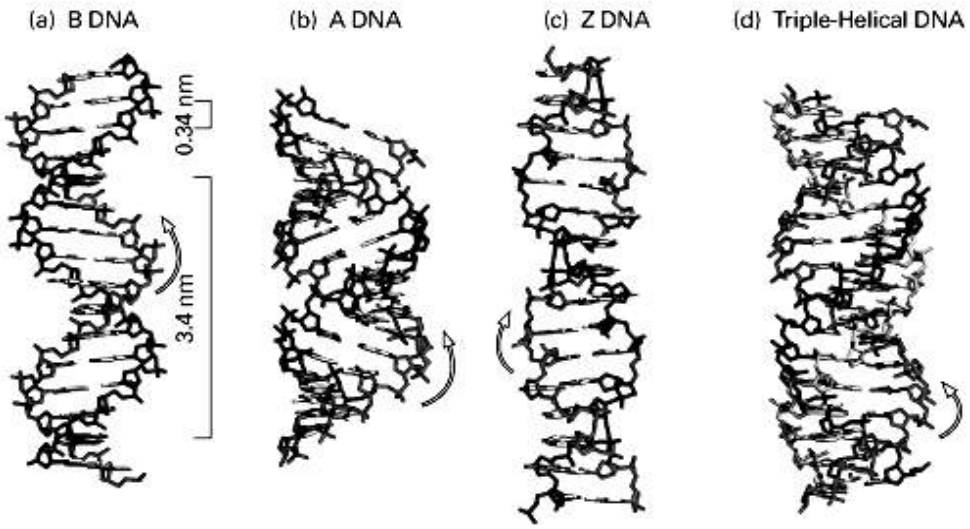

Fig. 5.9 - Different morphological forms of DNA

5.4 DNA AS GENETIC MATERIAL

Today, it is a well known fact that DNA is the genetic material. But initially, when the structure of DNA was studied several experiments were conducted by different scientists which proved that DNA is the genetic material. There are three major experiments conducted which established DNA to be the genetic material which can be transferred from one cell to another and also is transferred from one generation to next.

(I) Griffith's experiment which proved presence of transforming molecule in the cell

The experiments conducted by **Griffith** on *Streptococcus pneumoniae* (bacteria which causes pneumonia), proved to be a milestone in molecular biology was the first experiment suggesting that bacteria are capable of transferring genetic information through a process known as transformation. These experiments by Griffith were performed in 1928 and mice were utilized as experimental material.

Griffith used two strains of pneumococcus (*Streptococcus pneumoniae*) bacteria which infect mice – a **type III-S** (smooth) which was virulent, and a **type II-R** (rough) strain which was non-virulent. The III-S strain synthesized a polysaccharide capsule that protected itself from the host's immune system, resulting in the death of the host, while the II-R strain did not have that protective capsule and was defeated by the host's immune system.

Griffith performed his experiments into four parts (Fig 5.10).

1. When Type III (pathogenic) S-strain of bacteria was injected into mice, disease was caused and mice died.
2. When Type II (non-pathogenic) R-strain of bacteria was injected into mice, no disease was caused and all the mice were alive after the injection of pathogenic bacteria strain.
3. When heat killed Type III (pathogenic) strain of bacteria were injected into mice, no disease was caused and none of the mice died
4. The fourth set of experiment was most crucial in which mixed culture of heat killed Type III (pathogenic) strain of bacteria and Type II (non-pathogenic) R-strain of bacteria was injected into the mice. It was observed that all the mice died of pneumonia.

Now if we see, when heat killed type III and type II strain of bacteria were injected into mice, no disease was caused but when these were mixed and injected, disease was caused and mice died. Also, living pathogenic (S) bacteria were recovered from dead mice. Thus, some factor of dead (S) bacteria transformed the live (R) bacteria into (S) type, due to which R (non-pathogenic) strain was transformed into S (pathogenic) strain. This is called transformation principle.

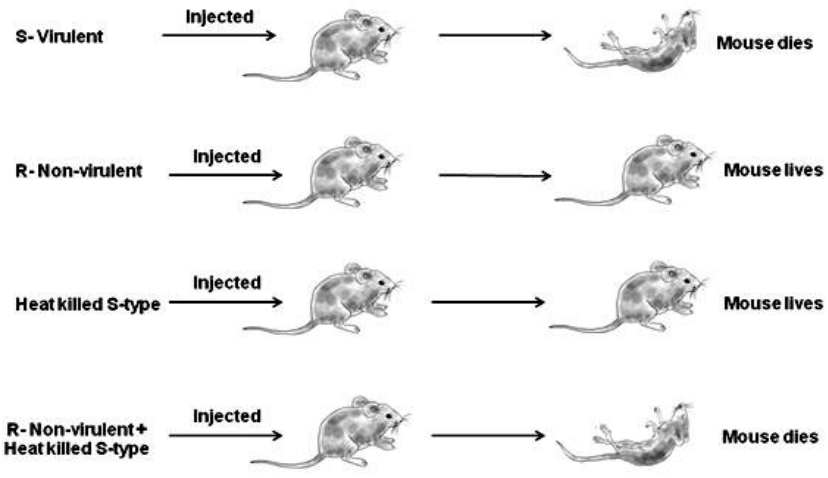

Fig. 5.10- Illustration of Griffith's experiment on transformation

(II) Avery–MacLeod–McCarty experiment to prove DNA is genetic material

Avery–MacLeod–McCarty repeated the experiment conducted by Griffith and confirmed the transforming molecule which transformed R strain into S strain was DNA. They performed their experiment in vitro conditions. To begin with their experiment, first of all extract of S (pathogenic strain of *S. Pneumonia*) cells was prepared. Now it is well known that cell contains three main types of biomolecules DNA, RNA and proteins. One of which could be the possible transforming molecule.

Now, they divided their experiment into sub experiments. In each of the experiment, one biomolecules (out of DNA, RNA and protein) was left intact and other two was destroyed. Each such extract of S strain was then mixed with R cells and incubated for sufficient time to allow transformation to occur. Now these extracts were treated with antibodies which caused aggregation of R cells. Now all the extracts were cultured onto Petri plates containing microbiological culture medium. Following results were obtained:

1. When extract of S cells was treated with RNase and DNase, the two enzymes destroyed DNA and RNA and proteins remained intact (undamaged). When this extract was mixed with R cells incubated and cultured no colonies were obtained. That is no transformation occurred which showed that protein was not the transforming molecule.
2. In another experimental setup, the extract of S cells was treated with protease and DNase which destroyed proteins and DNA and RNA remained intact. When this extract was mixed with R cells and incubated and cultured no colonies were obtained. That is no transformation occurred which showed that RNA was not the transforming molecule.
3. In another setup, extract of S cells was treated with protease and RNase which destroyed proteins and RNA and DNA remained as such. Now when this extract was incubated and cultured onto microbiological medium bacterial colonies of S type cells were obtained. This showed that R cells were transformed into S cells. It clearly proved that DNA was the transforming (genetic) material.

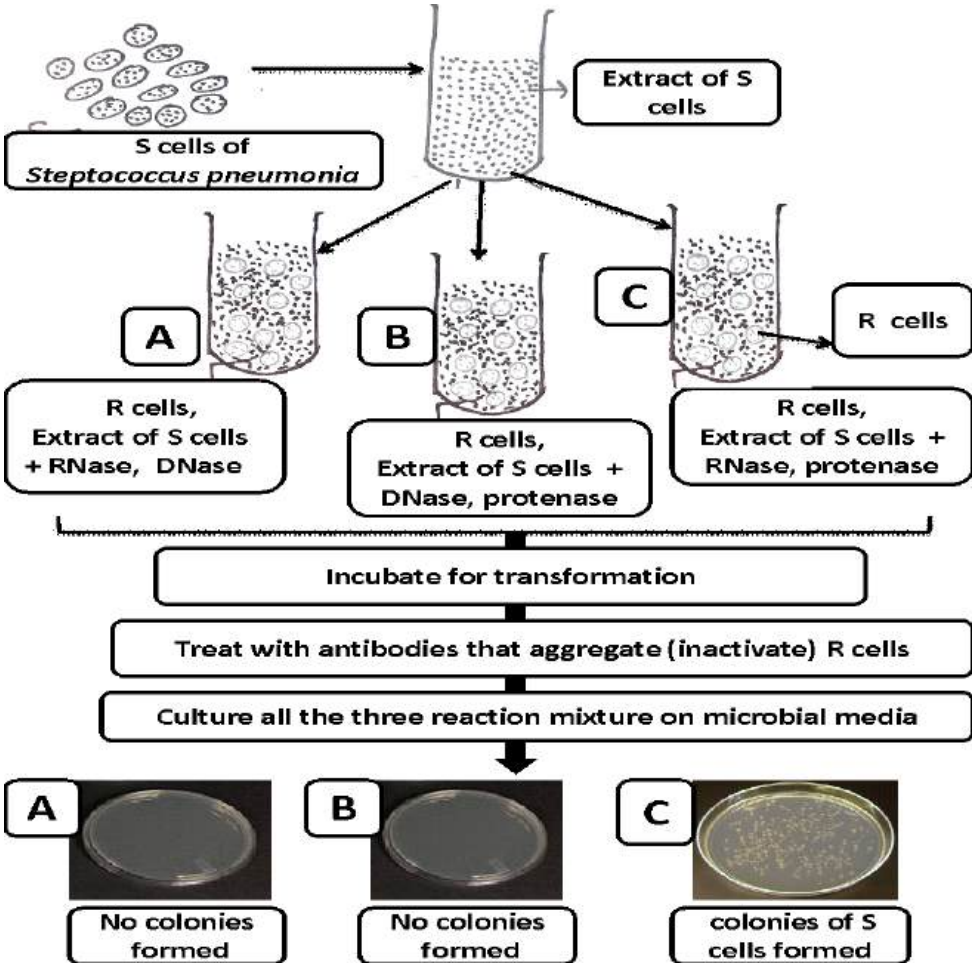

Fig. 5.11 - Avery–MacLeod–McCarty experiment to prove DNA is genetic material

(III) Hershey and Chase experiment to prove DNA is genetic material

In their experiment **Alfred D. Hershey** and **Martha Chase** (1953), utilized T_2 bacteriophage and *E. coli* bacteria to prove that DNA is genetic material. T_2 virus contains a DNA core and a protective coat made up of a number of protein molecules. They grew culture of T_2 viruses in radioactive sulphur ^{35}**S** medium. As a result of this, radioactive sulphur was incorporated by bacteriophages into their outer protein coat (because S is present in protein coat). Similarly, in another culture of bacteriophage T_2 viruses was cultured in medium containing radioactive phosphorus ^{32}**P**. Now in this case, radioactive P got incorporated into DNA of these viruses because P is present in DNA. These isotopes were specifically used as DNA contains phosphorous and no sulphur while proteins contain sulphur in their outer coat and no phosphorus. Both the types of labelled viruses were allowed to infect two unlabelled separate cultures of *E. coli* bacteria. After about 30 minutes, the bacteria were subjected to centrifugation and two fractions were separated. The heavier infected bacterial cells settled at the bottom, while the phage particles remained floating as supernatant.

Now in the experiment in which bacterial cells were infected with bacteriophage whose DNA was labelled with radioactive P, both the infected bacteria*l* cells as well as newly synthesized phage particles were found to contain radioactive labeled P.

In another experimental setup where bacterial cells were infected with bacteriophage whose protein coat was labelled with radioactive S, both the infected bacterial cells as well as newly synthesized phage particles were found to contain insignificant or negligible amount of radioactive labeled S because all the protein were left outside the bacterial (*E.coli*) cells. This experiment further proved that genetic material is DNA and is transferred from one generation to another. Fig. 5.4 (c) depicts illustration of Hershey and Chase experiment.

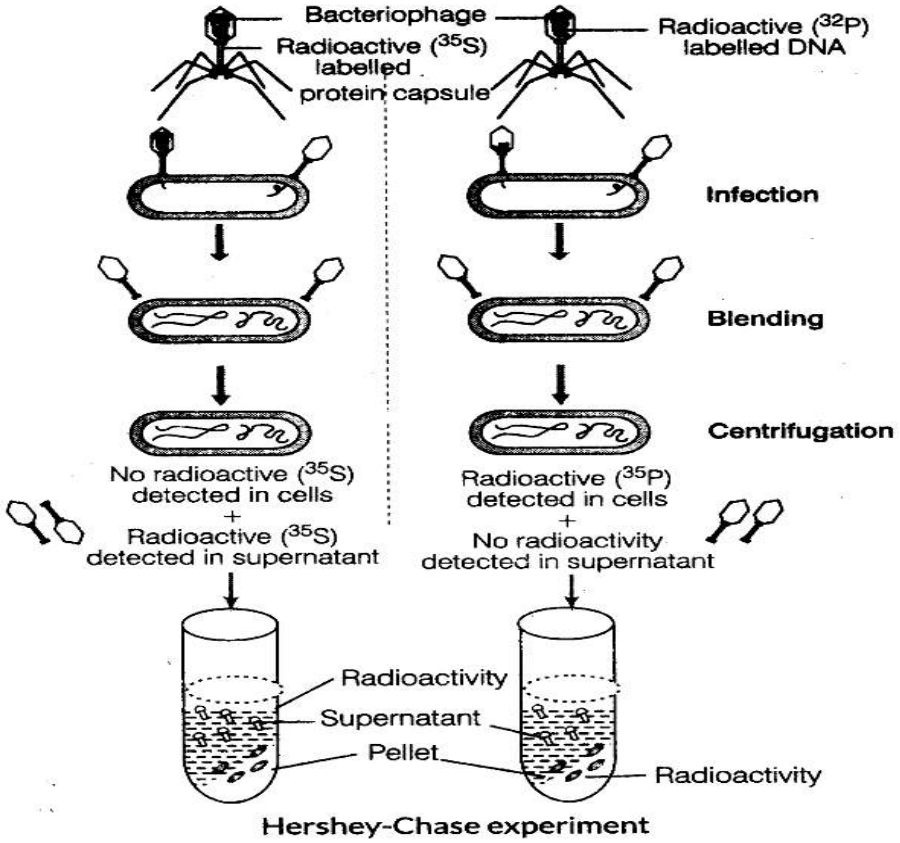

Fig. 5.12- Hershey and Chase experiment to prove DNA as genetic material

5.5 DNA REPLICATION

When a cell divides to form two daughter cells, the cellular content gets divided almost equally into two daughter cells. As, every cell has only one nucleus, so how is it possible that one nucleus be given to two daughter cells. Hence, in cell cycle, before a cell divides into two, it replicates its DNA in S phase of cell cycle which gets separated into two nuclei in a process

called karyokinesis (which has four phases known a prophase, metaphase, anaphase and telophase) and then the cell divides into two by a process of cytokinesis.

DNA replication is a process in which original DNA molecule (present in nucleus of eukaryotes and cytoplasm of prokaryotes) called as parent DNA is replicated to form two new DNA molecules called as daughter DNA molecules. Parental strands act as template (guide) strand for the synthesis of daughter strands.

Three different hypotheses had been proposed for the mode of DNA replication (Fig. 5.5 -a).

1. Conservative Mode

In this model, the two strands of DNA unwind from each other, and each acts as a template for synthesis of a new, complementary strand. This results in two DNA molecules with one original strand and one new strand. An entirely new molecule is synthesized from a DNA template and no change occurs in template (parent) DNA i.e. it remains conserved.

2. Semi-Conservative Model

In this model, DNA replication results in one molecule that consists of both original DNA strands (identical to the original DNA molecule) and another molecule that consists of two new strands (with exactly the same sequences as the original molecule). It means after replication each DNA molecule has one parental strand and one newly synthesized strand.

3. Dispersive Model

In the dispersive model, DNA replication results in two DNA molecules that are mixtures, or "hybrids," of parental and daughter DNA. In this model, each individual strand is a patchwork of original and new DNA. New molecules are made up of segments of new and old DNA.

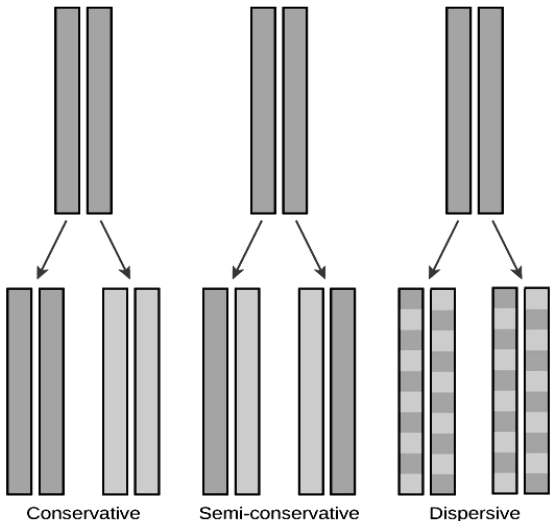

Fig. 5.12 - Different modes of DNA replication

Meselson and Stahl Experiment: DNA Replication is Semi-Conservative

Watson and Crick model suggested that DNA replication is semi-conservative. It implies that half of the DNA is conserved. Only one new strand is synthesized, the other strand is the original DNA strand (template) that is retained. Each parental DNA strand serves as a template for one new complementary strand. Each of these strands of the double helix contains one original parental strand and one newly formed strand.

Semi-conservative replication of DNA was proved by the work of **Mathew Meselson** and **Franklin Stahl** (1958). In their experiment, *Escherichia coli* was grown for several generations on a medium containing heavy isotope of nitrogen ^{15}N, till the bacterial DNA became completely labelled with heavy isotope. The labelled bacteria were then cultured onto fresh medium with normal nitrogen ^{14}N. DNA was isolated from each generation of bacteria and tested for the presence of heavy isotope of nitrogen through density gradient centrifugation using cesium chloride (CsCl). When centrifuged at high speed the salt forms a density gradient with heaviest region at the bottom and less concentrated lighter one towards the surface.

Meselson and Stahl found that DNA of the **first generation** was hybrid or intermediate (^{15}N and ^{14}N). It settled in CsCl solution at a level higher than the fully labelled DNA of parent bacteria ($^{15}N^{15}N$). Because *N*-15 has one extra neutron, it's slightly heavier than *N*-14 and therefore makes the DNA molecule denser. The **second generation** of bacteria after 40 minutes contained two types of DNA, 50% light ($^{14}N^{l4}N$) and 50% intermediate ($^{15}N^{l4}N$). The **third generation** of bacteria after 60 minutes contained two types of DNA, 25% intermediate ($^{15}N^{14}N$) and 75% light ($^{14}N^{14}N$) in 1 : 3 ratio.

This observation is possible only if the two strands of DNA duplex separate at the time of replication and act as a template for the synthesis of new complementary strands of DNA having normal or ^{14}N. This will produce two DNA duplexes with one old strand (^{15}N) and one new strand (^{14}N). In each of the daughter DNA molecule one strand is parental and another strand is newly synthesized DNA strand. Hence the process of DNA replication is semi-conservative.

The complete process of DNA replication can be divided into three steps:

1. Initiation

The first step occurs when DNA helicase unwinds the double helix by breaking the hydrogen bonds between the parent strands of DNA at locations called replication origins. This opens up the DNA molecule to form a Y-shape structure, which is called as **replication fork**. Single-stranded binding proteins (SSB) work with helicase to keep the parental DNA helix unwound. Before the synthesis of daughter DNA strands, an RNA primer is made by the enzyme called RNA Primase. RNA Primase is the enzyme that builds an RNA primer on the parent strand to initiate DNA replication. Once the RNA primer is built, then the next enzyme, DNA polymerase, begins synthesis of DNA strands. During the process of replication DNA polymerase is positioned behind the RNA primer. The "Topoisomerase" proteins surround the unzipping strands and relax the twisting that might damage the unwinding DNA.

2. Elongation

After separation of helix separated and primer synthesis DNA polymerase starts adding complementary nucleotides and synthesis of daughter strand begin. Addition of complementary nucleotides means that if A is present on parental strand then nucleotide with base T will be added on daughter strand, if G is present on daughter strand then nucleotide with base C will be added on daughter strand and vice versa. Now at this stage where two parent strands of DNA are unwound or separated, one strand is oriented in the 5' to 3' direction and the other strand has opposite orientation in the 3' to 5' direction.

Single DNA polymerase will catalyze replication of both the strands. DNA polymerase functions only in 5' to 3' direction. This feature makes synthesis of daughter strands through different methods, one adding nucleotides one by one in the direction of the replication fork, the other able to add nucleotides only in chunks. The first strand, which replicates nucleotides one by one, is called the **leading strand**; the other strand, which replicates in chunks, is called the **lagging strand**.

(a) The Leading Strand: Because DNA polymerase moves along the parent strand in the 5' to 3' direction, replication can occur very easily on the leading strand. Addition of nucleotides occurs in the 5' to 3' direction. Triggered by RNA Primase, which adds the initial nucleotides (in form of primer) to the new chain, the DNA polymerase moves along the fork and keeps on adding complementary nucleotides one after the other according to the sequence of nucleotides present on parental strand. Synthesis of leading strand is a continuous process.

(b) The Lagging Strand: Whereas the DNA polymerase III on the leading strand can simply follow the replication fork, because DNA polymerase III must move in the 5' to 3' direction, on the lagging strand the enzyme must move away from the fork. The lagging strand replicates in small segments, called Okazaki fragments. These fragments are stretches of 100 to 200 nucleotides in humans (1000 to 2000 in bacteria) that are synthesized in the 5' to 3' direction away from the replication fork.

For synthesis of each Okazaki fragment a new primer is made before the segment is replicated. After replication of over these primers are degraded. Now in the vacant spaces DNA is synthesized by DNA polymerase I. These fragments are then stitched together by DNA ligase, creating a continuous strand. The synthesis of lagging strand is called discontinuous

3. Termination

After elongation is complete, two new double helices have been made from the original parental DNA molecule. The process of DNA replication is summarized in Fig.5.13.

Replication Enzymes

DNA replication would not occur without enzymes that catalyze various steps in the process. Enzymes that participate in the eukaryotic DNA replication process include:

1. DNA helicase: Unwinds and separates double stranded DNA as it moves along the DNA. It forms the replication fork by breaking hydrogen bonds between nucleotide pairs in DNA.

2. DNA primase: A type of RNA polymerase that generates RNA primers. Primers are short RNA molecules that act as templates for the starting point of DNA replication.

3. DNA polymerases: Synthesize new DNA molecules by adding nucleotides to leading and lagging DNA strands.

4. Topoisomerase or DNA Gyrase: unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.

5. **Exonucleases:** group of enzymes that remove nucleotide bases from the end of a DNA chain.

6. **DNA ligase**: joins DNA fragments together by forming phosphodiester bonds between nucleotides.

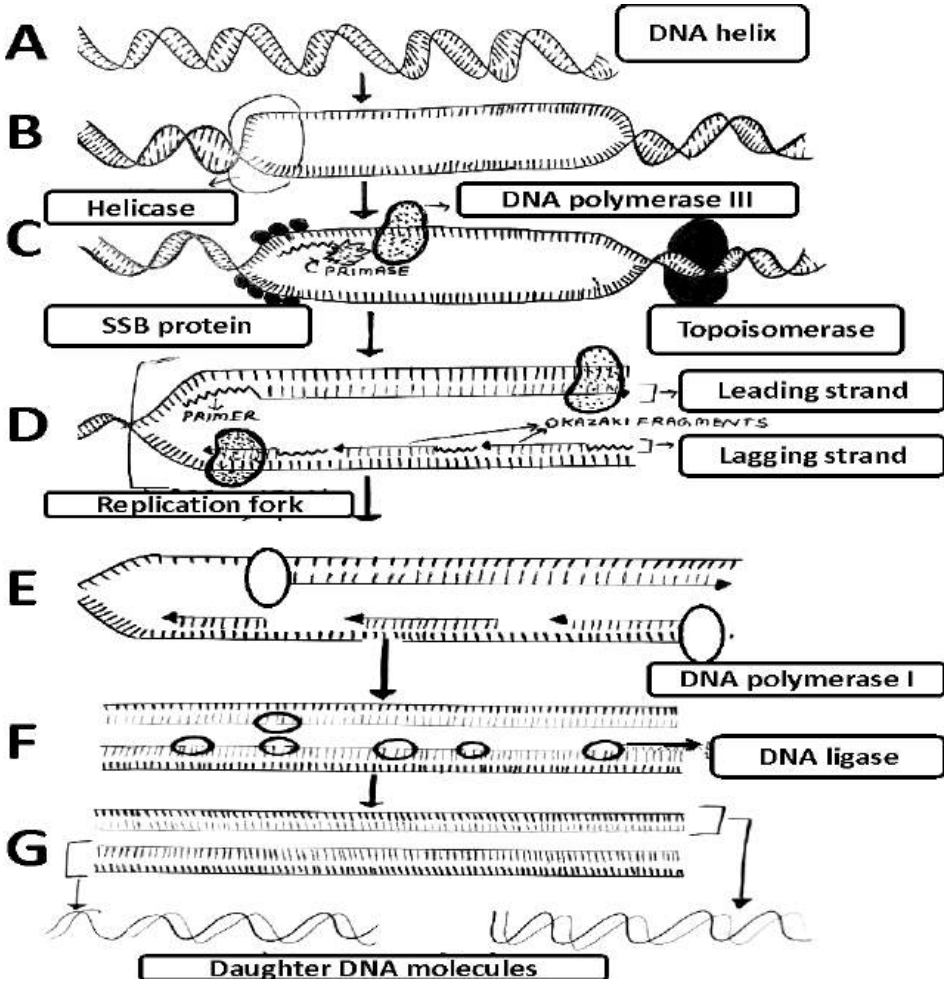

Fig. 5.13- Summary of DNA replication: (A) Parent DNA helix, **(B)** Helicase unwind DNA, (**C)** SSB binds to separated strands**,** Primase synthesizes primer, (**D)** DNA Polymerase-III starts synthesis of leading and lagging strand, (**E)** Complete primers are removed after replication (**F)** DNA Polymerase-I synthesize DNA in the gaps left after removal of primer and Ligase joins different DNA segments. (**G)** Two daughter DNA molecules are produced.

5.6 DNA PROTEIN INTERACTION

DNA-protein interactions occur when protein binds to DNA molecules. Such interactions generally occur to regulate the biological function or activity of DNA. It means that some protein may bind to DNA and initiate expression of a gene and there are proteins which bind to DNA and inhibit the expression of genes. Regulation of gene expression is most common role of DNA protein interaction.

DNA Protein interactions play very vital roles in any living cell. It controls various cellular processes which are very essential for living beings, viz. replication, transcription, recombination, DNA repair etc. There are several types of proteins found in a cell. But only those proteins interact with DNA, which have the DNA binding domains. Each DNA binding domain has at least one motif, which is a conserved amino acid sequence of this protein, which can potentially recognize a double stranded or a single stranded DNA. These DNA binding domains possess an affinity to bind to either double stranded or single stranded DNA.

There are mainly two broad types of DNA protein interactions:
1) Sequence specific DNA binding and
2) Sequence non-specific DNA binding.

In case of sequence specific DNA protein interactions, a DNA binding protein binds to a DNA on a site having a specific nucleotide sequence, as for example, the sequence specific DNA protein interaction is found to occur in case of transcription. But in case of sequence non-specific DNA protein interactions, the DNA binding protein can bind to a DNA in a random position on the DNA.

In non-specific interaction, binding of protein to DNA does not depend upon the sequence of nucleotides. Non-specific DNA protein interaction occurs in replication, between functional group of protein and sugar phosphate backbone of DNA and it is considered to be electrostatic. During replication the DNA double strand is melted by Helicase enzyme, and a replication fork is made. A special kind of protein called single strand binding protein or **SSB binds** to the melted single strand of DNA and stabilizes the system by preventing them to be re-natured. The best example of non-specific DNA-protein interaction is the association of histone proteins to DNA during packaging of DNA molecules to form nucleosomes.

Specific DNA protein interaction is much stronger as compared to non-specific. Specific DNA protein interaction is mediated by:

(a) Hydrogen bonding: In case of hydrogen bonding there can be direct hydrogen bonding between DNA and protein or it may occur through water molecules.

(b) Ionic Interaction: Ionic interaction in the form of salt bridge formation or interaction between side chain of protein and DNA backbone, occur during specific DNA protein interaction.

Beside above two, ***van der Waal forces*** and **hydrophobic interaction** are also involved in specific DNA protein interaction. Some of the examples of proteins which show specific DNA-protein interaction are Zinc Finger, Leucine Zipper. Initiation of the process of transcription (formation of mRNA from DNA) is one of the most suitable examples to understand specific DNA protein interaction. To initiate the process of transcription enzyme, RNA polymerase binds to a specific base sequence known as **promoter**. Here, when protein binds to specific DNA sequence, only then, the process of transcription initiate.

There are proteins which specifically bind to operator region of DNA and then enhance or inhibit the process of transcription. Now we have well understood that DNA protein interaction occurs when proteins bind to DNA molecule. We also know from structure of DNA, that there are two types of grooves present in structure of DNA called as major and minor grooves. Generally proteins bind to major grooves; however, there are few exceptions. The DNA protein complexes formed, which serves three types of functions i.e., structural, regulatory and enzymatic. When DNA and proteins interact to form a specific type of structure, the interaction is called as **structural DNA protein interaction**. Binding of histones and DNA during packaging of DNA into chromosomes, is an example of structural DNA protein interaction during which a structure called as nucleosome is formed which initiates process of DNA packaging.

When DNA protein complex serve regulatory function, such DNA protein interaction is called as **regulatory**. Many proteins called as **transcription factors** associate with DNA during the process of transcription to regulate it. The **transcription factors** are a special kind of DNA binding proteins. They can only recognize a specific DNA sequence. The third type of DNA protein interaction is one in which protein attached to DNA act as enzymes. For example DNA polymerase, RNA polymerase when binds to DNA, they bring out the process of DNA replication and DNA transcription respectively.

5.7 SUMMARY

Deoxyribonucleic acid, or DNA, is a biological macromolecule that carries hereditary information in many organisms. DNA is necessary for the production of proteins, the regulation, metabolism, and reproduction of the cell. Large compressed DNA molecules with associated proteins, called **chromatin**, are mostly present inside the nucleus. Some cytoplasmic organelles like the mitochondria also contain DNA molecules. DNA is usually a double-stranded polymer of nucleotides, although single-stranded DNA is also known. Nucleotides in DNA are molecules made of deoxyribose sugar, a phosphate and a nitrogenous base. The nitrogenous bases in DNA are of four types, **adenine, guanine, thymine and cytosine**. The phosphate and the deoxyribose sugars form a backbone-like structure, with the nitrogenous bases extending out like rungs of a ladder. Each sugar molecule is linked through its 3^{rd} and 5^{th} carbon atoms to one phosphate molecule each.

DNA is composed of two **polynucleotide strands**, the polymers of nucleotides. The Nitrogenous Bases in DNA store the instructions for making polypeptide chains, essentially coding for every feature of the entire organism. The two polynucleotide strands run 'antiparallel' to each other, with Nitrogenous Bases projecting inwards. The term 'antiparallel' means that the strands run in opposite directions, parallel to one another. The antiparallel strands twist in a complete DNA structure, forming a Double Helix. There are about 10 base pairs present per turn of DNA helix. Distance between two successive base pairs is 3·4 Å and one turn of helix measures 34 Å. The Two DNA strands in a helix are anti-parallel to each other. One strand has orientation 5'-3' and other 3'-5'.

The strands are held together by hydrogen bonds between the **nitrogenous bases** that are opposite each other. Bases bonded together are termed 'paired', and are very specific as to which base they will join to. A Purine will only pair with a Pyrimidine. Not only that, but the Adenine Purine will only pair with the Thymine Pyrimidine (A-T), and the Guanine Purine will only pair with the Cytosine Pyrimidine (G-C). These base pairings are termed **Complementary Base Pairings**. The reason that Purines will only bond with Pyrimidines is that Purines are larger molecules (composed of a double ring structure), so in order to ensure that the polynucleotide strands are equally spaced apart, the larger bases must pair with the smaller bases. The root for the specific Complementary Base Pairings is the number of hydrogen bonding sites available. Adenine and Thymine have two sites each, whereas Guanine and Cytosine have three sites each. There are different forms of DNA which are A-DNA, B-DNA, Z-DNA, etc. All these forms of DNA differ from each other in number of base pair present per turn of helix, diameter of helix etc. All the forms of DNA are right handed except Z-DNA which is left handed. The helical structure of DNA described by Watson and crick is of B-DNA.

A DNA strand can act as a template for synthesis of a new nucleic acid strand in which each base forms a hydrogen-bonded pair with one on the template strand (G with C, A with T, or A with U for RNA molecules). The new sequence is thus complementary to the template strand. The copying of DNA molecules to produce more DNA is known as **DNA Replication**. DNA replication takes place at a Y-shaped structure called a **replication fork**. A self-correcting DNA polymerase enzyme catalyzes nucleotide polymerization in a 5′-to-3′ direction, copying a DNA template strand with remarkable fidelity. Since the two strands of a DNA double helix are antiparallel, this 5′-to-3′ DNA synthesis can take place continuously on only one of the strands at a replication fork (the leading strand). On the lagging strand, short DNA fragments must be made by a "backstitching" process. Because the self-correcting DNA polymerase cannot start a new chain, these lagging-strand DNA fragments are primed by short RNA primer molecules that are subsequently erased and replaced with DNA. During replication one strand is synthesized continuously and is known as leading strand and another strand is synthesized discontinuously in small fragments. This strand is known as lagging strand and its fragments are called as **Okazaki fragments**. Synthesis of only one primer is needed for leading strand and separate primer is synthesized for each Okazaki fragments. After the replication is completed these primers are removed by enzyme nuclease. DNA polymerase I added nucleotides from where primers have

been removed. DNA ligase enzyme joins the DNA fragments to from a continuous DNA strands. Each daughter DNA molecule produced after the process of DNA molecule has one parental strand and one newly synthesized DNA strand. Hence, DNA replication is semi-conservation.

Griffith's in his experiments on *Diplococcus pneumonia*e and mice proved presence of transforming molecule in DNA. Avery–MacLeod–McCarty proved that transforming molecule in Griffith's experiment was DNA. Hershey and Chase onto their experiment on bacteriophage and *E.coli* also proved DNA to be genetic material. DNA replication is a process in which parental DNA molecule is replicated DNA molecule is replicated to form two daughter DNA molecules. Several enzymes are involved in the process of DNA replication. Enzyme Helicase unwinds the DNA helix and SSB proteins helps in keeping the separated strands in an unwind state (i.e., prevents them from rejoining) so that replication can occur. Enzyme Topoisomerase functions to remove supercoils during the process of DNA replication. Primase enzyme makes a RNA primer and when primer is made DNA polymerase-III starts adding nucleotide in the daughter strand. Nucleotides are added in the daughter strands have complementary bases to the bases present in parental DNA strand.

Several proteins are found to interact with DNA molecules; such interactions are called DNA protein interactions. These interactions can be specific or non specific. In specific DNA protein interaction protein binds to a particular (specific) segment of DNA molecule i.e., such proteins identifies a specific sequences of bases present on DNA strand. Binding of RNA polymerase to promoter, regulatory, protein to operator is examples of specific DNA-protein interaction. In non specific DNA protein interaction, binding of protein to DNA does not occurs to a specific DNA sequence of nitrogenous base. For example binding of histone protein to DNA does not require any specific sequence of nitrogenous bases.

5.8 GLOSSARY

A-DNA: One of three biologically active double helical structures along with B-DNA and Z-DNA. It is a right-handed double helix fairly similar to the more common B-DNA form, but with a shorter, more compact helical structure whose base pairs are not perpendicular to the helix-axis as in B-DNA.

Bacteriophage: The viruses that infect bacteria are called bacteriophage.

B-DNA: The typical form of double helix DNA in which the chains twist up and to the right around the front of the axis of the helix.

Biomolecules: A molecule that is involved in the maintenance and metabolic processes of living organisms.

Crystallographic: The experimental science of determining the arrangement of atoms in crystalline solids

Cytokinesis: The division of the cell cytoplasm that usually follows mitotic or meiotic division of the nucleus.

Deoxyribose: Any of certain carbohydrates derived from ribose by the replacement of a hydroxyl group with a hydrogen atom.

Exon: A section of a gene that contains the instructions for making a protein.

Genetic code: The biochemical instructions, that translate the genetic information present as a linear sequence of nucleotide triplets in messenger RNA into the correct linear sequence of amino acids for the synthesis of a particular peptide chain or protein.

Genomic: Relating to the haploid set of chromosomes in a gamete or microorganism, or the complete set of genes in a cell or organism.

Glycosidic bond: A type of covalent bond that joins a carbohydrate (sugar) molecule to another group, which may or may not be another carbohydrate.

Helicase: Any of the enzymes that use the energy derived from the hydrolysis of nucleoside triphosphates to unwind the double-stranded helical structure of nucleic acids.

Histone: Any of a group of five small basic proteins, occurring in the nucleus of eukaryotic cells, that organize DNA strands into nucleosomes by forming molecular complexes around which the DNA winds.

Intron: A section of a gene that does not contain any instructions for making a protein. Introns separate exons (the coding sections of a gene) from each other.

Karyokinesis: The series of active changes that take place in the nucleus of a living cell in the process of division.

Mitochondrial DNA: Mitochondria have their own independent genome, separate to a cell's nuclear DNA. The genome is usually circular, replicates independently and is comprised mainly of genes involved with production of adenosine triphosphate (ATP), the main source of cellular energy.

Nitrogenous bases: The purines (adenine and guanine) and pyrimidines (thymine, cytosine, and uracil) that comprise DNA and RNA molecules.

Nucleobase: Also known as nitrogenous bases or often simply bases are nitrogen-containing biological compounds that form nucleosides, which in turn are components of nucleotides, with all of these monomers constituting the basic building blocks of nucleic acids.

Nucleoid: The central region in a prokaryotic cell, as a bacterium, that contains the chromosomes and that has no surrounding membrane.

Nucleotide: Any of a group of molecules that, when linked together, form the building blocks of DNA or RNA, composed of a phosphate group, the bases adenine, cytosine, guanine, and thymine, and a pentose sugar, in RNA the thymine base being replaced by uracil.

Okazaki fragment: They are short, newly synthesized DNA fragments that are formed on the lagging template strand during DNA replication. They are complementary to the lagging template strand, together forming short double-stranded DNA sections.

Phosphodiester bond: The covalent chemical bond that holds together the polynucleotide chains of RNA and DNA by joining a specific carbon in the phosphate group in a sugar having five carbons, such as ribose, to a specific carbon in the hydroxyl group of the five-carbon sugar in the adjacent nucleotide.

Polymerase: An enzyme that catalyzes the addition of multiple subunits to a substrate molecule
Polynucleotide: A DNA polymer composed of multiple nucleotides
Purine: One of several purine derivatives, especially the bases adenine and guanine, which are fundamental constituents of nucleic acids. They are heterocyclic aromatic organic compound that consists of a pyrimidine ring fused to an imidazole ring.
Pyrimidine: One of several pyrimidine derivatives, especially the bases cytosine, thymine, and uracil, which are fundamental constituents of nucleic acids.
Replication fork: A structure that forms within the nucleus during DNA replication. It is created by helicases, which break the hydrogen bonds holding the two DNA strands together. It is the area where the replication of DNA will actually take place.
Semi-conservative replication: During DNA duplication, each strand of a parent DNA molecule is a template for the synthesis of its new complementary strand. Thus, one half of a preexisting DNA molecule is conserved during each round of replication.
Template: An RNA or single-stranded DNA molecule upon which a complementary nucleotide strand is synthesized.
Topoisomerase: An enzyme which alters the supercoiled form of a DNA molecule.
Transcription: The process of transcribing or making a copy of genetic information stored in a DNA strand into a complementary strand of RNA (messenger RNA or mRNA) with the aid of RNA polymerases.
X-ray crystallography: A technique used for determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of incident X-rays to diffract into many specific directions.
Z-DNA: A form of DNA that has a different structure from the more common B-DNA form. It is a left-handed double helix wherein the sugar-phosphate backbone has a zigzag pattern due to the alternate stacking of bases in anti-conformation and syn conformation.

5.9 SELF ASSESSMENT QUESTION

5.9.1 Choose the most appropriate option:

1. Circular DNA is found in?
(a) Plasmid (b) Mitochondria
(c) Chloroplast (d) All of the above

2. Primers is a
(a) Small RNA segment (b) Small DNA segment
(c) Small segment of nucleosides (d) All of the above

3. DNA replication in eukaryotes commences
(a) From both ends of a chromosome simultaneously
(b) Several sites along DNA of a chromosomes simultaneously

(c) From centromere to either end
(d) From one end of chromosome to another

4. DNA replication is
(a) Conservative and discontinuous (b) Semi-conservative and discontinuous
(c) Semi-conservative and semi-discontinuous (d) Conservative

5. Which of the following is correct pair of pyrimidines?
(a) Adenine and Guanine (b) Guanine and cytosine
(c) Adenine and Thymine (d) Thymine and cytosine

6. Which of the following is correct according to Chargaff's rule?
(a) A+T=G+C (b) A=C
(c) G=T (d) A+T/ C+G = 1

7. In DNA replication leading strand is synthesized in
(a) 5'-3' direction continuously (b) 5'-3' direction discontinuously
(c) 3'-5' direction continuously (d) 3'-5' direction discontinuously

8. Similarity between DNA and RNA is that both have
(a) Similar sugar (b) Similar mode of replication
(c) Similar pyrimidines (d) Polymers of nucleotides

9. Enzyme that catalyze union of DNA fragments is
(a) Polymerase (b) Ligase
(c) Helicase (d) Lipase

10. Hershey and Chase, by their experiment onto bacteriophage and *E. coli* proved
(a) DNA is genetic material (b) RNA is genetic material of viruses
(c) Bacteriophage attacks bacteria like *E.coli* (d) Protein is the genetic material

11. Read (P) to (S) and find the correct option
(P) Nitrogen base is linked to pentose sugar through N-glycosidic linkage
(Q) Phosphate group is linked to 5' - OH of a nucleoside through phosphodiester linkage
(R) Purine of one DNA strand are linked to purine of another strands by Hydrogen bond
(S) The two strands of DNA are anti-parallel
(a) P, Q, and R is wrong (b) S alone is wrong
(c) R and S are wrong (d) R alone is wrong

12. Double helix model of Watson and Crick is
(a) C-DNA (b) Z-DNA

(c) B-DNA (d) D-DNA

13. Condensation product of adenine, deoxyribose and phosphoric acid is
(a) Adenylic acid (b) Adenine phosphate
(c) Adenosine (d) None of the above

14. Purine possess nitrogen at
(a) 1, 2, 4 and 6 positions (b) 1, 3, 5 and 7 positions
(c) 1, 3, 7 and 9 positions (d) 1, 2, 6 and 8 positions

15. Circular DNA is found in
(a) Viruses (b) Bacteria, mitochondria and chloroplast
(c) Chloroplast and mitochondria alone (d) All of the above

16. Nucleotide arrangement in DNA can be seen by
(a) X-ray crystallography (b) Electron microscopy
(c) Ultracentrifuge (d) Light microscope

17. Okazaki fragments give rise to
(a) Leading strand (b) lagging strand
(c) m-RNA (d) both a and b

18. Type of coiling in Z-DNA is
(a) Right handed (b) Spiral
(c) Opposite (d) Left handed

19. The area where unwinding and separation of DNA strands begins during replication is called?
(a) Origin (b) initiation point
(c) Primer (d) replication fork

20. How many carbon atoms are in a pyrimidine ring?
(a) Two (b) Three
(c) Four (d) Six

21. Which is not a function of pyrimidine?
(a) Hereditary material (b) energy source
(c) Anti-epilepsy drugs (d) vitamin B

22. Which nitrogenous base does uracil bind to?

(a) Thymine (b) Guanine
(c) Cytosine (d) Adenine

23. Which of these statements about DNA is NOT true?
(a) In eukaryotes, DNA is present exclusively in the nucleus
(b) DNA is the genetic material for some viruses
(c) DNA replication is semi-conservative
(d) None of the above

24. Which of these scientists designed an experiment to show that DNA replication was semi-conservative?
(a) James Watson (b) Meselson
(c) Linus Pauling (d) All of the above

25. If DNA of a particular species was analyzed and it was found that it contains 27 percent A, what would be the percentage of C?
(a) 27 percent (b) 30 percent
(c) 23 percent (d) 54 percent

5.9.2 State whether the following statements are "True" or "False"

1. The two DNA strands in DNA helix are anti parallel.
2. Adenine and Thymine are two pyrimidines found in DNA.
3. Deoxyribose sugar is linked to nitrogenous base by glycosidic linkage.
4. There are about 10 base pairs present in each turn of helix.
5. Backbone of each DNA strand is made up of deoxyribose sugar and phosphoric acid.
6. Nucleosides are formed by joining of deoxyribose sugar and phosphoric acid.
7. Adenine always pairs with thymine by formation of three hydrogen bonds.
8. DNA molecules are negatively charged.
9. Pyrimidines are made up of one purine ring attached to one imidazole ring.
10. DNA replication is semi conservative.
11. There is multiple origin of replication of prokaryotes.
12. Leading strand comprises of large number of Okazaki fragments.
13. Replication bubble is not formed in prokaryotic DNA replication.
14. DNA ligase seals the nicks and fills the gap left after removal of primer.
15. Extra chromosomal DNA present in bacteria is called as plasmids.

5.9.3 Fill up the blanks:

1. _____ are double ringed nitrogenous bases present in DNA.
2. The two nucleotides are linked together by _____bond.

3. _____protein are responsible for unwinding of DNA helix.
4. Chromosomal DNA of bacteria dispersed in cytoplasm is called as _____.
5. _____removes supercoiling during the process of DNA replication.
6. Double helix model of DNA was given by _____ and _____.
7. DNA polymerase synthesis complementary strand in _____direction.
8. The _____strand must proceed in chunks, called as Okazaki fragments.
9. Nitrogenous bases in DNA are joined by _____bonds.
10. _____is a nitrogenous base found in DNA and not in RNA.

5.9.4 Very Short answer type question:

1. Mention different components of Nucleotide?
2. Why is B-DNA called wet DNA?
3. Which form of DNA is found under normal conditions in the cell?
4. What is genetic code?
5. Mention the function of DNA ligase during the process of DNA replication?
6. What are Okazaki fragments?
7. Draw only structure of guanine and cytosine?
8. How does formation of phosphodiester bond takes place during formation of DNA polynucleotide chain?
9. What holds one strand against the other in the double helix?
10. Under what cellular conditions A-DNA is found to occur?
11. Name eukaryotic cellular organelles which contain circular DNA?
12. What is the function of mRNA?
13. One of the DNA strand in helix has sequence 5'-ATCGGATACAGAT-3'. What will be the sequence of the other strand in the helix?
14. Name different enzymes involved in replication of DNA.
15. Give two main differences between prokaryotic and eukaryotic DNA replication?

5.9.1 Answers Key: 1-(d), 2-(a), 3-(b), 4-(c), 5-(b), 6-(d), 7-(a), 8-(d), 9-(b), 10-(a), 11-(d), 12-(c), 13-(a), 14-(c), 15-(b), 16-(a), 17-(b), 18-(d), 19-(a), 20-(c), 21-(b), 22-(d), 23-(a), 24-(b), 25-(c)

5.9.2 Answers Key: 1-True, 2-False, 3-False, 4-True, 5-True, 6-False, 7-False, 8-True, 9-False, 10-True, 11-False, 12-False, 13-True, 14-True, and 15-True

5.9.3 Answers Key: **1**-Purines, 2-Phosphodiester, **3**-Helicase, **4**-Nucleoid, **5**-Topoisomerase, **6**-Watson and Crick, **7**-5'-3', **8**-Lagging, **9**-Hydrogen, **10**-Thymine

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5.12 TERMINAL QUESTIONS

5.12.1 Short answer questions

1. Describe Chargaff's rule of base equivalence in DNA?
2. Two DNA strands are not identical but complementary. Explain?
3. What are Okazaki fragments?
4. Describe about the contribution of Griffith in establishing DNA as genetic material?
5. Two DNA strands are anti-parallel. Explain the meaning of the statement?
6. Briefly describe about the structure of nucleotide?
7. How and where does formation of phosphodiester bond and glycosidic bond formation occur in polynucleotide chain?

8. Differentiate between Z-DNA and B-DNA?
9. What is the function of histones in DNA packaging?
10. DNA replication is semi conservative. Explain?
11. Write a short notes on any two of the following:
    (a) Topoisomerase (b) Ligase (c) Helicase (d) DNA polymerase III

5.12.2 Long answer type questions

1. What background information did Watson and Crick have made available for developing a model of DNA? What was their contribution? Also mention the salient features of Watson and crick DNA model?
2. What are chemical building blocks of DNA? Give structural formula of each of them. Also name the bonds by which these monomers are linked in a polynucleotide chain?
3. Differentiate between the following:
    (a) Polymerase and ligase
    (b) Nucleoside and nucleotide
    (c) Purine and Pyrimidine
4. How does DNA replication in eukaryotes differ from prokaryotes?
5. Give an account of Hershey and Chase experiment. What did it conclusively prove? If both DNA and proteins contained phosphorus and sulphur do you think the result would have been the same?
6. With suitable diagram explain the process of DNA replication? Also enlist the role of different enzymes involved in DNA replication?
7. Explain about different known morphological forms of DNA?
8. Define transformation in Griffith's experiment. Discuss how it helps in the identification of DNA as the genetic material.
9. Describe about different experimental approaches which established DNA to be the universal genetic material?
10. What do you understand by DNA-protein interaction? Explain its different types with suitable examples?

UNIT-6 STRUCTURE AND COMPOSITION OF RNA

6.1 OBJECTIVES

After reading this unit students will be able-

- To study about structure of RNA.
- To understand about different types of RNA and their specific function.
- To have preliminary idea about synthesis of RNA.
- What are the structural and functional differences between DNA and RNA?

6.2 INTRODUCTION

Cells of living organisms contains two types of nucleic acids, DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid). Both the nucleic acids are made up of pentose sugar (deoxyribose in DNA and ribose in RNA), phosphoric acid and nitrogenous bases. RNA is involved in gene expression, the process of building a protein molecule coded by a gene. Gene expression involves two processes, transcription and translation. **Transcription** is the process by which a DNA sequence is copied to produce a complementary RNA segment. Translation is the process of synthesis of proteins with the help of three different types of RNA′s i.e., r-RNA, m-RNA, t-RNA.

RNA is a single stranded molecule, formed by the process of transcription from DNA, by an enzyme called as ***RNA polymerase***. In prokaryotes, there is a single RNA polymerase which catalyzes transcription of all the three types of RNA, whereas, in eukaryotes there are three different types of RNA which bring out transcription of different types of RNA′s. **RNA polymerase-I** catalyzes transcription of rRNA, **RNA polymerase-II** transcribes m-RNA and some snRNA, and **RNA polymerase-III** is responsible for transcription of t-RNA and 5S rRNA.

RNA is mainly found in cytoplasm and nucleolus. Inside cytoplasm, RNA can be found freely or it may be present bound to ribosomes. Along with this, RNA can also be found in chloroplast and mitochondria. Although, DNA is universal genetic material, but some plant and animal virus, contains RNA as genetic material. Such RNA, is known as genetic RNA, whereas, in organism where DNA, is present as genetic material, the different type of RNA found are called **non-genetic RNA**. It means that m-RNA, t-RNA and r-RNA present in living organisms are non-genetic RNA.

Research on RNA has led to many important biological discoveries and numerous Nobel Prizes. Nucleic acids were discovered in 1868 by **Friedrich Miescher** (Swiss physician and biologist), who called the material '**nuclein**' since it was found in the nucleus. It was later discovered that prokaryotic cells, which do not have a nucleus, also contain nucleic acids. The role of RNA in protein synthesis was suspected already in 1939. **Severo Ochoa** (Spanish-American physician and biochemist), won the 1959 Nobel Prize in Medicine after he discovered an enzyme that can synthesize RNA in the laboratory. However, the enzyme discovered by

Ochoa (polynucleotide phosphorylase) was later shown to be responsible for RNA degradation, not RNA synthesis. In 1956 **Alex Rich** (American biologist and biophysicist) and **David Davies** (Structural biologist) hybridized two separate strands of RNA to form the first crystal of RNA whose structure could be determined by X-ray crystallography.

During the early 1970s, retroviruses and reverse transcriptase were discovered, showing for the first time that enzymes could copy RNA into DNA (the opposite of the usual route for transmission of genetic information). For this work, **David Baltimore**, **Renato Dulbecco** and **Howard Temin** were awarded a Nobel Prize in 1975. In 1976, **Walter Fiers** and his team determined the first complete nucleotide sequence of an RNA virus genome, that of bacteriophage MS2.

6.3 RNA (RIBONUCLEIC ACID)

Ribonucleic acid (RNA) is typically single stranded and is made of ribonucleotides that are linked by phosphodiester bonds. A ribonucleotide in the RNA chain contains ribose (the pentose sugar), one of the four nitrogenous bases (A, U, G, and C), and a phosphate group. The subtle structural difference between the sugars gives DNA added stability, making DNA more suitable for storage of genetic information, whereas the relative instability of RNA makes it more suitable for its more short-term functions. The key difference in RNA structure is that the ribose sugar in RNA has a hydroxyl (-OH) group which is absent in DNA. RNA plays a very crucial role in the gene expression pathway by which genetic information in DNA is coded into proteins that determine cell function. The RNA-specific pyrimidine uracil forms a complementary base pair with adenine and is used instead of the thymine used in DNA. Even though RNA is single stranded, most types of RNA molecules show extensive intramolecular base pairing between complementary sequences within the RNA strand, creating a predictable three-dimensional structure essential for their function.

RNA is a polymeric molecule, essential in various biological roles in coding, decoding, regulation, and expression of genes. RNA and DNA are nucleic acids, and, along with lipids, proteins and carbohydrates constitute the four major macromolecules essential for all known forms of life. Like DNA, RNA is assembled as a chain of nucleotides, but unlike DNA it is more often found in nature as a single-strand folded onto itself, rather than a paired double-strand. Cellular organisms use messenger RNA (mRNA) to convey genetic information (using the nitrogenous bases of guanine, uracil, adenine, and cytosine, denoted by the letters G, U, A, and C) that directs synthesis of specific proteins. Many viruses encode their genetic information using an RNA genome.

Some RNA molecules play an active role within cells by catalyzing biological reactions, controlling gene expression, or sensing and communicating responses to cellular signals. One of these active processes is protein synthesis, a universal function where RNA molecules direct the assembly of proteins on ribosomes. This process uses transfer RNA (tRNA) molecules to deliver

amino acids to the ribosome, where ribosomal RNA (rRNA) then links amino acids together to form proteins.

Messenger RNA (mRNA) is the RNA that carries information from DNA to the ribosome, the sites of protein synthesis (translation) in the cell. The coding sequence of the mRNA determines the amino acid sequence in the protein that is produced. Many RNAs do not code for protein, however about 97% of the transcriptional output is non-protein-coding in eukaryotes). These so-called non-coding RNAs ("ncRNA"), can be encoded by their own genes (RNA genes), but can also derive from mRNA introns. The most prominent examples of non-coding RNAs are transfer RNA (tRNA) and ribosomal RNA (rRNA), both of which are involved in the process of translation. There are also non-coding RNAs involved in gene regulation, RNA processing and other roles. Certain RNAs are able to catalyse chemical reactions such as cutting and ligating other RNA molecules, and the catalysis of peptide bond formation in the ribosome, known as ribozymes.

6.3.1 Structure and composition of RNA

RNA is one of the three major macromolecules (along with DNA and proteins) that are essential for all known forms of life. The chemical structure of RNA is very similar to that of DNA, with two differences i.e., RNA contains the sugar ribose while DNA contains the slightly different sugar deoxyribose (a type of ribose that lacks one oxygen atom), and RNA has the nucleobase uracil, while DNA contains thymine (uracil and thymine have similar base-pairing properties). RNA, like deoxyribonucleic acid (DNA), is composed of nucleic acids that are found in the nucleus of plants and animals. Nucleic acids consist of high molecular weight macromolecules, which are made up of hundreds or thousands of smaller single unit molecules called nucleotides, all bound together. These molecules are the storehouse and delivery system of genetic traits and represent an organism's instruction manual for its protein–comprised manufacturing system. RNA, unlike DNA, is also found in other parts of the cell other than the nucleus. In fact, the majority of the RNA is present in the cytoplasm in various forms. Nuclear RNA is comprised of single stranded sequences and has a lower molecular weight than DNA.

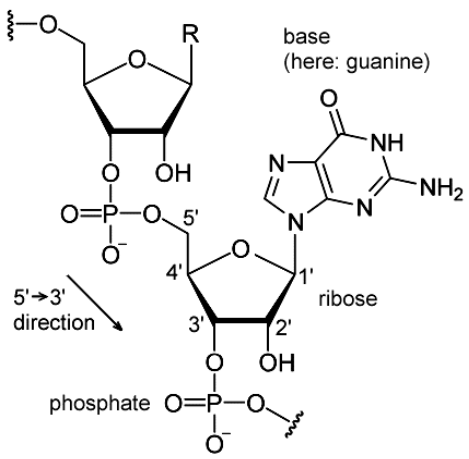

Fig.6.1Chemical structure of RNA

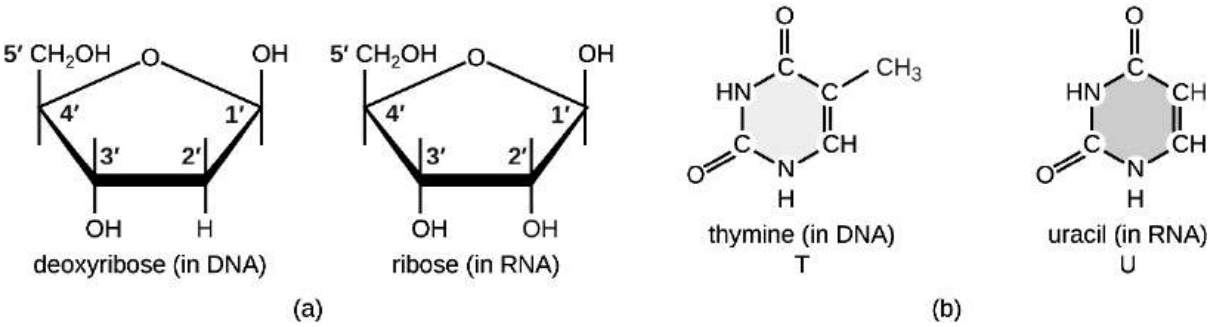

Fig.6.2: (a) Ribonucleotides contain the pentose sugar ribose instead of the deoxyribose found in deoxy-ribonucleotides; (b) RNA contains the pyrimidine-uracil in place of thymine found in DNA.

Each nucleotide molecule consists of a sugar group, a phosphate group, and an amino (nitrogen containing) group. The main difference between RNA and DNA is that in RNA the sugar is ribose (a five carbon sugar); while in DNA the sugar is deoxyribose. The prefix deoxy means that one oxygen atom is missing from the ribose. RNA is built from the same nucleotides as DNA just as proteins are built up from amino acids. There are only four bases that makeup RNA: adenine, cytosine, guanine, and uracil (A, C, G, and U, respectively). DNA contains thymine (T) instead of U. Structurally; the backbone consists of alternating sugar and phosphate parts, while the amino groups stick out like branches from the backbone. This coiled backbone in RNA if stretched out, would resemble a stretched out slinky.

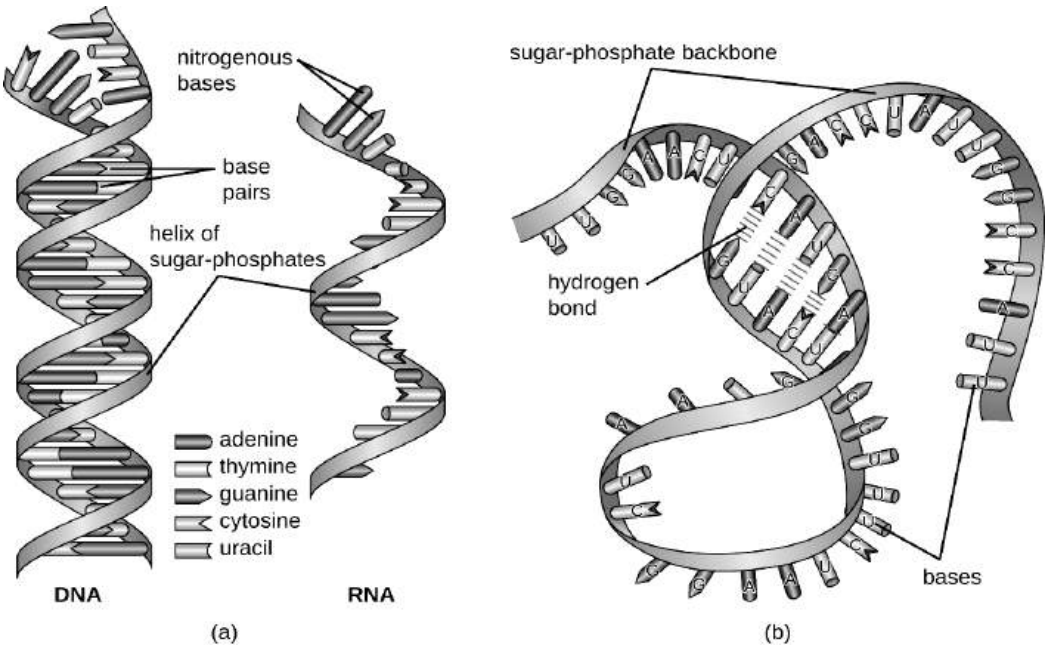

Fig.6.3: (a) DNA is typically double stranded, whereas RNA is typically single stranded. (b) Although it is single stranded, RNA can fold upon itself, with the folds stabilized by short areas of complementary base pairing within the molecule, forming a three-dimensional structure.

Ribonucleic acid (RNA) is a polymer, made up of large number of nucleotides. A structure called nucleoside, formed by joining of sugar molecules with a nitrogenous base. Now when a phosphate group is added to nucleoside it becomes a nucleotide **Fig.6.4.** Nucleotides of RNA are also known as ribonucleotides. In RNA molecules one nucleotide is linked to another nucleotide by 3'-5' phosphodiester bond. Large number of nucleotides gets linked to form single stranded unbranched polynucleotide RNA chain**.** Each nucleotide is made up of three subunits which are ribose sugar, phosphate and nitrogenous base. **Fig.6.5** shows how nucleotides are linked to form RNA.

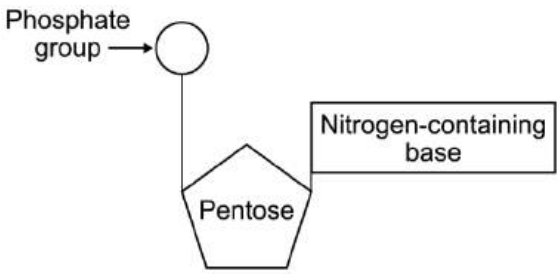

Fig.6.4 Structure of single nucleotide

Sugar present in RNA is ribose sugar. It is identical in structure to deoxyribose sugar present in DNA with only one difference that in ribose sugar an OH group is present at carbon number 2 whereas in deoxyribose sugar carbon number 2 contains H atom **Fig.6.6.** Other than this structure of both the sugars (ribose and deoxyribose) is same. Both are pentose sugar with 5 carbon atom (carbon number 1′ to 5′), out of which four carbon atoms with an oxygen atom forms a five-member ring and the fifth carbon is present outside the ring as a part of CH_2 group

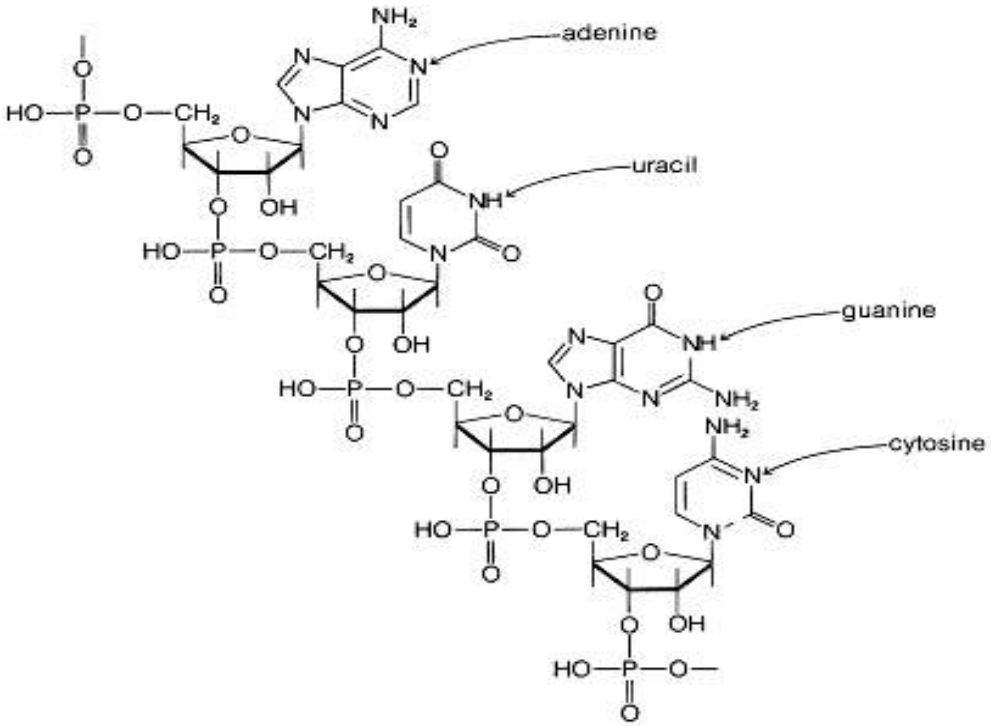

Fig.6.5 Structure of ribonucleic acid

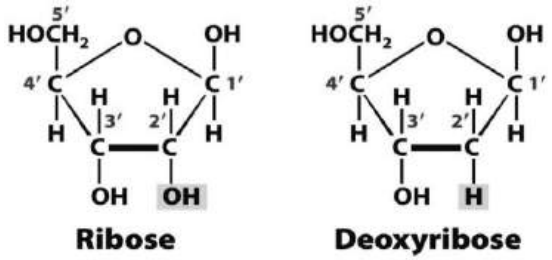

Fig.6.6 Structure of ribose and deoxyribose sugar

Table-1: Comparison between Deoxyribose and Ribose sugar

S.NO.	Deoxyribose sugar	Ribose sugar
1	Deoxyribose sugar is found to be present in DNA and does not contain OH group at C_2. Deoxyribose sugar is formed by removal of OH group from C_2 of ribose sugar.	Ribose sugar is present in RNA and contains OH group present at C_1 and C_2.
	Ribose sugar was discovered by **Phoebus Levene** (an American biochemist)	Ribose sugar was discovered by **H. Emil Fischer (**German chemist**)**
2	Chemical formula of deoxyribose is **$C_5H_{10}O_4$**	Chemical formula of deoxyribose is **$C_5H_{10}O_5$**
3	Molecular mass of ribose sugar is 134.13g/mol	Molecular mass of ribose sugar is 150.13g/mol
4	DNA molecules exhibit significant amount of stability	RNA molecules are comparatively less stable and short lived

A base (adenine, guanine, cytosine or thymine) is attached to carbon number 1 of each ribose sugar. A phosphate group functions to join two nucleotides together as one phosphate is linked to carbon number 3 of one ribose sugar and carbon number 5 of another ribose sugar. There are four nitrogenous bases found in RNA, two purines and two pyrimidines. Pyrimidines are single ringed nitrogen compound in which N atom is present at position 1 and 3 of six-membered ring.

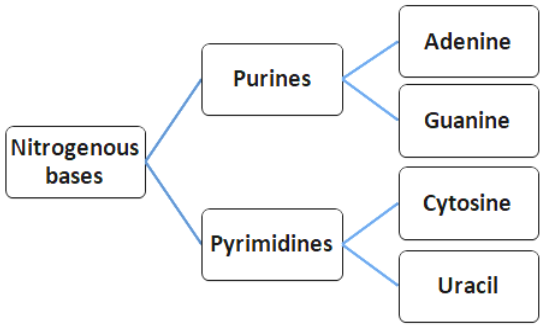

Fig.6.7 Types of nitrogenous bases present in RNA

Uracil and cytosine are the two pyrimidines present in RNA. Purines are double ringed nitrogen

compounds in which a five-membered imidazole ring is linked to pyrimidine ring. Adenine and guanine are the two purines present in RNA Fig.6.8.

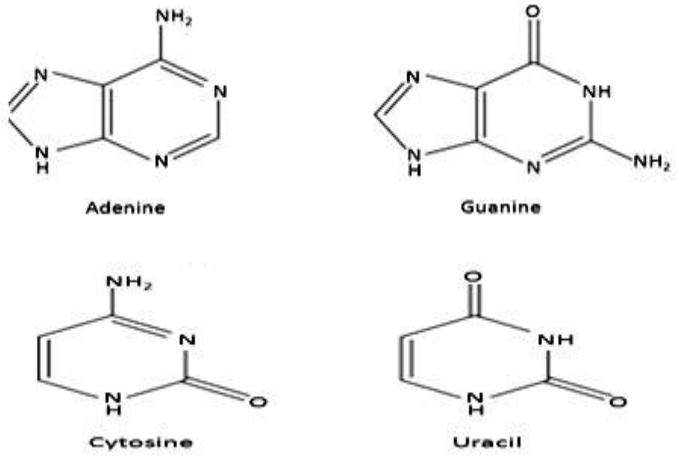

Fig.6.8 Structure of nitrogenous bases present in RNA

Comparison between DNA and RNA

While studying the structure of RNA you can observe that there exist numerous structural differences between DNA and RNA. One of the major differences is quite clearly visible by just observing their structure as DNA a double stranded helical structure while RNA is single stranded (except for few regions where loops are formed). Beside this DNA contains ribose sugar. DNA contains four nitrogenous bases adenine, guanine, cytosine and thymine. In RNA thymine is replaced by uracil while other three bases (A, G, C) are same as in DNA. In DNA purine and pyrimidines are present in equal proportion (this is known as ***Chargaff's rule***) where as in RNA no such rule is followed i.e., proportion of purine is not equal to pyrimidines. DNA is universal genetic material found to be present in almost all living organisms except plant viruses, some animal virus and several bacteriophages which have RNA as their genetic material (Table-2). When, RNA is present as genetic material, mostly it is single stranded but in some cases it can be double stranded. When, RNA is present in double stranded form, pairing between complementary bases occurs in the same way as it occurs in DNA i.e., Adenine pairs with Uracil and Guanine pairs with Cytosine.

Table-2: Represent viruses with RNA as genetic material

Type of Virus	Type of RNA	Example
Plant Virus	Single stranded Double stranded	Tobacco mosaic virus Wound tumour
Animal Virus	Single Stranded Double Stranded	Influenza Virus Reo virus
Bacteriophage	Single Stranded	MS2, F2

Table-3: Difference between DNA and RNA

S.No.	DNA	RNA
1	DNA is a genetic material found in all living organisms (except RNA virus).	RNA as genetic material found in some viruses.
2	DNA is a double-stranded molecule consisting of a long chain of nucleotides.	RNA is single stranded (mostly) except some viruses like retrovirus which has double stranded RNA. Relatively short chain (of nucleotides)
3	DNA contains deoxyribose sugar and Hydroxyl group absent	RNA contains oxyribose sugar and Hydroxyl group present
4	Types of DNA- Nuclear DNA, Mitochondrial DNA	Types of RNA found- mRNA, tRNA, and rRNA (mainly). Other types are hnRNA, snRNA, snoRNA, miRNA, siRNA
5	Nitrogenous bases found in DNA are adenine, cytosine, guanine and thymine.	Nitrogenous bases found in RNA are adenine, cytosine, guanine and uracil.
6	In DNA, proportion of Purines is equal to pyrimidine (Chargaff's rule).	In RNA, Purine is not equal to pyrimidine.
7	In DNA, complementary pairing of bases occurs throughout the length of molecule.	In RNA, Pairing between bases occurs only in specific region. Most of the RNA is linear and single stranded.
8	There are few number of uncommon bases found in DNA.	As compared to DNA, RNA contains more uncommon bases.
9	Genetic messages are usually encoded in DNA.	The usual function of RNA is translating messages encoded in DNA into protein.
10	DNA consists of large number of nucleotides.	RNA consists of lesser number of nucleotides as compared to DNA.

6.3.2 Types of RNA

The three major types of RNAs with their respective cellular composition are given below:

1. Ribosomal RNA (rRNA): 50-80%
2. Messenger RNA (mRNA): 5-10%
3. Transfer RNA (tRNA): 10-20%

Besides the three RNAs referred above, other RNAs are also present in the cells. These include heterogeneous nuclear RNA (hnRNA), small nuclear RNA (snRNA), small nucleolar

RNA (snoRNA) and small cytoplasmic RNA (scRNA). The major functions of these RNAs are given in Table-6.4.

Table-4: Cellular RNAs and their function(s)

Types of RNA	Abbreviation	Function(s)
Messenger RNA	mRNA	Transfers genetic information from genes to ribosomes to synthesized proteins.
Heterogeneous nuclear RNA	hnRNA	Serves as precursor for mRNA and other RNAs.
Transfer RNA	tRNA	Transfer amino acid to mRNA for protein biosynthesis.
Ribosomal RNA	rRNA	Provides structural framework for ribosomes.
Small nuclear RNA	snRNA	Involved in mRNA processing.
Small nucleolar RNA	snoRNA	Plays a key role in the processing of rRNA molecules.
Small cytoplasmic RNA	scRNA	Involved in the selection of proteins for export.
Transfer messenger RNA	tmRNA	Mostly present in bacteria. Adds short peptide tags to proteins, to facilitate the degradation of incorrectly synthesized proteins.

The RNAs are synthesized from DNA, and are primarily involved in the process of protein biosynthesis. The RNAs vary in their structure and function. A brief description on the major RNAs is given.

(1) Ribosomal RNA (rRNA): The ribosomes are the factories of protein synthesis. The eukaryotic ribosomes are composed of two major nucleoprotein complexes- 60S subunit and 40S subunit. The 60S subunit contains around 35 different proteins and possesses three different rRNA namely; 28S rRNA (4700 bases long), 5S rRNA (12 base long) and 5.8S rRNA (160 base long) while the 40S subunit contain 50 proteins and possesses only one type of rRNA i.e. 18S rRNA, which is about 1900 bases long. 5S r-RNA has a separate gene which is transcribed to form the RNA, whereas, all other RNA are synthesized as a single transcript, which after formation is cleaved to form other RNA molecules. The function of rRNAs in ribosomes is not clearly known. It is believed that they play a significant role in the binding of mRNA to ribosomes and protein synthesis.

However, beside this r-RNA can also act as enzymes. Such RNA molecules are known as ribozymes. The main known role of ribozymes is in the process of splicing of introns. In eukaryotes formation of r-RNA occurs from a small region of DNA. Ribosomal RNA is a single strand which might be twisted up in some regions. Prokaryotic ribosomes are also made up of

two subunits 30S and 50S. 30S subunit has 16S r-RNA and 50S subunit contains 23S and 5S r-RNA**.** In different types of r-RNA mentioned "S" stands for **sedimentation coefficient.**

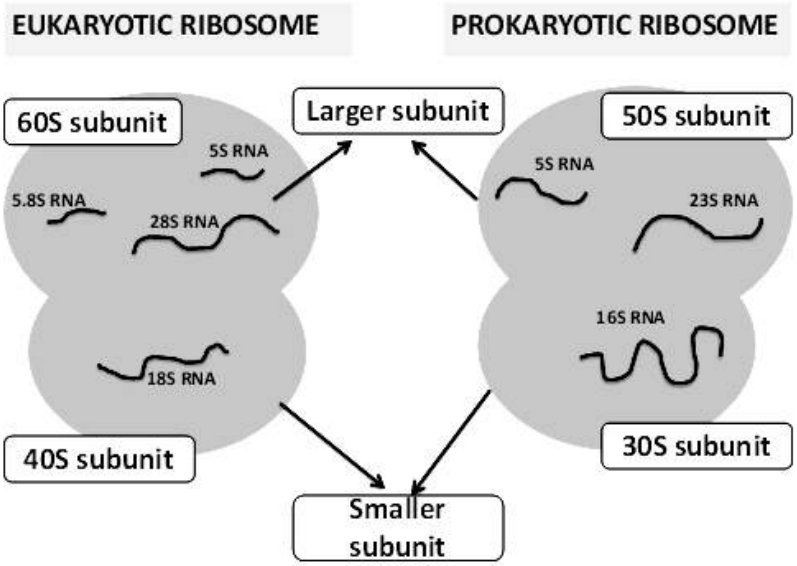

Fig. 6.9: Different RNA present in prokaryotic and eukaryotic ribosomes

(2) Messenger RNA (m-RNA): The term messenger RNA was coined by **Francois Jacob** (French biologist) and **Jacques Monad** (French biochemist). Messenger RNA is a linear molecule formed inside the nucleus by the process of transcription from DNA. The sequence of mRNA is complementary to the template DNA strand. The nitrogenous bases on mRNA strand are arranged in form of codons, made up of three nitrogenous bases. Messenger RNA carries genetic information from nucleus (chromosomal DNA) to cytoplasm for protein synthesis.

After being transported to cytoplasm m-RNA combines with ribosomes to form polyribosomes or polysomes. Each polysome contains several ribosomes (normally five) which form a complex with m-RNA. Generally, each gene transcribes its own m-RNA therefore there are approximately as many types of m-RNA molecules as there are genes. The characteristic feature of m-RNA is its heterogeneous nature. Messenger RNA significantly differs in their size and molecular weight. This difference arises due to difference in size and number of cistrons.

Life span of m-RNA

Messenger RNA formed in eukaryotes is much more stable and has a longer life span as compared to the m-RNA formed in prokaryotes which survive for a very short time period probably for about one minute or even less than a minute. Messenger RNA might have short life span but to compensate it they possess high turnover number. Messenger RNA comprises about 10% of total cellular RNA. Immediately after formation it is transported from nucleus into cytoplasm where it gets deposited on ribosomes.

Messenger RNA has been classified as monocistronic and polycistronic. Monocistronic m-RNA is a type of m-RNA which codes for only one protein whereas polycistronic m-RNA can synthesis more than one type of protein from same m-RNA molecules. This is because monocistronic m-RNA contains codons of single cistron whereas polycistronic m-RNA contains codons for more than one cistron. Monocistronic m-RNA is characteristic feature of eukaryotes while prokaryotes contain polycistronic m-RNA.

Table-5: Differences between monocistronic and polycistronic m-RNA

S.NO.	Monocistronic m-RNA	Polycistronic m-RNA
1	Contains codon of more than one cistron.	Contains codons of a single codon.
2	Codes for more than one protein.	Codes for single protein.
3	Present in prokaryotes.	Present in eukaryotes.
4	Transcribed from more than one gene.	Transcribed from single gene.

Components of structure of m-RNA

1. Messenger RNA molecule comprises of several segments namely 5′ cap, non-coding region, initiation codon, coding region, termination codon, another non coding region and 3′poly(A). Messenger RNA strand begins with a cap region present at 5′end of m-RNA strand. Presence of cap has been found to be associated with binding of m-RNA molecules to ribosomes.
2. The next segment present in m-RNA sequence is a non coding region. The length of this region is about 10-100 nucleotides. As its name suggests it is non coding region i.e., it does not codes for any protein.
3. Non-coding region is followed by **initiation codon** (AUG). Initiation codon marks the beginning of protein synthesis. Next segment in m-RNA strand is coding region which is translated to form a protein.
4. A termination codon is present at end of each coding segment. When this termination codon is reached the process of protein synthesis is terminated as these codons do not code for any amino acids. **Termination codon** is also called stop codon. There are three termination codons, UAA, UAG and UGA.
5. Coding region is followed by another non coding segment. This segment is about 50-150 nucleotides long and contains a specific sequence AAUAAA.
6. The last segment in m-RNA strand is poly (A) sequence i.e., large number of AAA..... (About 200-250) nucleotides are present. The addition of poly (A) sequence in m-RNA occurs inside nucleus before; m-RNA is transported from nucleus to cytoplasm.

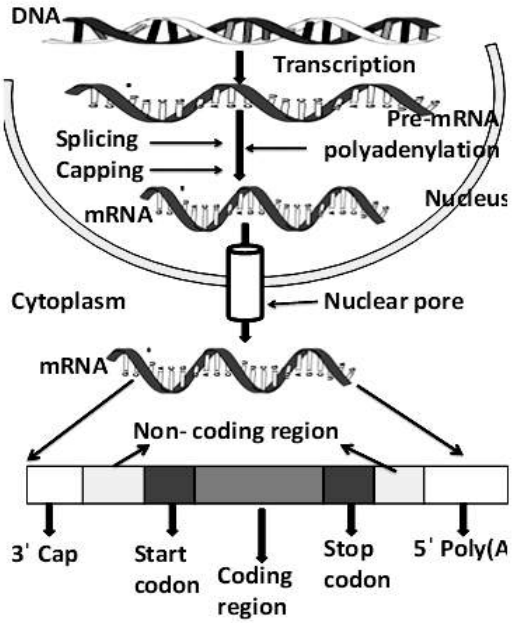

Fig.6.10: Outline of RNA processing and structure of m-RNA

Table-6: Difference between prokaryotic and eukaryotic mRNA

S.NO.	Prokaryotic mRNA	Eukaryotic mRNA
1	Translation begins when the mRNA is still being transcribed on DNA.	Translation begins when the transcription is completed.
2	Prokaryote mRNAs are very short lived. It constantly undergoes breakdown to its constituent ribonucleotide by ribonuclease.	Eukaryotic mRNAs are long lived. Thus are metabolically stable.
3	In prokaryotes mRNAs are polycistronic.	Eukaryotic mRNAs are monocistronic.
4	The mRNAs undergo very little processing after being transcribed.	The mRNA undergoes several processing after being transcribed such as polyadenylation, capping and methylation.
5	Prokaryotic m-RNA do not have poly (A) tail.	Eukaryotic m-RNA has Poly (A) tail.

(3) Transfer RNA (t-RNA)

Transfer RNA (soluble RNA) molecule contains 71-80 nucleotides (mostly 75) with a molecular weight of about 25,000. There are at least 20 species of tRNA corresponding to 20 amino acids present in protein structure. The structure of tRNA (for alanine) was first elucidated by **Holley**. The structure of tRNA depicted in Fig.6.3.2 (c) resembles that of a clover leaf. Transfer RNA molecule consists of a single polynucleotide chain folded to form 5 arms. These five arms are named as acceptor arm, D-arm, anticodon arm, TΨC arm and Variable arm. Transfer RNA contains mainly four arms, each arm with a base paired stem. Except acceptor arm, other arms have a stem and loop it means that this arm has two segments one called as stem in which complementary base pairing occurs and another part as a loop in which there is base pairing. As it has already been stated that acceptor arm has only stem and no loop. Beside this variable arm may or may not have a stem.

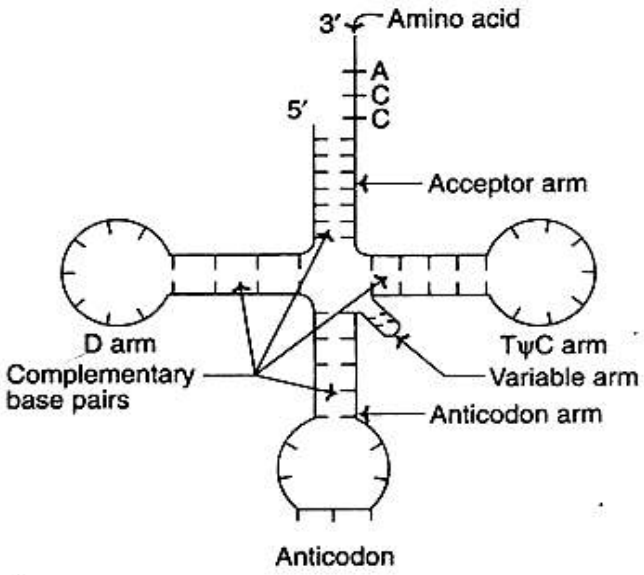

Fig. 6.11 Structure of transfer RNA

1. **The acceptor arm:** This arm is capped with a sequence CCA (5′ to 3′). The amino acid is attached to the acceptor arm.
2. **The anticodon arm:** This arm, with the three specific nucleotide bases (anticodon), is responsible for the recognition of triplet codon of mRNA. The codon and anticodon are complementary to each other.
3. **The D arm:** It is so named due to the presence of dihydrouridine.
4. **The TΨC arm:** This arm contains a sequence of T, pseudouridine (represented by psi, Ψ) and C.
5. **The variable arm:** This arm is the most variable in tRNA. Based on this variability, tRNAs are classified into 2 categories:

(a) **Class I tRNAs:** The most predominant (about 75%) form with 3-5 base pairs length.
(b) **Class II tRNAs:** They contain 13-20 base pair long arm.

Base pairs in tRNA: The structure of tRNA is maintained due to the complementary base pairing in the arms. The four arms with their respective base pairs are given below:

1. The acceptor arm – **7 bp**
2. The TΨC arm – **5 bp**
3. The anticodon arm – **5 bp**
4. The D arm – **4 bp**

Table-7: Summary of features of different arms of t-RNA

Arm	Characteristic Feature
Acceptor arm	Have 7 paired nucleotides and 4 unpaired nucleotides. 3′ end of this arm has characteristic CCA sequence to which amino acid attaches. This site is also known as **amino acid binding site**. 5′ end of acceptor arm is either G or C.
D arm	It has a total of 15-18 nucleotides, 3-4 paired bases and 7-11 unpaired nucleotides (in loop region). Loop of D arm is called as **D loop** or **DHU** (dihydrouridine) loop because of presence of high proportion of dihydrouridine in loop region.
Anti codon arm	Anti codon stem has 5' base pair. Anti codon loop has 7 unpaired nucleotides. Out of these 7 unpaired nucleotides, the middle three forms the anticodon. Anticodon recognizes complementary codon on m-RNA.
Variable arm	There are two types or variable arm in t-RNA. In one type, loop is present with 4-5 bases but there is no stem and in another type, both loop and stem are present.
TΨC arm	In this arm stem is made up of 5 base pairs and loop contains 7 unpaired nucleotides. TΨC loop contains a constant TΨC sequence. TΨC loop contains **ribosome recognition site**.

Transfer RNA is comparatively smaller RNA molecule made up of 73-93 nucleotides. The nucleotide sequence of t-RNA was given by **Holley et al** in 1965 for yeast. Transfer RNA molecule is also known as **soluble RNA**. Synthesis of t-RNA molecule occurs inside nucleus from DNA template strand. However, very small percent of total DNA (about 0.025%) codes for t-RNA. This feature is quite similar to r-RNA because r-RNA is also formed from a small portion of DNA. By this we can understand that both t-RNA as well as r-RNA does not show any obvious base relationship to DNA, because both (r-RNA and t-RNA) are formed from small portion of DNA. Contrary to this m-RNA exhibits an expected base relationship with DNA because m-RNA is synthesized from larger portion of DNA. The basic function of t-RNA is to carry amino acid to m-RNA during the process of protein synthesis. Each t-RNA carries a specific amino acid (out of a total 20 amino acids).

Transfer RNA differs from m-RNA and r-RNA as it contains some unusual bases beside the normal bases A, G, C and U. Many of the unusual bases present in m-RNA are formed by methylation of usual bases. For example Methylation of guanine forms methylguanine, Methylation of cytosine forms methylcytosine.

During t-RNA synthesis, first a precursor t-RNA is formed which is processed to form mature t-RNA. Precursor t-RNA contains normal bases and it is when precursor mRNA is modified to mature RNA, some usual bases is also modified to unusual bases. Presence of unusual bases is considered important because they protects t-RNA molecule from degradative action of enzymes. Some other unusual bases present in t-RNA are pseudouridine, dihydrouridine, methylinosine, dimethylguanine etc. All the three type of RNA are involved in the process of protein synthesis. Each type of RNA serve a specific function as mentioned in Table-8.

Table-8: Role of different RNA in translation

Type of RNA	Function of translation
m-RNA	It carries instruction for protein synthesis from nucleus to ribosome.
t-RNA	It carries amino acids to ribosomes and matches them to coded m-RNA message. It serves as adapter molecule in protein synthesis and transfer m-RNA codons into amino acids.
r-RNA	Forms an important part of both subunits of ribosome. It plays catalytic and structural role in ribosomes.

Other types of RNA

In recent years new types of RNA have been discovered besides the three classically known RNA.

(a) Small nuclear RNA (snRNA): SnRNA are found to be present in multiple copies in nucleus. Many of the snRNA are involved in the process of splicing. Small nuclear RNA is also known as U-RNA because they are rich in uridylic acid. Small nuclear RNA molecules are involved in editing of other RNA molecules. U1, U2, U5, U4 and U6 are the snRNA involved in the process of splicing, snRNA are always associated with proteins forming a complex called as small nuclear ribonucleoproteins snRNPs (pronounced "snurps").

(b) Short interfering RNA (siRNA): Short interfering is double stranded RNA molecules and is involved in RNA interference. The length of such RNA is about 20-25 base pairs. It interferes with his expression of specific genes by degrading mRNA after transcription; as a result translation of proteins is prevented.

(c) Micro RNA (miRNAs): A micro RNA (miRNA) is a small non-coding RNA molecule. It is about 22 nucleotides long. Micro RNA is found in plants, animals and some viruses that functions in RNA silencing and post-transcriptional regulation of gene expression. Micro RNAs show a base pairing with complementary sequences in mRNA molecules. As a result of this base pairing, the mRNA molecules are silenced, by one or more of the following processes:

1. Cleavage of the mRNA strand into two pieces,
2. Shortening of its poly (A) tail resulting in destabilization of mRNA

3. Improper translation of the mRNA.

(d) Heterogeneous nuclear RNA (hnRNA): Unlike prokaryotic mRNA, eukaryotic mRNAs are monocistronic. The primary transcript in eukaryotes is much larger than the mature mRNA and is called **Heterogeneous nuclear RNA (hnRNA)**. As its name suggests, hnRNA is a term that encompasses various types and sizes of RNAs found in the eukaryotic cell nucleus. It contains unique sequences and has about 10 times as many sequences as the mature mRNA. The hnRNA undergoes processing and finally the mRNA is produced and therefore, it is called "mRNA precursor" or "pre-mRNA". RNAs exist in many forms and carry out a wide range of functions.

The majority of hnRNAs are **pre-mRNAs**, newly synthesized mRNAs often made up of two types of segments: exons and introns. The exon segments are joined together to produce a mature mRNA that encodes a protein; the non-coding intron segments are removed by splicing. HnRNP proteins stabilize pre-mRNAs by allowing them to form a unique secondary structure. Different hnRNP proteins recognize and bind to different sequences along the pre-mRNA. HnRNP-bound pre-mRNAs are then spliced by spliceosomes (large macromolecular structures made up of polypeptides and RNA) to produce mature mRNAs. And the mature mRNAs in turn serve as the templates translation machinery use to produce their encoded polypeptides.

Main characteristics of hnRNA are as follows:

1. Size is not uniform and sedimentation values range from 20S to 100S.
2. Base composition of hnRNA is similar to DNA from which it is transcribed.
3. Mostly the hnRNA is located outside the nucleolus.
4. mRNA is derived from hnRNA through enzymatic processing.

Catalytic RNAs Ribozymes

In certain instances, the RNA component of ribonucleoproteins (RNA in association with protein) is catalytically active. Such RNAs are termed as **ribozymes**. At least five distinct species of RNA that act as catalysts have been identified. Three are involved in the self-processing reactions of RNAs, while the other two are regarded as true catalysts (RNase P and rRNA). Ribonuclease P (RNase P) is a ribozyme containing protein and RNA component. It cleaves tRNA precursors to generate mature tRNA molecules.

RNA molecules are known to adapt tertiary structure just like proteins (i.e. enzymes). The specific conformation of RNA may be responsible for its function as biocatalyst. It is believed that ribozymes (RNAs) were functioning as catalysts before the occurrence of protein enzymes, during the course of evolution.

Functions of RNA in Protein Synthesis

Cells access the information stored in DNA by creating RNA to direct the synthesis of proteins through the process of translation. Proteins within a cell have many functions, including building

cellular structures and serving as enzyme catalysts for cellular chemical reactions that give cells their specific characteristics. The three main types of RNA directly involved in protein synthesis are messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA).

In 1961, French scientists **François Jacob** and **Jacques Monod** hypothesized the existence of an intermediary between DNA and its protein products, which they called messenger RNA. Evidence supporting their hypothesis was gathered soon afterwards showing that information from DNA is transmitted to the ribosome for protein synthesis using mRNA. If DNA serves as the complete library of cellular information, mRNA serves as a photocopy of specific information needed at a particular point in time that serves as the instructions to make a protein.

The mRNA carries the message from the DNA, which controls all of the cellular activities in a cell. If a cell requires a certain protein to be synthesized, the gene for this product is "turned on" and the mRNA is synthesized through the process of transcription. The mRNA then interacts with ribosomes and other cellular machinery (Fig. 6.3.2-d) to direct the synthesis of the protein it encodes during the process of translation (see Protein Synthesis). Messenger RNA is relatively unstable and short-lived in the cell, especially in prokaryotic cells, ensuring that proteins are only made when needed.

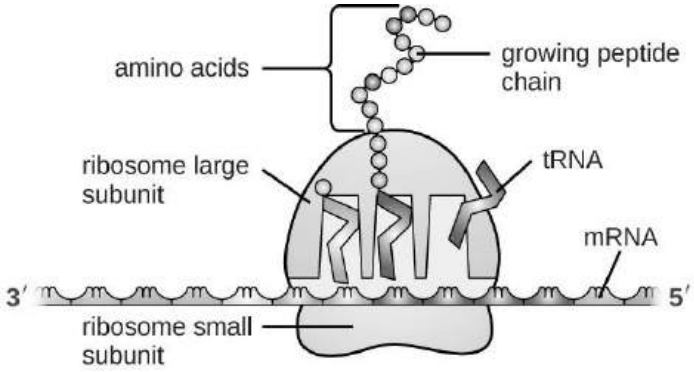

Fig.6.12: A generalized illustration of how mRNA and tRNA are used in protein synthesis within a cell.

The rRNA and tRNA are stable types of RNA. In prokaryotes and eukaryotes, tRNA and rRNA are encoded in the DNA, and then copied into long RNA molecules that are cut to release smaller fragments containing the individual mature RNA species. In eukaryotes, synthesis, cutting, and assembly of rRNA into ribosomes takes place in the nucleolus region of the nucleus, but these activities occur in the cytoplasm of prokaryotes. Neither of these types of RNA carries instructions to direct the synthesis of a polypeptide, but they play other important roles in protein synthesis.

Ribosomes are composed of rRNA and protein. As its name suggests, rRNA is a major constituent of ribosomes, composing up to about 60% of the ribosome by mass and providing the location where the mRNA binds. The rRNA ensures the proper alignment of the mRNA, tRNA, and the ribosomes; the rRNA of the ribosome also has an enzymatic activity (Peptidyl

transferase) and catalyzes the formation of the peptide bonds between two aligned amino acids during protein synthesis. Although, rRNA had long been thought to serve primarily a structural role, its catalytic role within the ribosome was proven in 2000. Scientists in the laboratories of **Thomas Steitz** (1940 US, biochemist, a Sterling Prof. of Molecular Biophysics and Biochemistry) and **Peter Moore** (1939 US, Prof. of Chemistry, Molecular Biophysics and Biochemistry) at Yale University were able to crystallize the ribosome structure from *Haloarcula marismortui*, a halophilic archaeon, isolated from the Dead Sea. Because of the importance of this work, Steitz shared the 2009 Nobel Prize in Chemistry with other scientists who made significant contributions to the understanding of ribosome structure.

Transfer RNA is the third main type of RNA and one of the smallest, usually only 70–90 nucleotides long. It carries the correct amino acid to the site of protein synthesis in the ribosome. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain being synthesized (Fig. 6.13). Any mutations in the tRNA or rRNA can result in global problems for the cell because both are necessary for proper protein synthesis.

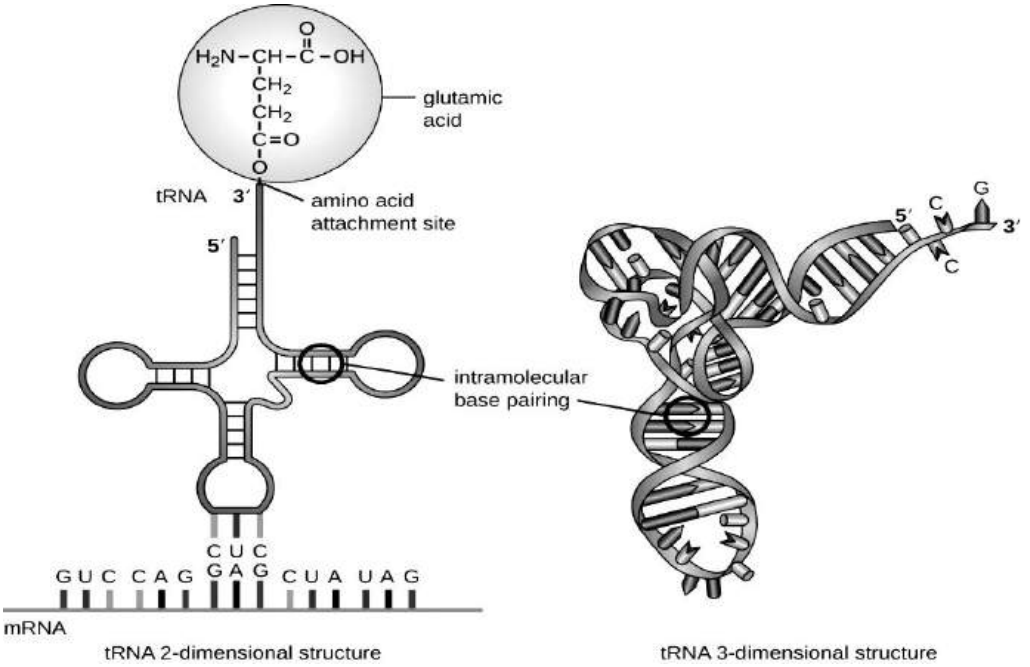

Fig.6.13: A tRNA molecule is a single-stranded molecule that exhibits significant intracellular base pairing, giving it its characteristic 3-D shape.

RNA as Hereditary Information

Although RNA does not serve as the hereditary information in most cells, RNA does hold this function for many viruses that do not contain DNA. Thus, RNA clearly does have the additional capacity to serve as genetic information. Although RNA is typically single stranded within cells, there is significant diversity in viruses. Rhinoviruses, which cause the common cold; influenza viruses; and the Ebola virus, are single-stranded RNA viruses. Rotaviruses, which cause severe

gastroenteritis in children and other immune-compromised individuals, are examples of double-stranded RNA viruses. Because double-stranded RNA is uncommon in eukaryotic cells, its presence serves as an indicator of viral infection.

6.4 SUMMARY

1. Ribonucleic acid (RNA) is typically single stranded and contains ribose as its pentose sugar and the pyrimidine uracil instead of thymine. An RNA strand can undergo significant intramolecular base pairing to take on a three-dimensional structure.
2. There are three main types of RNA, all involved in protein synthesis. Genetic information copied from DNA is used to build three types of RNA which are Ribosomal RNA (rRNA), Messenger RNA (mRNA), and Transfer RNA (tRNA).
3. Ribosomal RNA (rRNA) is a type of stable RNA that is a major constituent of ribosomes. It ensures the proper alignment of the mRNA and the ribosomes during protein synthesis and catalyzes the formation of the peptide bonds between two aligned amino acids during protein synthesis.
4. Ribosomal RNA (rRNA) is a structural component of ribosomes that binds mRNA and tRNA together (most RNA in a cell is rRNA).
5. In prokaryotic cells which do not have nucleus and cytoplasm compartments, mRNA can bind to ribosomes while it is being transcribed from DNA. Eukaryotic ribosomes contain four different rRNA molecules: 18S, 5.8S, 28S and 5S rRNA.
6. Messenger RNA (mRNA) serves as the intermediary between DNA and the synthesis of protein products during translation. Messenger RNA (mRNA) *transcribes* the code from DNA and takes it from the nucleus into the cytoplasm at the ribosome.
7. After synthesis, m-RNA immediately diffuses out of the nucleus into the cytoplasm, where it gets associated with the ribosomes.
8. Messenger RNA has a short life span and withers away after few translations. It has a high turnover. There is one mRNA for each polypeptide chain. Because of great variation in the size of mRNA molecules, mRNA is also called heterogeneous nuclear RNA or hnRNA.
9. Based on the number of cistrons, two types of mRNA have been recognized, monocistronic and polycistronic.
10. Monocistronic mRNA molecule contains the codons of a single cistron. Polycistronic mRNA molecule contains the codon for more than one cistron. The 28S and 5S molecules occur in large subunit (60S subunit) of ribosome, whereas 18S molecules are present in the small subunit.
11. Transfer RNA (tRNA) is a small type of stable RNA that carries an amino acid to the corresponding site of protein synthesis in the ribosome. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain being synthesized.

12. Transfer RNA (tRNA) *translates* the message by transferring amino acids from the cytoplasm to the ribosomes based on the instructions in the mRNA.
13. Each type of tRNA molecule can be attached to only one type of amino acid but because the genetic code contains multiple codons that specify the same amino acid, tRNA molecules bearing different anticodons may also carry the same amino acid.
14. Clover leaf models have five different regions which can be recognized in the structure of tRNA are Acceptor Arm, Anticodon Arm, Dihydrouridine Loop, TΨC Loop, and Variable Arm.
15. Acceptor arm is for amino acid attachment and an anticodon region for codon recognition that binds to a specific sequence on the messenger RNA chain through hydrogen bonding.
16. In addition to the usual bases of RNA, each tRNA molecule has several unusual bases. Some of them are pseudouridine, methylguanine, methyl aminopurine etc.
17. In addition to these three of RNAs, other types of RNAs are also found in cell Small Nuclear RNAs (snRNAs), micro RNA (miRNA), etc.
18. Although, RNA is not used for long-term genetic information in cells, many viruses do use RNA as their genetic material.

6.5 GLOSSARY

Adenine: A purine base, $C_5H_5N_5$, one of the fundamental components of nucleic acids, as DNA, in which it forms a base pair with thymine, and RNA, in which it pairs with uracil.

Chromosome: A thread-like structure of nucleic acids and protein found in the nucleus of most living cells, carrying genetic information in the form of genes.

Cistron: a section of a DNA or RNA molecule that codes for a specific polypeptide in protein synthesis

Codon: a sequence of three nucleotides which together form a unit of genetic code in a DNA or RNA molecule.

Codon: A triplet of adjacent nucleotides in the messenger RNA chain, those codes for a specific amino acid in the synthesis of a protein molecule.

Cytosine: A pyrimidine base, $C_4H_5N_3O$ that is one of the fundamental components of DNA and RNA, in which it forms a base pair with guanine.

DNA: (Deoxyribonucleic acid), the chemical molecule that is the basic genetic material found in all cells.

Enzymes: A protein that accelerates the rate of chemical reactions. Enzymes are catalysts that promote reactions repeatedly, without being damaged by the reactions.

Eukaryotes: A eukaryote is any organism whose cells contain a nucleus and other organelles enclosed within membranes.

Exon: a segment of a DNA or RNA molecule containing information coding for a protein or peptide sequence.

Gastroenteritis: Inflammation of the stomach and intestines.

Gene: A unit of heredity which is transferred from a parent to offspring.

Genome: A full set of chromosomes; all the inheritable traits of an organism.

Guanine: A purine base, $C_5H_5N_5O$ that is a fundamental constituent of DNA and RNA, in which it forms base pairs with cytosine.

Introns: Introns are non-coding sections of an RNA transcript, or the DNA encoding it, that are spliced out before the RNA molecule is translated into a protein.

Monocistronic: Describing a type of messenger RNA that can encode only one polypeptide per RNA molecule. In eukaryotic cells virtually all messenger RNAs are monocistronic.

M-RNA: A single-stranded molecule of RNA that is synthesized in the nucleus from a DNA template and then enters the cytoplasm, where its genetic code specifies the amino acid sequence for protein synthesis.

Nucleic acid: a complex organic substance present in living cells, especially DNA or RNA, whose molecules consist of many nucleotides linked in a long chain.

Nucleolus: A conspicuous, rounded body within the nucleus of a cell.

Nucleoprotein: Any of the class of conjugated proteins occurring in cells and consisting of a protein combined with a nucleic acid, essential for cell division and reproduction.

Nucleotides: Nucleotides are the building blocks of nucleic acids; they are composed of three subunit molecules: a nitrogenous base, a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group.

Polycistronic: Describing a type of messenger RNA that can encode more than one polypeptide separately within the same RNA molecule. Bacterial messenger RNA is generally polycistronic.

Prokaryotes: A prokaryote is a unicellular organism that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle.

Purine: One of several purine derivatives, especially the bases adenine and guanine, which are fundamental constituents of nucleic acids.

Pyrimidine: One of several pyrimidine derivatives, especially the bases cytosine, thymine, and uracil, which are fundamental constituents of nucleic acids.

Ribonuclease: Any of the class of enzymes that catalyze the hydrolysis of RNA.

Ribozymes: Ribozymes (ribonucleic acid enzymes) are RNA molecules that are capable of catalyzing specific biochemical reactions, similar to the action of protein enzymes

RNA: Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation, and expression of genes.

Splicing: splicing is the editing of the nascent precursor messenger RNA (pre-mRNA) transcript. After splicing, introns are removed and exons are joined together

Thymine: A pyrimidine base, $C_5H_6N_2O_2$ that is one of the principal components of DNA, in which it is paired with adenine.

Transcription: Transcription is the first step of gene expression, in which a particular segment of DNA is copied into RNA.

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

T-RNA: A small RNA molecule, consisting of a strand of nucleotides folded into a clover-leaf shape that picks up an unattached amino acid within the cell cytoplasm and conveys it to the ribosome for protein synthesis.

Uracil: A pyrimidine base, $C_4H_4N_2O_2$ that is one of the fundamental components of RNA, in which it forms base pairs with adenine.

6.6 SELF ASSESSMENT QUESTIONS

6.6.1 Choose the most appropriate option for the following objective questions:

1. Most abundant RNA of the cell is?
(a) t-RNA (b) m-RNA
(c) r-RNA (d) Si-RNA

2. Purines of RNA are?
(a) Guanine and adenine (b) Adenine and cytosine
(c) Uracil and thymine (d) Uracil and guanine

3. DNA and RNA show similarity in having?
(a) Similar sugars (b) Double Strands
(c) Similar pyrimidines (d) Polymers of nucleotides

4. Messenger RNA (mRNA) is a polymer of?
(a) Deoxy ribonucleotides (b) Ribonucleotides
(c) Ribonucleosides (d) Deoxyribonucleosides

5. The smallest RNA is
(a) m-RNA (b) r-RNA
(c) t-RNA (d) Chromosomal RNA

6. Ribozyme is
(a) RNA with enzyme activity (b) RNA without phosphate
(c) RNA without sugar (d) RNA with extra phosphate

7. Maximum amount of RNA is found in
(a) Chloroplasts (b) Ribosomes
(c) Nucleolus (d) Cytoplasm

8. RNA that picks up specific amino acid from amino acid pool of cytoplasm to carry it to ribosome during protein synthesis is
(a) m-RNA (b) r-RNA

(c) t-RNA (d) g-RNA

9. t-RNA recognises ribosome by
(a) TψC loop (b) Anticodon
(c) DHU loop (d) AA-site

10. Prokaryotic mRNA differs from eukaryotic m RNA
(a) In not having Poly (A) tail (b) being short lived
(c) Being polycistronic (d) All of the above

11. The three termination codons are
(a) UAA, UAG, UCC (b) UAA, UAG, UGA
(c) UAA, TAG, UGA (d) UAA, UAG, UGG

12. t-RNA is also called
(a) Soluble RNA (b) Messenger RNA
(c) Microsomal RNA (d) Informosome

13. When m-RNA is synthesised on DNA the unwanted DNA regions are removed and regions coding for amino acids are joined together. This process is referred to as
(a) Lysogeny (b) Replication
(c) Splicing (d) Regulation

14. DNA and RNA differ by
(a) N-bases and phosphate groups (b) N- bases and sugars
(c) Numbers of C- atoms in sugars (d) Sugar and phosphate

15. Polycistronic m-RNA is found in
(a) Prokaryotes only (b) Prokaryotes and unicellular eukaryotes
(c) Eukaryotes only (d) Both a and b

16. m-RNA's is heterogeneous in nature due to difference
(a) Size of m-RNA (b) Molecular Weight
(c) Both a and b (d) None of the above

17. Double stranded RNA is found as genetic material in which of the following
(a) Tobacco mosaic virus (b) Reo virus
(b) Bacteriophage (d) All the above

18. 60S subunit of ribosomes of vertebrates contains
(a) 28S r-RNA only (b) 5.8S and 5S r-RNA

(c) 28S and 5.8S r-RNA (d) 5S, 5.8S and 28S r-RNA

19. One of the main functions of ribozymes is in
(a) Replication of DNA molecules (b) Splicing of RNA molecules
(c) Splicing of DNA molecules (d) Replication of RNA molecules

20. Match the two columns given in the table below and choose the correct option

1.	m-RNA	(i) Carries amino acids to ribosomes
2.	t-RNA	(ii) Plays catalytic and structural role in ribosomes.
3.	r-RNA	(iii) Regulation of gene expression
4.	i-RNA	(iv) Carries information from chromosomal DNA to ribosomes.

(a) 1-(i), 2-(ii), 3-(iii),4-(iv) (b) 1-(iv), 2-(i), 3-(iii),4-(ii)
(c) 1-(i), 2-(iii), 3-(ii),4-(iv) (d) 1-(iv), 2-(i), 3-(ii),4-(iii)

21. Which of the following types of RNA codes for a protein?
(a) dsRNA (b) mRNA
(c) rRNA (d) tRNA

22. A nucleic acid is purified from a mixture. The molecules are relatively small, contain uracil, and most are covalently bound to an amino acid. Which of the following was purified?
(a) DNA (b) mRNA
(c) rRNA (d) tRNA

23. Which of the following types of RNA is known for its catalytic abilities?
(a) dsRNA (b) mRNA
(c) rRNA (d) tRNA

24. Ribosomes are composed of rRNA and what other component?
(a) Protein (b) Polypeptides
(c) DNA (d) mRNA

25. Which of the following may use RNA as its genome?
(a) Bacterium (b) Archaeans
(c) Virus (d) Eukaryote

6.6.2 State whether following statements are "True" or "False"

1. In RNA proportion of purines is not necessarily equal to pyrimidine.
2. Messenger RNA is most abundant among different RNA found in a cell.
3. Eukaryotic mRNA is long lived as compared to prokaryotic mRNA.
4. Transfer RNA possesses more amounts of universal bases as compared to r-RNA and mRNA.

5. Formation of RNA from DNA is known as translation.
6. RNA is made up of deoxyribose sugar, phosphoric acid and nitrogenous bases.
7. Eukaryotic mRNA is more stable than prokaryotic mRNA.
8. During RNA formation splicing occurs in prokaryotes and eukaryotes to remove introns.
9. DNA can form a complex three dimensional structure whereas RNA cannot.
10. The three types of RNA are synthesized during different stages of development.
11. RNA can act as catalyst also whereas DNA cannot.
12. In RNA adenine pairs with thymine with two hydrogen bond and cytosine pairs with guanine with three hydrogen bonds.
13. Monocistronic m-RNA codes for a single protein whereas polycistronic m-RNA codes for more than one protein.
14. Ribosomal RNA carries genetic information from nucleus to cytoplasm for protein synthesis.
15. Messenger RNA is heterogeneous in nature.
16. Each t-RNA molecule contains 4 arms and 5 loops.
17. Presence of unusual bases occurs in t-RNA only.
18. Nucleotide is the monomer unit of RNA.
19. Messenger RNA contains 5ˈpoly AAA---- tail and a 3ˈcap.
20. Transfer RNA combines with ribosomes to form polyribosomes.

6.6.3 Fill in the blanks

1. RNA is single stranded except in some _____.
2. Ribosomal RNA is derived from _____ DNA.
3. The term mRNA was proposed by _____.
4. _____acts as adapter for attaching amino acids to mRNA template.
5. Monocistronic mRNA is found in _____.
6. The r-RNA and t-RNA are found to be located in _____.
7. _____are catalytic RNA enzymes.
8. _____is the process of formation of RNA from DNA.
9. Removal of introns during RNA Formation is known as _____.
10. The r-RNA in cytoplasm combines with protein to form _____.
11. 40S subunit of eukaryotic ribosome contains _____r-RNA.
12. _____ plays a role in editing of other class of RNA.
13. Clover leaf model of _____ was given by _____.
14. All the arms of t-RNA have loop except _____arm.
15. Nucleotides are linked to each other by _____ bond.

6.6.4 Very short answer type questions:

1. What are ribozymes?
2. What do you understand by the term splicing?

3. Why is mRNA heterogeneous in nature?
4. Name different purines and pyrimidines found in RNA?
5. Give two examples each of plant and animal viruses where RNA is present as genetic material?
6. Name two unusual bases present in t-RNA?
7. Where does translation take place?
8. Name the five arms present in structure of t- RNA?
9. Name the enzyme involved in the process of transcription?
10. Give two examples of unusual bases present in t-RNA?
11. What are polyribosomes?
12. What is the function of stop codon?
13. What do you understand by genetic and non-genetic RNA?
14. What is initiation codon?
15. What is anticodon?

6.6.1 Answer Key: 1-(c), 2-(a), 3-(a), 4-(b), 5-(a), 6-(a), 7-(a), 8-(a), 9-(a), 10-(d), 11-(b), 12-(a), 13-(c), 14-(b), 15-(a), 16-(c), 17-(b), 18-(d), 19-(b), 20-(d), 21-(b), 22-(d), 23-(c), 24-(a), 25-(c).

6.6.2-Answer Key: (1)-True, (2)-False, (3)-True, (4)-True, (5)-False, (6)-False, (7)-True, (8)-False, (9)-False, (10)-True, (11)-True, (12)-True, (13)-True, (14)-False, (15)-True, (16)-False, (17)-True, (18)-True, (19)-True, (20)-False

6.6.3-Answer Key: (1) Viruses, (2) Nuclear, (3) Francis Jacob and Jacques Monod, (4) tRNA, (5) Prokaryotes, (6) Cytoplasm, (7) Ribozymes, (8) Transcription, (9) Splicing, (10) Ribosomes, (11) 18S, (12) SnRNA, (13) t-RNA, Holley, (14) Acceptor (15) 3'-5' phosphodiester bond.

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6.9 TERMINAL QUESTIONS

6.9.1 Short answer type questions

1. What are nucleotides? How are they linked to form nucleic acids?
2. Name different types of nucleic acid found in eukaryotes. Mention structural and functional differences between them?
3. Write short note on structure and function of r-RNA?
4. How does the enzyme know where to start and stop transcription?
5. Mention the role of three different types of RNA in the process of protein synthesis?
6. Classify the nitrogenous bases present in RNA?
7. What is the function of the 5' cap on an mRNA? What about the 3' poly (A) tail?
8. What are ribonucleotides? Explain giving relevant structures?
9. Briefly describe about siRNA and miRNA?
10. Describe about characteristic feature of m-RNA molecule?

6.9.2 Long answer type questions:

1. What is RNA, and how is it different from DNA? What are the three kinds of RNA, and what is the purpose of each?
2. How is protein made from mRNA? Write about the function of RNA in protein synthesis? Why is it important for translation that ribosomes contain three sites for tRNA and not just one?
3. Differentiate between:
    (a) Monocistronic and Polycistronic mRNA.
    (b) Different types of RNA
4. Describe about synthesis and function of mRNA. Also mention how does prokaryotic mRNA differ from eukaryotic mRNA?
5. With the help of well labelled diagram explain the structure of t-RNA. Elaborate the role of t-RNA in protein synthesis?
6. What are the different components present in structure of RNA? How are they arranged to form RNA?
7. Write a short note on following:
    (a) Ribosomal RNA (rRNA)
    (b) Small nuclear RNA (snRNA)
    (c) Micro RNA (miRNA)
8. Write about the characteristic features of different tRNA arms.
9. Giving the structure of ribose and deoxyribose sugar mention the similarities and difference between them?
10. Why is RNA necessary to act as a messenger? Why can't the code be taken directly from DNA? Also discuss the RNA as hereditary information.

UNIT-7 MODERN CONCEPT OF GENE AND GENETIC CODE

7.1 OBJECTIVES

Main objective of this unit is to make student:

- To understand the concept of gene in light of classical, neoclassical and modern concept.
- Concept of operon and how it is regulated
- How Lac and tryptophan operon work in *E.coli*
- Organization and characteristics feature of genetic code.

7.2 INTRODUCTION

Genetics is the branch of science which deals with study of heredity and variation i.e. how genes are inherited and transmitted from one generation to another. Existence of anything like genes was first of all conceived by **Gregor Johann Mendel** (Father of Genetics), in 1860's. However he never used the term gene, instead he utilized the term ***factors*** (elements which are transferred from one generation to another). Ever since the concept of gene came, the concept of gene has been continuously evolving with the findings and discovers made in the field of Genetics, Molecular biology, Biochemistry etc. It was in 1905, when Danish botanist, plant physiologist and geneticist **Wilhelm Johannsen** introduced the term ***gene***. However, early work done by several workers proposes various hypotheses to explain the exact nature of genes. In 1906, **W. Bateson** and **R.C. Punnett** reported the first case of linkage in sweet pea and proposed the presence or absence theory. According to them the dominant character has a determiner, and the recessive character lacks determiner.

From Mendel's experiment on hybridization it is understand that genes are hereditary units transmitted from one generation to other generation through the germ cells and associated with carrying the characters. After **Johannsen,** several workers experimentally proved that genes are the fractions or part of DNA molecule which regarded as the genetic material. **Sutton** and **Boveri** independently suggested that chromosome is the container of hereditary units. In 1926, **Thomas Hunt Morgan** discarded all the previous existing theories and put forth the particulate gene theory. He thought that genes are arranged in a linear order on the chromosome and look like '*beads on a string'*. He proposed the gene theory which state that:

- Chromosomes are bearers of hereditary units and each chromosome carries hundreds or thousands of genes.
- The genes are arranged on the chromosomes in the linear order and on the special regions or locus.

In 1928, **Belling** proposed that the chromosome that appeared as granules would be the gene. This theory of gene was well accepted by the cytologists. In 1933, Morgan was awarded Nobel Prize for advocating the theory of genes. After the discovery of DNA as carrier of genetic information's, the Morgan's theory was discarded. Therefore, it is necessary to understand both, the classical and modern concepts of gene.

7.3 GENE CONCEPT

The concept of the gene has always been constantly evolving, and is happening. In order to provide a structure of understanding the concept, its history is divided into classical, neoclassical, and modern periods (or concept of gene). **Classical concept** considered gene as a unit of inheritance, which explained phenotypic (and genotypic) similarities and differences, between parents and offspring. The classical view prevailed into the 1930's, and conceived the gene as an indivisible unit of genetic transmission, recombination, mutation, and function. **Neoclassical concept** of gene came into 1940's with discovery of intragenic recombination and recognition of DNA as physical basis of inheritance. In this view the gene (or cistron, as it was called then) was subdivided into its constituent parts, mutons and recons, identified as nucleotides. Each cistron was believed to be responsible for the synthesis of m-RNA and eventually protein. This colinearity hypothesis prevailed from 1955 to the 1970s.

Modern concept of gene began during 1970's, starting from the early 1970s, DNA technologies have led to the modern period of gene conceptualization, wherein none of the classical or neoclassical criteria are sufficient to define a gene. With further studies aided with modern research, concept of gene discoveries include those of repeated genes, split genes and alternative splicing, assembled genes, overlapping genes, transposable genes, complex promoters, multiple polyadenylation sites, polyprotein genes, editing of the primary transcript, and nested genes. All the research and finding done related to genetics or gene can be divided broadly into classical genetics and modern genetics. All the research, events, findings before **Central Dogma Concept,** which came in 1941 are considered to be classical genetics; whereas events and studies done in the field of genetics and molecular biology after 1941 are considered as neo-classical and modern concept of gene.

Classical concept of gene

The renowned work of Mendel on *Pisum sativum* (1865); rediscovery of Mendel's work by three scientists - **Carl Correns**, **Hugo de Vries** and **Erich von Tschermak** in 1990, basically belong to classical genetics. Actual formation of classical concept of gene is credited to work done by **Thomas Hunt Morgan**, along with **Calvin Blackman Bridges**, **H.J. Muller** and **Alfred Sturtevant**. They proposed the chromosomal theory of inheritance according to which genes are arranged in chromosomes in similar way as beads are present on a string. Concept of linkage given by T.H Morgan and his workers is a milestone in field of genetics. Work done by **Sutton** and **Boveri** (1903) is also significant contribution to classical genetics. Their work also supports chromosomal theory of inheritance. They also proposed that Mendel's Laws are explained or justified by behavior of chromosomes during meiosis. Gene concept as per **classical genetics** can be summarized as follows:

1. Genes can be transmitted from parent to off springs.

2. Genes are present in pairs and having fixed number of chromosomes (every species has a definite number of chromosome pairs.
3. Theses chromosome pairs are regarded as linkage groups. Large number of genes is present on each chromosome. All the genes present on one chromosome are called **linked genes**.
4. The genes are arranged in a single linear order like beads on a string. Each gene occupies specific position on a particular chromosome and such position (on which genes are present) is called **locus**. If the position of gene changes, the character also get changes.
5. Genes are discrete particles inherited in Mendelian fashion that occupies a definite locus in the chromosome and responsible for expression of specific phenotypic character. Two or more genes pairs may interact to influence expression of a character.
6. As already states genes are present in pairs (alleles), Each pair of gene separates during gamete formation and only one gene of each pair enter gamete (Mendel's law of segregation).
7. Gene pairs located on different chromosomes exhibits independent assortment during inheritance. Number of genes in each organism is more than the number of chromosomes; hence several genes are located on each chromosome.
8. Genes present on a chromosome pair may shift from one homologous pair to another (non sister chromatids) due to crossing over or translocation.
9. Genes may undergo for sudden changes in position and composition called **mutation**. They are arranged in a linear fashion on chromosomes and their order of arrangement remains unchanged until a mutation occurs.
10. Genes are capable of self duplication producing their own exact copies. They combined together or can be replicated once during a cell division.

After classical concept came neoclassical concept during which comprehensive research on nature of genetic material was done. **Oliver** (1940) and **Lewis** (1941) observed intragenic recombination in *Drosophila melanogaster*. The advent of this phenomenon (intragenic recombination) marked the beginning of new classical concept of gene. **Dounce** (1952) and **Gamow** (1954) individually presented a concept called colinearity hypothesis, according to which linear structure of DNA determines the linear structure of protein.

In a significant study, **Avery *et al*** (1944) experimentally proved that material or substance responsible for transformation in bacteria was DNA. **Griffith** in 1928 discovered the process of transformation. **Hershey** and **Chase** (1952) also demonstrated that it is only viral DNA which is transferred from viral to bacterial host cell.

7.3.1 Modern Concept of Gene

From central dogma to today's molecular genetics, comprise modern genetics. Some of the main milestones of modern genetics include **Beadle** and Tatum's ***one gene one enzyme*** hypothesis, **Watson** and **Crick** DNA model, **Paul Berg's** genetic engineering work etc. The works done by

Benzer remain a landmark in the field of molecular biology. While working on bacteriophage he could identify and map hundreds of mutations.

S. Benzer (1957) coined different terms for different nature of gene and genetic material in relation to the chromosome on the basis of genetic phenomena to which they involve. He postulated three fundamentals units namely Cistron, muton and recon.

1. Genes as unit of transmission or Cistron:

The part of DNA specifying a single polypeptide chain is termed as cistron. Cistron is a segment of DNA which represents unit of function. A cistron can have 100 nucleotide pairs in length to 30,000 nucleotide pairs. It transmits characters from one generation to other as unit of transmission. Cistron of *E.coli* may contain up to 1500 base pairs, some cistrons are made up of large number of nucleotides. Beginning of each cistron is marked by initiated codon and each cistron ends with termination codon. Each cistron can be transcribed to form a functional mRNA.

2. Gene as unit of mutation or Muton:

The shortest chromosomal unit capable of undergoing mutation has been called the muton. It is the smallest unit of mutation. A change in muton is sufficient enough to cause a change in phenotype. It means that muton is that segment of genetic material (DNA) which can undergo mutation and lead to a mutant phenotype. Thus muton is delimited to a single nucleotide. The muton consists of one or many pairs of nucleotides within the DNA molecule.

3. Genes as unit of recombination or Recon:

The smallest segment of DNA capable of being separated and exchange with other chromosome is called recon. Recon has been recognized as smallest unit of recombination. It is a unit of genetic material which can be separated from other units (recon) by means of genetic recombination. A recon consists of not more than two pairs of nucleotides. Thus, each recon is generally made up of one to two nucleotides.

With the development and utilization of modern techniques in research, classical and neoclassical genetics was further extended by discovering what is known as modern concept of gene. Modern concept of gene identifies below mentioned forms of genes, however classical concept (Mendel's findings, Morgan's work) still remains undisputedly correct and is foundation of modern genetics.

Forms of genes identifies as a part of modern genetics

1. **Constitutive Genes (House-keeping Genes):** A constitutive gene is a gene that is transcribed continually as opposed to a facultative gene, which is only transcribed when needed. They are those genes which are constantly expressing themselves in a cell because their products are required for the normal cellular activities, e.g. genes for glycolysis, ATPase.

A housekeeping gene is typically a constitutive gene that is transcribed at a relatively constant level. The housekeeping gene's products are typically needed for maintenance of the cell. It is generally assumed that their expression is unaffected by experimental conditions. Examples include actin, GAPDH and ubiquitin.

2- Repeated Genes: Also known as repetitive elements (or repeats) are patterns of nucleic acids (DNA or RNA) that occur in multiple copies throughout the genome, e.g. histone gene, tRNA genes, rRNA genes, actin genes. Repetitive DNA was first detected because of its rapid reassociation kinetics. There are 3 major categories of repeated sequence or repeats are:

(i) Long terminal repeats (LTRs): They are identical sequences of DNA that repeat hundreds or thousands of times found at either end of retrotransposons or proviral DNA formed by reverse transcription of retroviral RNA. They are used by viruses to insert their genetic material into the host genomes.

(ii) Tandem repeats: The copies which lie adjacent to each other, either directly or inverted. This class consists of DNA sequences that are repeated many times, one copy following another in tandem array. By transcribing all of the copies in these tandem clusters simultaneously, a cell can rapidly obtain large amounts of the product they encode. The genes present in a tandem cluster are very similar in sequence but not always identical; some may differ by one or a few nucleotides. An example would be: ATTCGATTCG ATTCG, in which the sequence ATTCG is repeated three times.

a. Satellite DNA - typically found in centromeres and heterochromatin

b. Minisatellite - repeat units from about 10 to 60 base pairs, found in many places in the genome, including the centromeres

c. Microsatellite - repeat units of less than 10 base pairs; this includes telomeres, which typically have 6 to 8 base pair repeat units

(iii) Transposons: Transposons are much longer than satellite DNA and exist in multiple copies scattered about the genome. In *Drosophila*, for example, more than 30 different transposons are known, most of them present at some 20 to 40 different sites throughout the genome. Mammalian genomes contain fewer kinds of transposons than the genomes of many other organisms.

3-Multigene Families: Many genes exist as parts of multigene families, groups of related but distinctly different genes that often occur together in a cluster. Multigene families differ from tandem clusters in that they contain far fewer genes (from three to several hundred) and those genes are much more different from one another than the genes in tandem clusters.

4-Dispersed Pseudogenes: Silent copies of a gene that have been inactivated by mutation are called Pseudogenes.

5- Split Genes: In most of eukaryotes, many non-coding sequences are present between coding sequences. The coding sequences of DNA of the genes are called exons. In between exons are present non-coding sequences called introns. Exons alternate with introns. Normally introns do not possess any genetic information and are not translated. Such genes are called split genes or interrupted genes.

6-Nested genes: A nested gene is a gene whose entire coding sequence lies within the bounds (between the start codon and the stop codon) of a larger external gene. The coding sequence for a nested gene differs greatly from the coding sequence for its external host gene. Typically, nested genes and their host genes encode functionally unrelated proteins, and have different expression patterns in an organism.

7-Overlapping genes: An overlapping gene is a gene whose expressible nucleotide sequence partially overlaps with the expressible nucleotide sequence of another gene. In this way, a nucleotide sequence may make a contribution to the function of one or more gene products.

On the basis of their behavior the genes may be categorized into the following types:

a) Basic genes: These are the fundamental genes that bring about expression of particular character.

b) Lethal genes: These bring about the death of their possessor.

c) Multiple gene: When two or more pairs of independent genes act together to produce a single phenotypic trait.

d) Cumulative gene: Some genes have additive effects on the action of other genes. These are called cumulative genes.

e) Pleiotropic genes: The genes which produce changes in more than one character are called pleiotropic gene.

f) Modifying gene: The gene which cannot produce a character by itself but interacts with other to produce a modified effect is called modifier gene.

g) Inhibitory gene: The gene which suppresses or inhibits the expression of another gene is called inhibitory gene.

7.3.2 Operon Concept

DNA is the universal genetic material present in nucleus of living cells. Depending upon species DNA may contain several hundred to several thousand genes. When we considered eukaryotic multicellular organisms such as plants and animals, they are made up of different types of cell which significantly vary in structure and function. However, genetic makeup (composition of genes) is same in all the cells of a living organism.

Now the question rises, when the cells are genetically identical how do they exhibit so much difference in structure and function. It is to be understood that a cell may contain thousands of genes to produce thousand's of enzymes but a cell will make only that protein or

enzyme which is required by the cell at a particular time and also only that amount or concentration of the protein will be made which is required by the cell.

This mean that at any given time in the cell, only the genes whose product (protein) is required are switched on, whereas, the remaining genes whose proteins are not required (at a particular time) are switched off. However, a gene which is switched off at a particular time may be switched on at another time interval when its protein is required. This process is called as **differential gene action** or **expression**. This control over gene expression is also essential so that the cell is not over flooded by synthesis of enzymes which are not required. Thus, synthesis of same enzymes may be induced at some time and repressed at another time depending upon the physiological requirement of the enzymes by the cell.

From the outgoing discussion it appears that a cell has auto control mediated by the gene itself. **Francois Jacob** and **Jacques Monad** (1961) first of all put forward a concept to explain this induction and repression of genes. The model proposed by them was named as ***operon model*** and is considered to be one of the leading biological discoveries. An operon is a cluster of functionally-related genes that are controlled by a shared operator. They consist of multiple genes grouped together with a **promoter** and an **operator**. Operons are present in prokaryotes (bacteria and archaea), but are absent in eukaryotes. In some situations multiple operons are controlled by the same regulatory protein; in these cases the operons form a **regulon**.

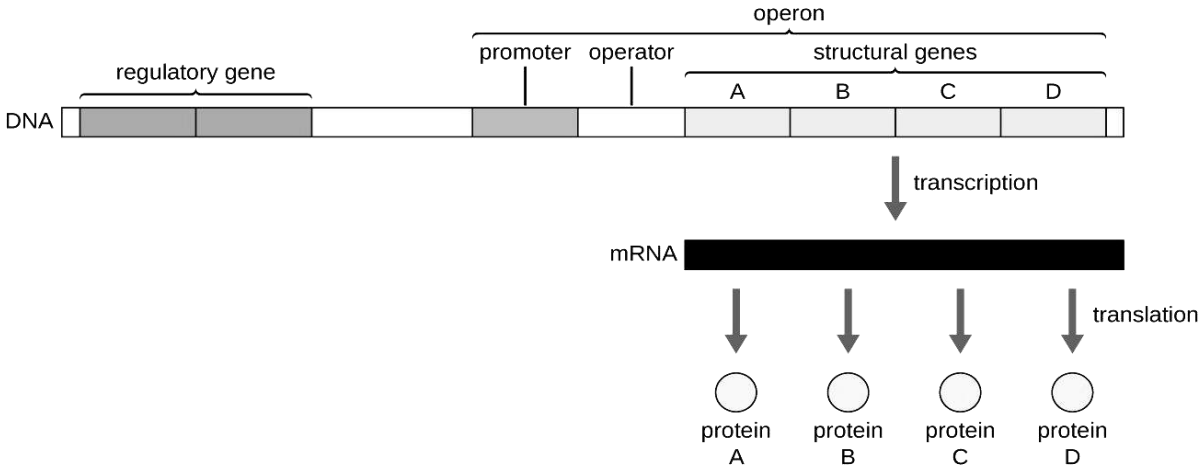

Fig.7.1 Structure of an Operon with their various components; Structural gene, Operator, Promoter and Regulatory gene

An operon can be defines as "*a set of structural genes whose transcription is controlled and regulated by coordinated action of regulator, operator, promoter*". A unit made up of linked genes which are thought to regulate other genes responsible for protein synthesis. An operon is a set of genes transcribed under the control of an operator gene. More specifically, an operon is a segment of DNA containing adjacent genes including structural genes, an operator gene, and a regulatory gene. An operon is thus a functional unit of transcription and genetic regulation. It can be said that operon consists of regulator gene, promoter gene, operator gene, structural gene.

Operons are characteristic feature of bacterial chromosome. They were first identified as a mode of gene expression control, by Jacob and Monod.

Operon = Regulator gene + Promoter gene + Operator gene + Structural genes

7.3.2.1 Components of an Operon

Genetic regulatory system found in bacteria and the viruses in which genes coding for functionally related proteins are clustered along the DNA. This feature allows protein synthesis to be controlled coordinately in response to the needs of the cell. By providing the means to produce proteins only when and where they are required, the operon allows the cell to conserve energy (which is an important part of an organism's life strategy). A typical operon consists of a group of structural genes, operator gene, and promoter gene.

1. Structural genes: Structural genes are those genes of an operon which when expressed leads to synthesis of cellular proteins. They are those genes which actually synthesize mRNAs. An mRNA controls metabolic activity of cytoplasm through the formation of protein or enzyme over the ribosomes. An operon has one or more structural genes.

Now if an operon contains many structural genes. There are two ways in which they may be controlled and expressed. In one way each structural genes may be controlled independently and transcribe separate mRNA molecule and in another method all structural genes are controlled or regulated in such a way that they lead to transcription (formation of mRNA from DNA) of single polycistronic or polygenic mRNA molecule.

Eukaryotes have only a single functional site for producing monogenic mRNA, whereas, in prokaryotes polygenic mRNAs are common. A small segment of DNA molecule in bacterium may have several structural genes which may transcribe one long polycistronic mRNA molecules. This controls the synthesis of many proteins. Formulation of polygenic mRNA molecule is quite common in bacteria.

The lactose or lac-operon of *Escherichia coli* contains three structural genes (Z, Y, A). They transcribe a polycistronic mRNA molecule that helps in the synthesis of three enzymes—**β-galactosidase** for hydrolyzing lactose or galactoside, lactose or **galactoside permease** for allowing entry of lactose from outside and **thiogalactoside acetylase** or **transacetylase** for metabolizing toxic thiogalactosides which are also allowed entry by lactose permease.

2. Operator gene: Operator gene is located adjacent to first structural gene. It determines whether the structural gene is to be repressed by the repressor protein (a product of regulator gene). It is a gene which directly controls the synthesis of mRNAs over the structural genes. Regulator gene is known to produce a small protein molecule called **repressor**. The operator gene is recognized by repressor protein which binds to the operator forming an operator-repressor complex and transcription (expression) of structural gene is inhibited.

It is switched off by the presence of a repressor. An inducer can take away the repressor and switch on the gene. The gene then directs the structural genes to transcribe. Operator gene of lac operon is made of only 27 base pairs. The operator genes as well as the group of genes that are under it are known as operons. The operator genes are carried in the chromosome and also show recombination.

3. Promoter gene: Promoter gene is continuous with the operator gene and is located just before operator (next to operon) on upstream side. Promoter region contains palindromic sequences i.e., region present on either side of a particular point are identical. It acts as an initiation signal which functions as recognition centre for RNA-polymerase provided the operator gene is switched on. RNA polymerase is bound to the promoter gene. When the operator gene is functional, the polymerase moves over it and reaches the structural genes to perform transcription.

The complete nucleotide sequence of control region in some bacteria extends from the termination codon (TGA on DNA or UGA on mRNA) of the structural gene and induces the promoter and operator genes. In 1975, **David Pribnow** proposed a model to recognize elements present in promoter. According to his model the promoter region has three essential elements, which are constant in position with respect to each other. These elements are:

(i) A recognition sequence,
(ii) A RNA-polymerase binding sequence, and
(iii) An mRNA initiation site.

The recognition site is located outside the polymerase binding site, i.e., the region of DNA protected from the action of DNase. RNA polymerase first binds to DNA by forming a complex with the recognition sequence. Then it binds with the binding sequence to produce the pre-initiation complex. The binding site consists of a group of 7 bases present in a constant position in the protected fragments. The mRNA initiation site starts the mRNA transcription on one or two bases near the binding sequence. It marks the point from where transcription starts. The starting sequence is located in the DNA fragment protected from DNase action.

4. Regulator Gene: As the name indicates regulator gene is the gene which regulates the synthesis of a protein, which may be either an active repressor or an inactive repressor (Aporepressor). In lac-operon, it is called **i -gene** because it produces an inhibitor or repressor. The repressor binds to operator gene and stops the working of the latter. It exerts a negative control over the working of structural genes. Repressor protein has one active site for operator recognition and the other active site for inducer. When inducer binds with repressor, it distorts the molecule so that operator site no longer recognizes the operator gene.

Active repressor protein has an affinity for the operator gene. In absence of an inducer protein the repressor binds to the operator gene and blocks the path of RNA polymerase. Thus the structural genes are unable to transcribe mRNA and consequently protein synthesis does not

occur. In presence of an inducer the repressor protein binds to the inducer to form an Inducer-repressor complex or co-repressor. The repressor when linked to an inducer undergoes a change which makes it ineffective and as a result it cannot bind to the operator gene and the protein synthesis is possible.

7.3.2.2 Control Mechanism

There are two proposed control mechanisms for synthesis of proteins or enzymes.

(a) Induction: There are several enzymes which are either absent or if present, they are in very less amount in the cell. However, their synthesis increases only in presence of an inducer molecule. Such enzymes are called as inducible enzymes and such system is called as **inducible system**. For e.g., **Lac operon** is a type of inducible system.

(b) Repression: It is a type of a system in which presence of a metabolic product inhibits synthesis of a particular enzyme. The substance which suppresses the synthesis of a protein is called as repressor and such system is called **repressible system**. For e.g., **Tryptophan operon** in *E.coli* is an example of Repression system.

Negative and positive control

(i) Negative control: As you have already seen that in both the control system inducible as well as repressible the structural genes are expressed only when the operator is free. But when operator is blocked by repressor protein, synthesis of proteins is inhibited. Such control mechanism for protein synthesis is called as **Negative type control system**. Lac operon model of *E.coli* is an example of negative control.

(ii) Positive control: Regulator protein produces repressor protein which binds to operator and blocks transcription and translation. However, in some cases regulator protein may act as an activator and enhances protein synthesis. The site at which activator attached are called **initiator site**.

Operator can also act as initiator site; however, it is not necessary that initiator site will always be the operator. Since, in the case where regulator protein enhances the synthesis of proteins, it is called Positive control system. **Arabinose operon** of *E.coli* is an example of positive control of gene regulation.

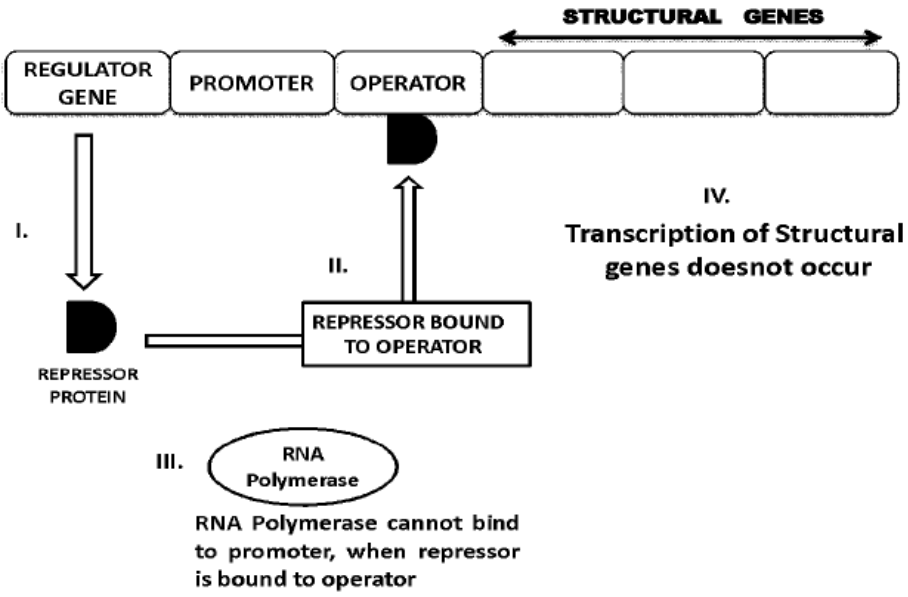

Fig 7.2 Effect of repressor on expression of structural genes in inducible system

When an inducer protein is present, it binds with repressor protein and an **inducer-repressor complex** is formed, as a result of this repressor protein fails to bind to operator. Now, RNA polymerase can catalyze normal transcription and mRNA from structural genes is formed which undergoes the process of translation and protein synthesis occurs. Each repressor molecule is believed to have one inducer binding site. Once bound to inducer, repressor protein undergoes a conformational change which renders (makes). The repressor protein to be inactive and it can no longer bind to operator (Fig. 7.3.2.2 -b).

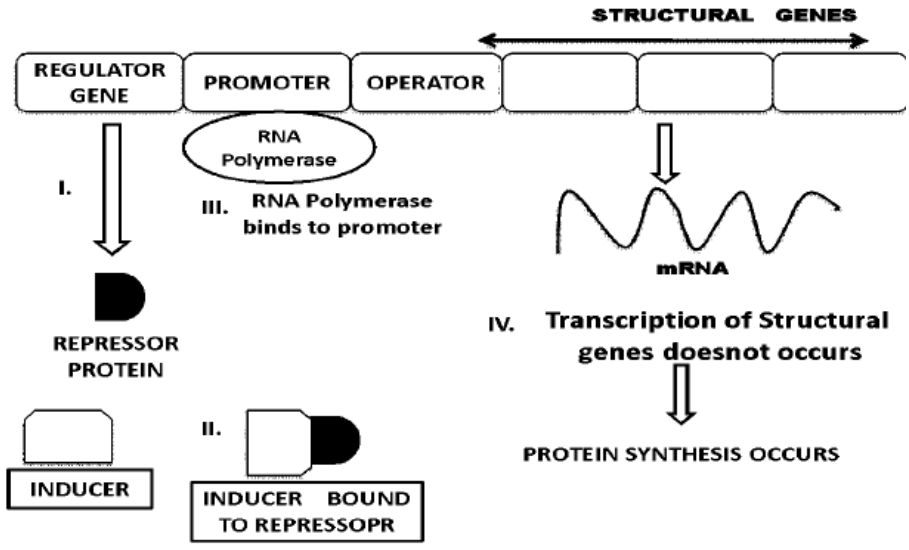

Fig 7.3 - Effect of inducer on repressor in inducible system

The mode of action of repressor protein is slightly different in repressible system. In this case the repressor protein formed by regulator gene is inactive and therefore cannot block the operator. Hence, if repressor protein is present alone in repressible system the transcription of structural genes is not blocked and normal transcription and protein synthesis from structural genes occurs (Fig. 7.4).

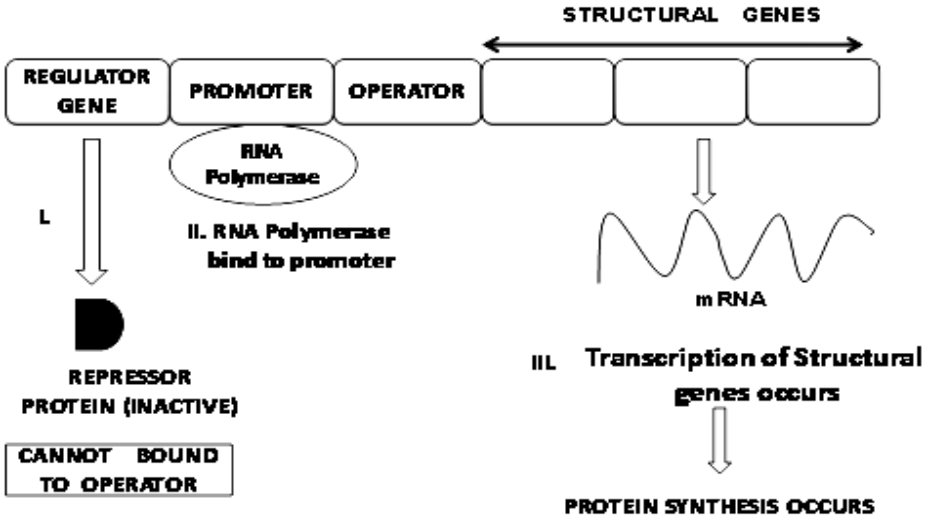

Fig 7.4 - Effect of repressor on expression of structural genes in repressible system

As already stated, that in repressible system the repressor protein is synthesized in inactive form. But this inactive repressor can be activated after binding to co-repressor. Hence, when corepressor is present, repressor is activated and it binds to operator, therefore, transcription and translation of structural genes is inhibited and no proteins are produced (Fig. 7.5).

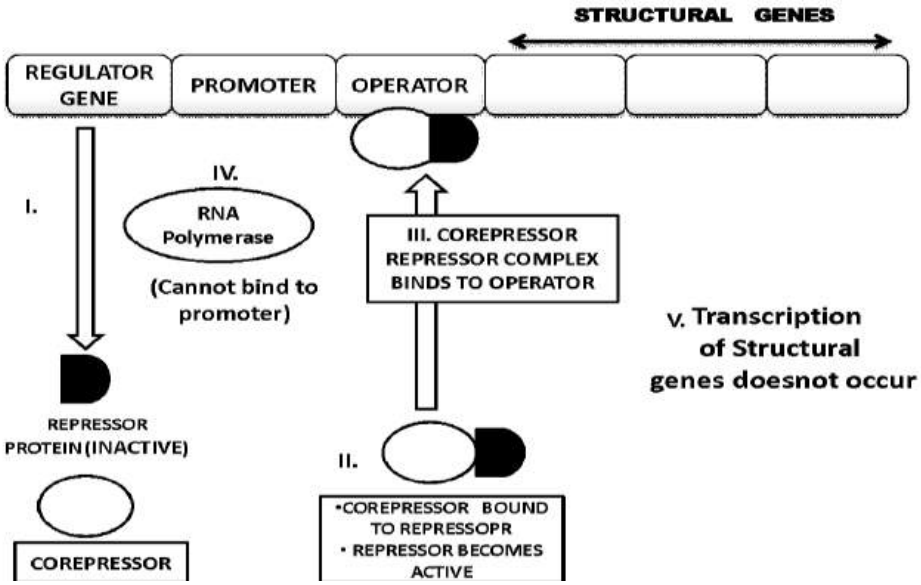

Fig 7.5 - Effect of co-repressor on repressor in repressible system

7.3.2.3 Lac Operon

Jacob and **Monod** (1961) gave the concept of operon model to explain the gene regulation in *E.coli.* Experiments on metabolization of glucose and lactose by *E.coli* were utilized in their study. The Lac operon is found to be present in *E. coli* and many other enteric bacteria. It is required for metabolism of lactose. It is a well known fact that glucose is the most preferred carbon source utilized by living organisms including bacteria. If a bacteria is cultured on a medium in which both glucose and lactose are present, bacteria will first of all metabolize (utilize) glucose as a carbon source.

When concentration of glucose will start decreasing (or when glucose will be completely utilized by bacteria) then bacteria will utilize lactose as a source of energy. Now to utilize lactose, bacteria will need specific enzyme called as **β-galactosidase**. Lac operon is the operon whose structural genes when expressed, it leads to production of enzyme β-galactosidase. Now it should be clear that β-galactosidase will be synthesized (from Lac operon) when lactose is present and glucose is absent.

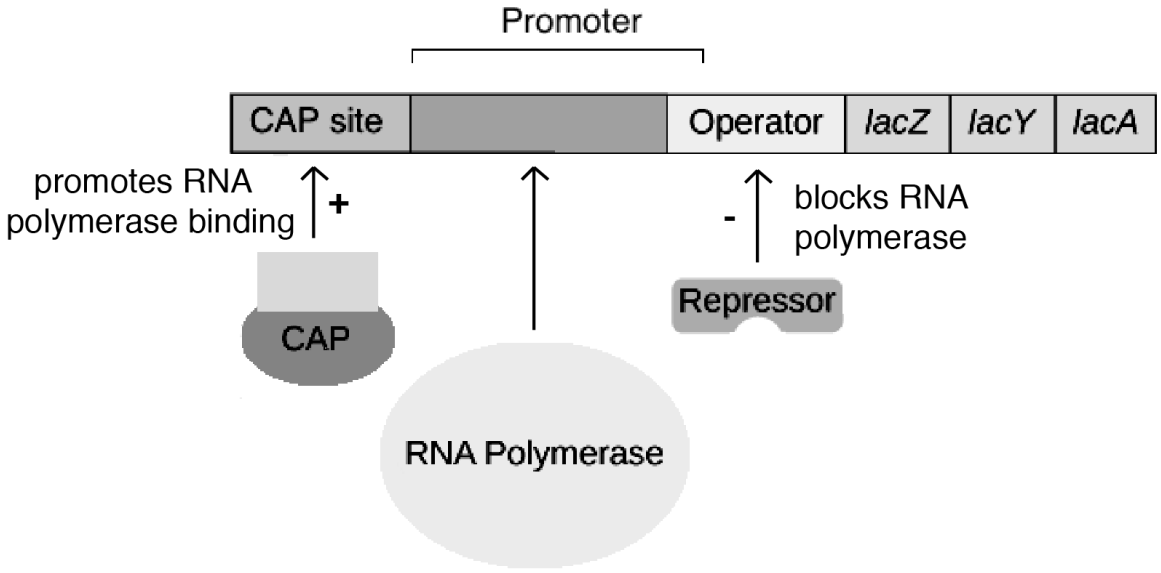

Fig. 7.6 - Organization of lac operon

Structure of the lac operon

The DNA of the lac operon contains (in order from left to right): CAP binding site, promoter (RNA polymerase binding site), operator (which overlaps with promoter), structural genes. There are three structural genes clustered together in Lac Operon, i.e. lacZ gene, lacY gene, and lacA gene Fig. 7.3.2.3-a. The activator protein CAP (catabolite activator protein), when bound to a molecule called **cAMP**, at the CAP binding site, then it promotes RNA polymerase binding to the promoter. The lac repressor protein binds to the operator and blocks RNA polymerase from binding to the promoter and transcribing the operon.

Table-1: Genetic components of lac operon

	Gene	Codes for
1.	Regulator (Lac I)	Repressor protein
2.	Operator	Binding site for regulatory proteins
3.	Promoter	Binding site for promoter
4.	Structural gene	
	(i) Lac Z	β-galactosidase
	(ii) Lac Y	β-galactoside Permease
	(iii)Lac A	β-galactoside transacetylase

Lac operon contain a regulator gene known as **'lac I'** which is present adjacent to structural gene and are responsible for regulation of expression of structural gene. Regulator gene which is responsible for the synthesis of protein, called as **repressor protein**. The repressor protein binds to specific DNA sequence (operator). When repressor protein binds to operator, the binding of **RNA polymerase** is prevented (we know that RNA polymerase is required for transcription of genes). Hence, there is no transcription of structural genes and synthesis of β-galactosidase is inhibited.

Therefore, the regulatory gene codes for the repressor of the lac operon. There are three structural genes present in Lac operon called as LacZ, LacY and LacA. **The LacZ** gene codes for β-galactosidase, which is primarily responsible for the hydrolysis of the disaccharide lactose into glucose and galactose (Fig.7.7). **LacY** codes for enzyme lactose permease, it is a protein which is required for transport of lactose into the cell through the cell membrane. LacA, the third structural gene codes for Galactoside O-acetyltransferase. Lac operon is an example of negatively regulator operon model and is under control of negative regulation.

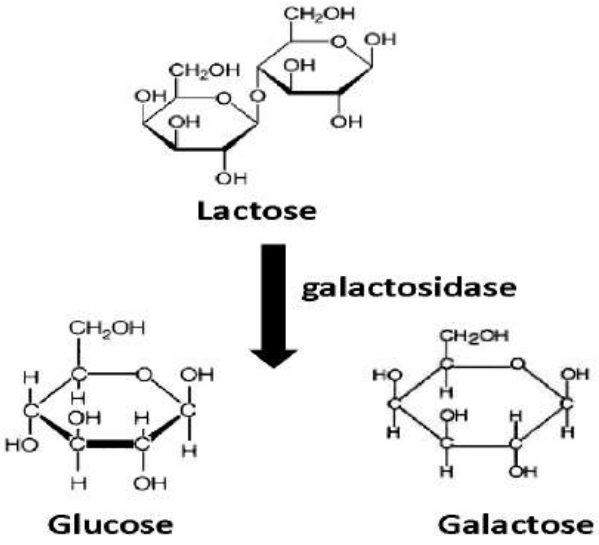

Fig. 7.7-Action of enzymes galactosidase in lac operon

A. When Lactose is absent in the cell

When a lactose is absent inside the cell, there is no need of expression of genes responsible for metabolism of lactose. Repressor protein is synthesized from regulator gene and repressor protein bind to operator. As a result, binding of RNA polymerase DNA is prevented and hence there is no transcription of genes responsible for lactose metabolism (Fig.7.8).

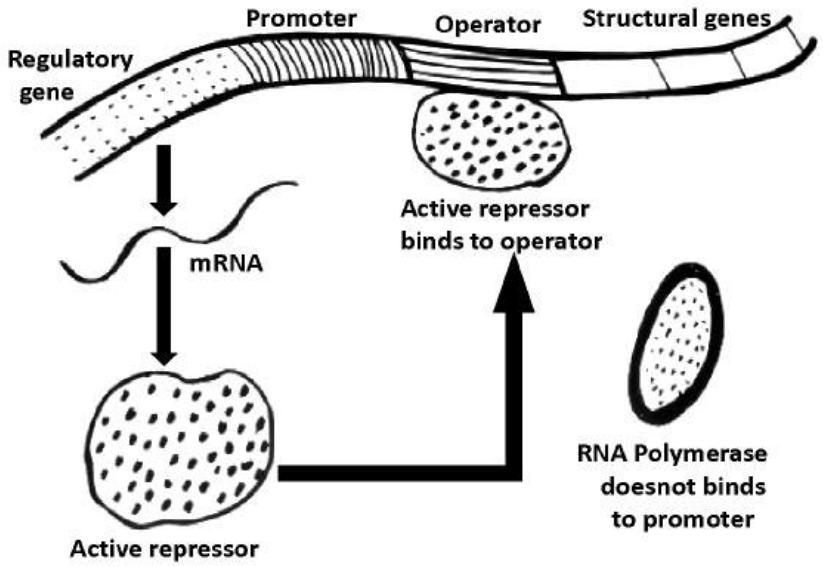

Fig. 7.8 - Action of repressor protein on lac operon in absence of Lactose

B. When Lactose is present in the cell

When lactose is present in the cell there is need of transcription of genes responsible for lactose metabolism. In this case, when regulator gene produces repressor protein, lactose molecule binds to repressor protein. Now, since repressor protein is bound to lactose, it cannot bind to operator. Hence, promoter site is free to be occupied by RNA polymerase, transcription of structural genes occurs and lactose is metabolized (Fig.7.9)

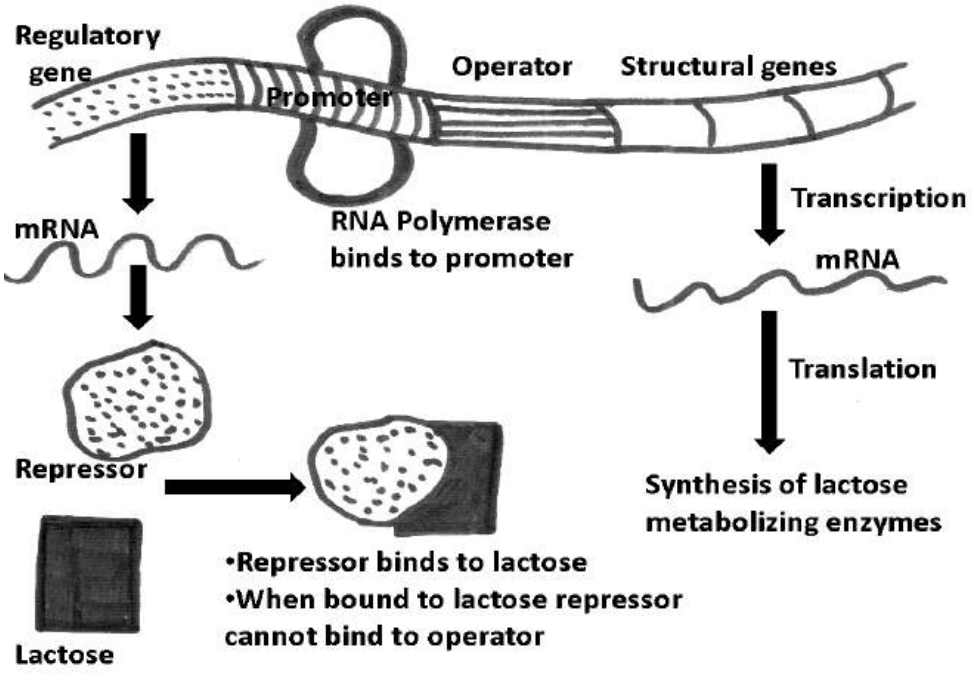

Fig. 7.9-Action of repressor protein on lac operon in presence of Lactose

Effect of cyclic AMP on Lac operon

CAP isn't always active (able to bind DNA). Instead, it's regulated by a small molecule called cyclic AMP (cAMP). The cAMP has been reported to have a positive control on lac operon. The cAMP is a "hunger signal" made by E. coli when glucose levels are low. **Catabolite activator protein** (CAP; also known as **cAMP Receptor Protein**, CRP) is a receptor protein found in *E.coli*, which binds to cAMP and forms a **CRP-cAMP complex**. This complex binds to promoter and facilitates binding of RNA polymerase to promoter.

The cAMP binds to CAP, changing its shape and making it able to bind DNA and promote transcription. Without cAMP, CAP cannot bind DNA and is inactive. Hence presence of cAMP helps in transcription of structural genes (Lac Z, Lac Y, Lac A) resulting in synthesis of enzymes required for lactose metabolism (Fig.7.10). CAP is only active when glucose levels are low (cAMP levels are high). Thus, the lac operon can only be transcribed at high levels when glucose is absent. This strategy ensures that bacteria only turn on the lac operon and start using lactose after they have used up the entire preferred energy source (glucose).

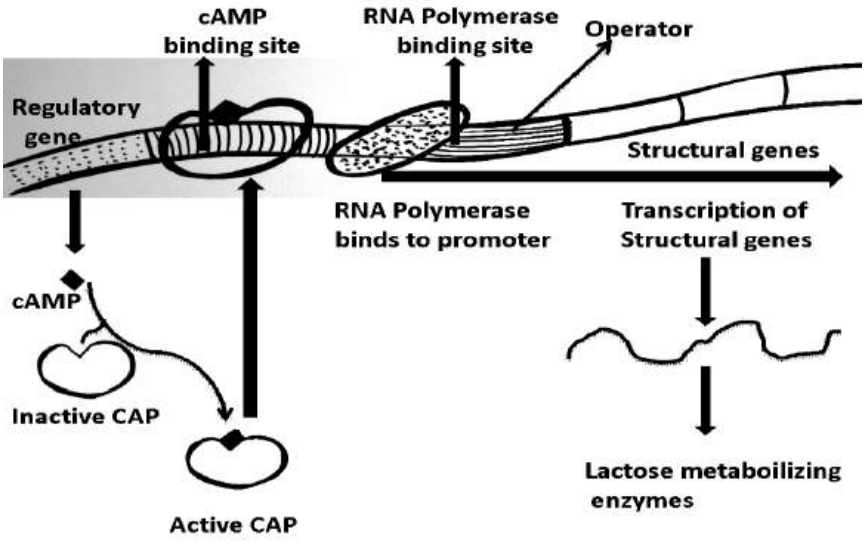

Fig. 7.10- Action of cAMP on lac operon

Catabolite repression

We have already seen that lac operon is under negative control. Beside this, it is also known that presence of glucose inhibits expression of lac operon. We have studied that when lactose is present, structural genes are synthesized (lac operon is on) but in presence of glucose, lac operon remains switched off whether lactose is present or not. This is known as **catabolite repression**.

Enzyme Induction is still considered a form of negative control because the effect of the regulatory molecule (the active repressor) is to decrease or down regulate the rate of transcription. Catabolite repression is a type of positive control of transcription, since a regulatory protein affects an increase (up regulation) in the rate of transcription of an operon.

The process was discovered in E. coli and was originally referred to as the "**glucose effect"** because it was found that glucose repressed the synthesis of certain inducible enzymes, even though the inducer of the pathway was present in the environment. The effect of the glucose catabolite is exerted on an important cellular constituent called **cyclic adenosine monophosphate** (cAMP).

When glucose is present in high concentrations, the cAMP concentration is low; as the glucose concentration decreases, the concentration of cAMP increases correspondingly. The high concentration of cAMP is necessary for activation of the lac operon. Mutants that cannot convert ATP into cAMP cannot be induced to produce β-galactosidase, because the concentration of cAMP is not great enough to activate the lac operon.

In addition, there are other mutants that do make cAMP but cannot activate the lac enzymes, because these mutants lack yet another protein, called **catabolite activator protein** (CAP), made by the ***crp* gene**. CAP forms a complex with cAMP, and it is this complex that is able to bind to the CAP site of the operon. The DNA-bound CAP is then able to interact physically with RNA polymerase and essentially increase the affinity of RNA polymerase for the lac promoter. In this way, the catabolite repression system contributes to the selective activation of the lac operon

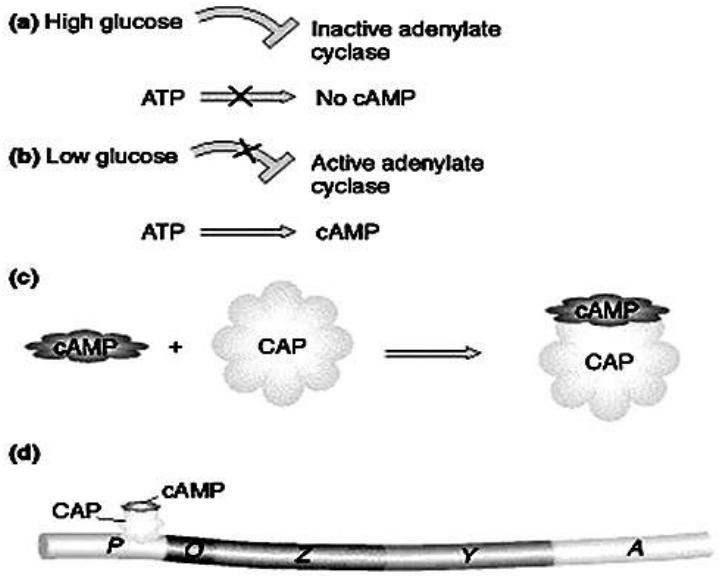

Fig: 7.11- Catabolite control of the lac operon

The operon is inducible by lactose to the maximal levels when cAMP and CAP form a complex. Under conditions of high glucose, a glucose breakdown product inhibits the enzyme adenylate cyclase, preventing the conversion of ATP into cAMP. Under conditions of low glucose, there is no breakdown product, and therefore adenylate cyclase is active and cAMP is formed. When cAMP is present, it acts as an allosteric effector, complexing with CAP. The cAMP-CAP

complex acts as an activator of lac operon transcription by binding to a region within the lac promoter.

7.3.2.4 Tryptophan Operon

Tryptophan is an aromatic amino acid, and is required for the synthesis of all proteins that contain tryptophan. If tryptophan is not present in the medium in adequate quantity, the bacterial cell has to make it, as it is required for the growth of the bacteria. It is known as repressible system. Also called a negative control system but forms a biosynthetic pathway. It works on the principle that when the amino acid tryptophan is present, there is no need to activate the tryptophan operon. The tryptophan operon of E. coli is depicted in Fig.7.12. This operon contains five **structural genes** (trpE, trpD, trpC, trpB, trpA), and the **regulatory elements;** primary promoter (trpP), operator (trpO), attenuator (trpA), Leader (trpL), and the terminator (trpt).

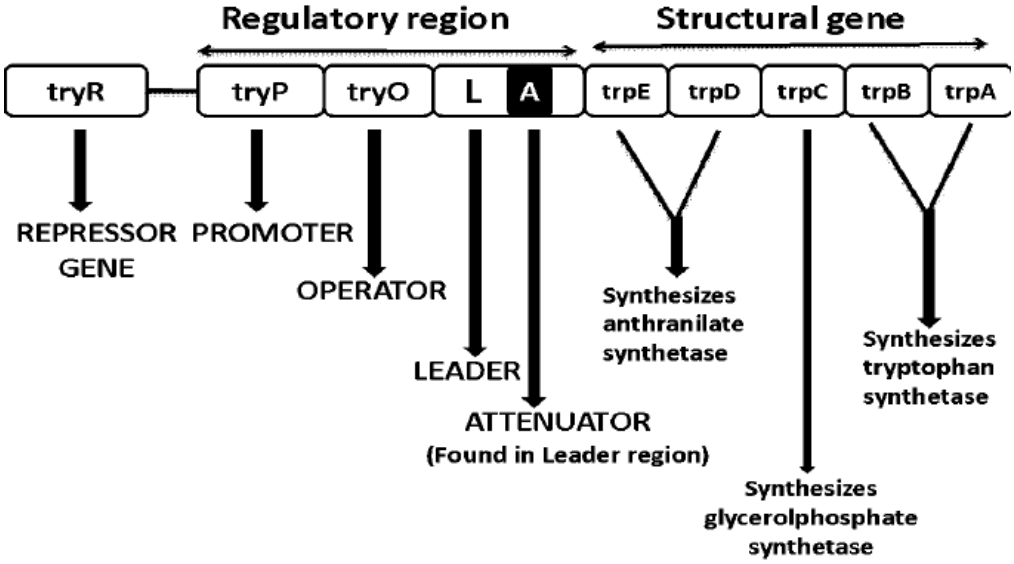

Fig.7.12-Organization of tryptophan operon

The five structural genes of tryptophan operon code for three enzymes (two enzymes contain two different subunits) required for the synthesis of tryptophan from chorismate. The tryptophan repressor is always turned on, unless it is repressed by a specific molecule called co-repressor. Thus lactose operon is inducible, whereas tryptophan operon is repressible. The tryptophan operon is said to be depressed when it is actively transcribed. The tryptophan operon is regulated by two processes:

1. By the help of repressor protein: When tryptophan is not present in the cell, repressor protein (produced in inactive form) is not able to bind to operator and there is no inhibitory effect of repressor protein. RNA polymerase binds to promoter, transcription of structural genes occur and all the three enzymes required for tryptophan synthesis occurs (Fig.7.13).

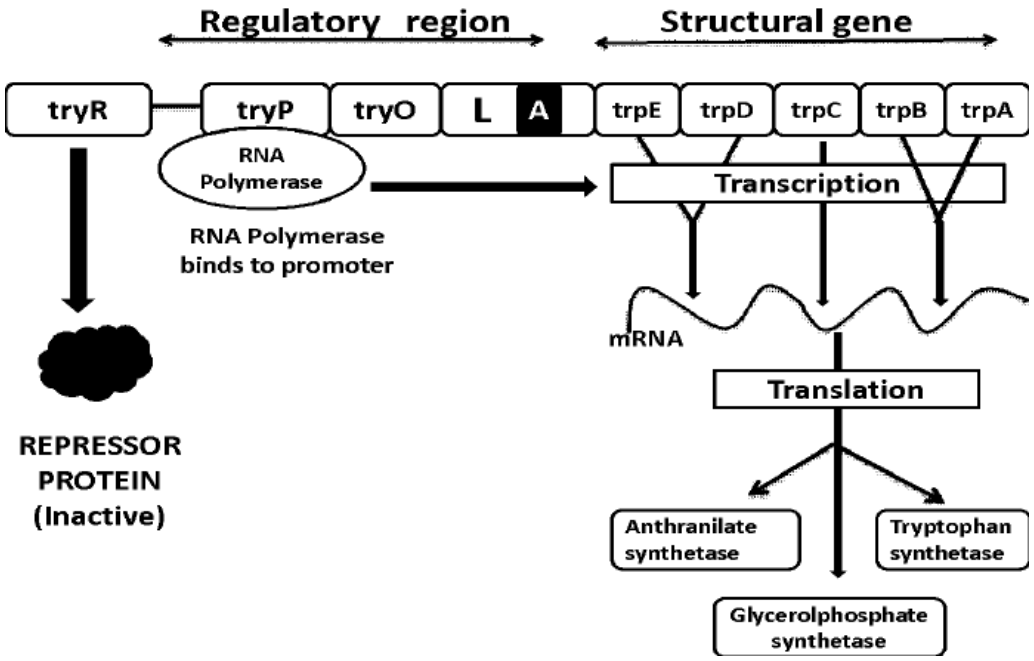

Fig.7.13- Regulation of try operon when tryptophan is absent

When tryptophan is present in the cell, try operon is negatively regulated, which means that repressor protein binds to tryptophan and gets activated. Active repressor protein binds to operator. This prevents binding of RNA polymerase to promoter and hence transcription of structural genes is blocked (Fig.7.14).

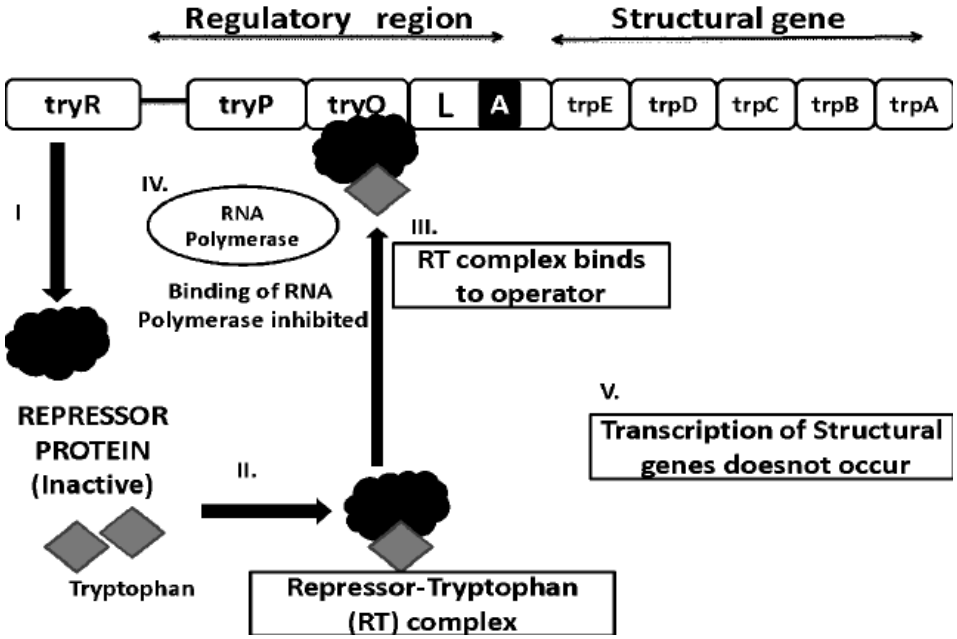

Fig. 7.14 - Regulation of try operon when tryptophan is present

2. By the process of attenuator: Attenuator is an intrinsic terminator present at the beginning of transcription unit. The phenomenon of attenuation controls the ability of RNA polymerase to read through attenuator, it depends on formation of loop or hair pin needed for termination (Fig. 7.15). If the tryptophan amino acid is not available the hair pin is not formed and transcription takes place, but if the tryptophan is present, hair pin forms and transcription stops before reaching structural genes. Leader sequence (trpL) play important role in attenuation. Leader sequence contains such a nucleotide sequence that mRNA transcribed from it contains four specific regions; Region 1, region 2, region 3 and Region 4. Region 3 is complementary to both region 2 and region 4. If region 3 and region 4 base pair with each other, they form a loop like structure called **attenuator** and it function as transcriptional termination.

If pairing occurs between region 3 and region 2, then no such attenuator form so that transcription continues. Region 1 is the most important region that determines whether to form loop between region 2-region 3 and region 3-region 4. The region 1 consists of sequence of 14 codons, out of which two codons are tryptophan codon (codon 10 and 11).

When tryptophan is high in cell than tRNA carrying tryptophan encodes codon 10 and 11. Such that ribosome encloses the region 2 which is near to the tryptophan codon. Hence region 3 base pair with region 4 to form attenuator as region 2 is not available for pairing. Consequently, transcription is halted. When tryptophan is low or absent in cell, then translation stops at the position of tryptophan codon.

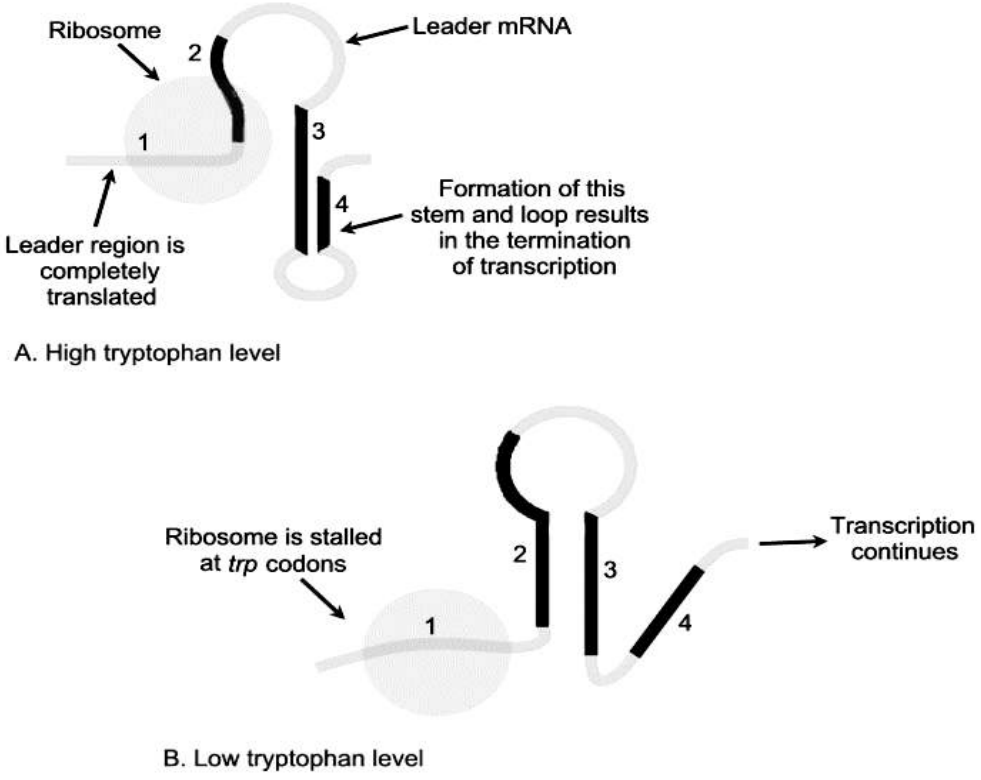

Fig. 7.15 - Attenuation of tryptophan operon

7.4 GENETIC CODE

The genetic code is the set of rules by which information encoded in genetic material (DNA or RNA sequences) is translated into proteins (amino acid sequences) by living cells. The genetic code, once thought to be identical in all forms of life, has been found to diverge slightly in certain organisms and in the mitochondria of some eukaryotes. Nevertheless, these differences are rare, and the genetic code is identical in almost all species, with the same codons specifying the same amino acids.

The genetic code consists of 64 triplets of nucleotides. These triplets are called codons. With three exceptions, each codon encodes for one of the 20 amino acids used in the synthesis of proteins. This produces some redundancy in the code; most of the amino acids being encoded by more than one codon.

7.4.1 Organization and Concept of Genetic Code

Arrangement of nitrogenous bases in DNA is said to determine by the sequence of amino acid in a protein molecule. Since, there are only 4 nitrogenous bases **Adenine** (A), **Cytosine** (C), **Guanine** (G) and **Thymine** (T). It is obvious that sequence of these four nitrogenous bases on DNA stand directs the synthesis of proteins.

Different theories have been proposed to predict the mechanism through which sequence of nitrogenous bases present in DNA transcribed into mRNA, which eventually determines the position of specific amino acids in protein. Theory proposed by **F.H.C Crick** remains widely accepted. The theory explains existence of genetic code and its smallest unit codon which codes for one amino acid. A codon is defined as nucleotide sequence in mRNA which codes for particular amino acid.

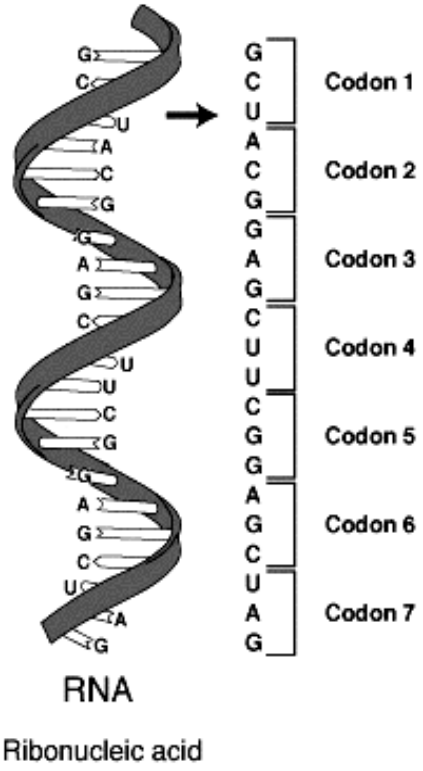

Fig.7.16-A series of codons within a part of an mRNA molecule

When it became clear that codon is a sequence of nucleotide which codes for amino acid, the next question arise was how many nucleotides are present in one codon i.e., it has to be determined the exact number of nucleotides in a codon which codes for an amino acid. Since, there are 20 amino acids and four nitrogenous bases in mRNA. Hence, there was requirement of sufficient number of codons which could code for 20 amino acids. If you consider that a codon consists of 1 nucleotide i.e., singlet code. Now in this case, since we have 4 nitrogenous bases the total

number of codon becomes 4, but how can four codons code for 20 amino acids. Hence, it was clear that each codon consists of more than one nucleotide. Singlet Code = 4x1 = 4 Codons (how can 4 codons code for 20 amino acids).

If we consider that a codon consists of 2 nucleotide each i.e., doublet code for each amino acid. Now, in this case the total number of codons becomes 16. Still the problem was not solved because by considering a codon to be a set of two nucleotides only 16 possible codons could be figured out and the amino acids were still 20. Doublet Code = 4x4 = 16 codons. Hence, it become clear that codon with one or two nitrogenous bases could not provide sufficient number of codon combinations to code for 20 known amino acids.

Gamow (1954) proposed possibility of a three letter code i.e., each codon consisting of three nitrogenous bases (Triplet code). Now, in this case the total possible combination of codon becomes 4x4x4= 64 (Table 2)

Since there are only 20 amino acids, hence, 64 codons are more than enough to code for amino acids. Here you should also note that now total no. of codons becomes 64 and amino acids for which they will code is 20. Hence, several different codons will code for same amino acids. Hence, genetic code is a nucleotide base sequence consisting of codons, each codon made up of three nitrogenous bases. Genetic code is translated into a sequence of amino acids which combine to form proteins.

Table-2: Composition of different codons comprising genetic code

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

Contribution of H.G. Khorana

Har Gobind Khorana(9 Jan 1922- 9 Nov 2011)

Dr. H.G. Khorana, was an Indian American biochemist, developed a technique for artificially synthesizing mRNA having repeated sequences of known nucleotides. Khorana was one of the first scientists to demonstrate the role of nucleotides in protein synthesis and helped crack the genetic code. He was awarded Noble prize in 1968 for his valuable contribution in Physiology or Medicine with **M. W. Nirenberg** and **R.W. Holley**. This they were awarded for their elucidation of the genetic code and its function in protein synthesis.

Khorana's work confirmed Nirenberg's finding that the chemical composition and function of a new cell is determined by how the four nucleotides are arranged on the spiral 'staircase' of a DNA molecule. He also demonstrated that the nucleotide code is always transmitted in groups of three, called codons, and that these codons instruct the cell to start and stop the production of proteins.

Khorana and his coworkers developed chains of polyribonucleotide (By using synthetic DNA) with repeated sequence of 2 or 3 nucleotides. One of the developed polyribonucleotide chain poly CUC UCU CUC UCU…, consists of two codons CUC and UCU codes for leucine & serine, hence, this synthesized polynucleotide chain codes for leucine – serine - leucine – serine.

Another polynucleotide chain synthesized was poly CUA, CUA, CUA, CUA, and CUA....., which is an example of homopolymer (chain of nucleotide consisting of repeating units of same codon). Since, CUA codes for leucine this polynucleotide chain codes for leucine-leucine-leucine-----leucine.

7.4.2 Types of Codon

(a) Sense Codon: Those codons that code for amino acids are called sense codons. There are 61 sense codons in the genetic code which code for 20 amino acids.

(b) Signal Codons: Those codons that code for signals during protein synthesis are known as signal codons. There are four codons which code for signal. These are AUG, UAA, UAG and UGA.

Signal codons are of two types:

1. Start codons, and
2. Stop codons.

1. Start Codon (Chain Initiation Codons): Codon with nucleotide sequence "AUG" is called as start codon. It codes for amino acid methionine in most organisms. The process of translation (protein synthesis) always begins with expression of start codon AUG.

However, codon AUG can occur later in mRNA also, then it will simply code for amino acid methionine. The triplets AUG and GUG, plays double roles in E. coli. When they occur in

between the two ends of a cistron (intermediate position), they code for the amino acids methionine and valine, respectively in an intermediate position in the protein molecule.

2. Stop Codon (Chain Termination Codons): Out of total 64 genetic codes, the 3 triplets UAA, UAG, UGA do not code for any amino acid. They were originally described as non-sense codons, as against the remaining 61 codons, which are termed as sense codons. During the process of protein Synthesis these codons function to terminate the process.

When any of the three codon is read, the ribosomes pauses and gets separated from mRNA and hence the process of protein synthesis is terminated. Due to this reason these codons are also called as termination codons. UAA, UAG and UGA are also known as ochre, Amber and Umber respectively, are believed to be used as signals which end the synthesis of a protein chain.

7.4.3 Characteristics of Genetic Code

1. Triplet nature: Each codon (genetic code) is made up of three nitrogenous bases hence the genetic code is a triplet codon. A triplet code could make a genetic code for 64 different combinations (4x4x4) genetic code and provide plenty of information in the DNA molecule to specify the placement of all 20 amino acids. When experiments were performed to crack the genetic code it was found to be a code that was triplet. These three letter codes of nucleotides (AUG, AAA, etc.) are called codons.

2. Non-overlapping: Non-overlapping codons means that each nitrogenous base in mRNA which be used for one codon only. One nitrogenous base cannot be a part of two codons or the same letter is not used for two different codons. In other words, no single base can take part in the formation of more than one codon. Hence genetic code is non-overlapping, i.e., the adjacent codons do not overlap.

3. Commaless code: A commaless code means that no nucleotide or comma (or punctuation) is present in between two codons. There is no signal to indicate the end of one codon and the beginning of the next. Therefore, code is continuous and commaless and no letter is wasted between two words or codons. Thus, the genetic code is commaless (or comma-free).

4. Non-ambiguity: A particular codon will always code for the same amino acid, i.e. genetic code is specific for e.g. UUU codes for amino acid Phenylalanine, it cannot code for any other amino acid. While the same amino acid can be coded by more than one codon (the code is degenerate), the same codon shall not code for two or more different amino acids. This property of genetic code makes them non ambiguous. However, there are some exceptions.

- AUG and GUG both may code for methionine although GUG codes for valine.
- GGA is another codon which codes for two amino acid glycine and glutamic acid.

5. Universality: Although the code is based on work conducted on the bacterium Escherichia coli but it is valid for other organisms. This important characteristic of the genetic code is called its universality. It means that the same sequences of 3 bases encode the same amino acids in all life forms from simple microorganisms to complex, multi-celled organisms such as human beings. All types of living organisms use same type of genetic code.

This means that codons specifying 20 amino acids are same in bacteria, fungi, plant and animal. Occurrence of similar genetic codes in diverse organisms indicates common origin of life on earth. However there are very few exceptions where same codon codes for different amino acid. Most prominent exception to universality of genetic code is seen in animal mitochondrial DNA where UGA codes for tryptophan however normally **UGA** is a stop codon.

Similarly in animal mitochondrial DNA, codon AGA and AGG are used as stop codons which are different from normal stop codons. As compared to animal mitochondrial DNA plant mitochondrial DNA utilizes universal (normal) gene code pattern. In Yeast (*Saccharomyces cerevisiae*), all codons begins with CU code for amino acid Threonine, whereas, in other organisms it codes for leucine.

Table-3: Enlists Exceptions to universal genetic code

DNA (Organism)	Genetic code	Universally codes for	Altered expression
Human Mitochondrial DNA	UGA	Stop	Tryptophan
	AUA	Isoleucine	Methionine
All vertebrate Mitochondrial DNA	AGA	Arginine	Stop
	AGG		
Yeast (*Saccharomyces cerevisiae*)	UGA	Stop	Tryptophan
	CUA	Leucine	Threonine
	CUG		
	CUU		
	CUA		
Plants	CGG	Arginine	Tryptophan
Nuclear DNA (*Paramecium)*	UAG	Stop	Glycine
Bacterial DNA (*Mycoplasma)*	UGA	Stop	Tryptophan

6. Degeneracy: The code is degenerate which means that the same amino acid is coded by more than one base triplet. Genetic code is degenerate i.e., more than one codon can code for same amino acid. For example, the three amino acids arginine, alanine and leucine each have six synonymous codons. There are a total of 64 codons and 20 known amino acids.

Hence, if you are asked to assign one amino acid to each codon then it is obvious that same amino acids will be assigned too many amino acids. Hence a single amino acid can be coded by many codons except tryptophan and methionine which are coded by one codon each. Degeneracy of genetic code is also known as **redundancy**.

Degeneracy of genetic code can be two types-

(a) Partial degeneracy: when the first two nitrogenous bases are same but the third base is different, the degeneracy is called partial degeneracy, e.g. CUU and CUC (codes for amino acid leucine).

(b) Complete degeneracy: complete degeneracy occurs when the third position in the genetic code can be taken by any of the four bases and the codon in each case codes for same amino acid. i.e., UCU, UCA, UCC, UCG (codons codes for serine).

Significance of degeneracy of genetic code

(i) It minimizes occurrence of lethal mutation. Mutation is inheritable change that occurs in DNA sequence. Lethal mutation leads to death of the organism.

(ii) Due to degeneracy of genetic code, same type of proteins and enzymes may be produced by microorganism which may have wide variation in their genetic code.

7. Genetic code has a polarity: The genetic code has polarity, that is, the code is always read in a fixed direction, i.e., in the 5′ → 3′ direction. If the genetic code is read in opposite or reverse direction different amino acid will be coded leading to formation of different protein.

8. Co-linearity: DNA is a linear polynucleotide chain and a protein is a linear polypeptide chain. The sequence of amino acids in a polypeptide chain corresponds to the sequence of nucleotide bases in the gene (DNA) that code for it. Change in a specific codon in DNA produces a change of amino acid in the corresponding position in the polypeptide. The gene and the polypeptide it codes for are said to be co-linear.

7.5 SUMMARY

1. Gene is unit of inheritance.
2. Work done by **J. Mendel** and **T.H. Morgan** basically belongs to classical genetics or era of classical concept of gene. Research done in the field of molecular biology and genetics after 1970's comprises modern genetics.
3. Nitrogenous bases in DNA are arranged in set of codons. Each codon is made up of three nitrogenous bases hence it is called as a **triplet**. Each codon codes for a particular amino acid.
4. There are a total of 64 codons which code for 20 amino acids. Genetic code is translated into a sequence of amino acids which combine to form proteins. Out of 64 codons **AUG** is known

as **start codon** or initiation codon. Process of protein synthesis becomes with expression of start codon.

5. Three codons UAA, UAG and UGA are known as termination or **stop codons**. Stop codons do not code for any amino acid. UAA is also known as **Ochre**, UAG as Amber and UGA as Opal.
6. Genetic code is non-overlapping. It means that one nitrogenous base cannot be a part of two codons. Genetic code is **commaless** there is no punctuation (Such as full stop, comma, colon, semicolon) between two codons.
7. Genetic code is **non-ambiguous**, each codon codes for a specific amino acid. Genetic code is universal i.e., same genetic codes (coding for specific amino acids) are found in all the living organisms.
8. There is little exception to universal nature of genetic code. UGA, AUA, AGC Codons of yeast; UGA, AUA of human mitochondrial DNA code for amino acid different from usual pattern (amino acid coded by these codons in other organisms).
9. Genetic code exhibits **degeneracy** it means that same amino acid can be coded by more than one codon. Degeneracy of genetic code can be partial or complete. Degeneracy of genetic code is important as it minimizes chances of lethal mutation. Genetic code is always read in **5'-3' direction**.
10. Expression of gene is strictly regulated and controlled. Only those genes are expressed in a cell a given time whose product (proteins) are required. At any given time some genes are switched on and some are switched off depending upon physiological requirement of the cell.
11. **Operon** is set of structural genes whose expression is controlled and regulated by other genes (regulator, promoter and operator).
12. Expression of structural genes results in synthesis of enzymes or protein coded by the genes.
13. **Operator gene** regulates the expression of structural gene. If a repressor protein is bound to operator. RNA polymerase does not bind to promoter and transcription of structural genes is blocked. If operator is free RNA polymerase binds to promoter and transcription of structural genes is carried out.
14. **Promoter** is a segment or gene to which RNA polymerase binds to start the process of transcription.
15. **Pribnow** (1975) identified three different elements present in promoter namely; Recognition sequences, Binding site and mRNA initiation site.
16. Regulator gene synthesizes repressor protein, it binds to operator and binding of RNA polymerase is prevented, hence, there is no transcription of structural genes.
17. When an **inducer protein** is present, it binds to with repressor protein and an **inducer-repressor complex** is formed, as a result of this repressor protein fails to bind to operator.
18. In **repressible system** the repressor protein formed by regulator gene is inactive and hence cannot block the operator. In repressible system, inactive repressor can be activated after binding to co-repressor.

19. **Jacob** and **Monod** (1961) gave the concept of operon model to explain the gene regulation in *E.coli*
20. **Lac operon** consists or regulator, operator, promoter and three structural genes. The three structural genes present in Lac operon called as LacZ, LacY and LacA. The **LacZ gene** codes for β-galactosidase, **LacY gene** codes for enzyme lactose permease, and **LacA gene** codes for galactoside O-acetyltransferase.
21. When a lactose is absent inside the cell, there is no need of expression of genes responsible for metabolism of lactose. When lactose is present in the cell there is need of transcription of genes responsible for lactose metabolism
22. Presence of glucose inhibits expression of lac operon. Presence of **cAMP induces** expression of lac operon.
23. The **tryptophan operon** in *E. coli* controls production or synthesis of amino acid tryptophan.
24. Tryptophan operon is formed by set up of three enzymes which are coded by five different genes; trp E, trp D, trp C, trp B, and trp A, which encode tryptophan synthetase.
25. Structural genes *E* and *D* produce the enzyme anthranilate synthetase. Structural gene C produces enzyme indole glycerolphosphate synthetase. Structural genes B and A synthesizes enzyme tryptophan synthetase.
26. The tryptophan operon is negatively regulated, which means that it is inhibited by repressor protein. The tryptophan operon is also regulated by attenuator, an intrinsic terminator present at the beginning of transcription unit. It depends on formation of hair pin needed for termination.
27. If the tryptophan amino acid is not available the hair pin is not formed and transcription takes place but if the amino acid is present hair pin forms and transcription stops before reaching structural genes.
28. The **genetic cod**e is the set of rules by which information encoded in genetic material is translated into proteins by living cells.
29. The genetic code consists of 64 triplets of nucleotides. These triplets are called codons. With three exceptions, each codon encodes for one of the 20 amino acids used in the synthesis of proteins.

7.6 GLOSSARY

Allele: Each of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome.

Ambiguous: Having more than one direction, development, or interpretation or meaning.

Aporepressor: A regulatory protein that, when combined with another corepressor, undergoes allosteric transformation, which allows it to combine with an operator locus and inhibit transcription of certain genes.

Attenuation: The regulation of transcription termination by interfering with mRNA elongation, a process restricted to prokaryotes. It requires coupled transcription and translation.

Catabolite repression: The decreased expression of an operon because of elevated levels of a catabolite of a biochemical pathway.

Central Dogma: The central dogma of molecular biology explains the flow of genetic information, from DNA to RNA, finally to a protein.

Cistron: The smallest unit of genetic material that must be intact to function as a transmitter of genetic information; as traditionally construed, approximately synonymous with gene.

Codon: A series of three adjacent bases in one polynucleotide chain of a DNA or RNA molecule, which codes for a specific amino acid.

Corepressor: In the field of molecular biology, a corepressor is a substance that inhibits the expression of genes. In prokaryotes, corepressor is small molecules whereas in eukaryotes, corepressor is proteins.

Degeneracy: The fact that several different triplet codons encode the same amino acid.

Genetic code: The set of DNA and RNA sequences that determine the amino acid sequences used in the synthesis of an organism's proteins. It is the biochemical basis of heredity and nearly universal in all organisms.

Genetics: The branch of biology that deals with heredity, especially the mechanisms of hereditary transmission and the variation of inherited characteristics among similar or related organisms.

Heredity: Heredity is genetic information passing for traits from parents to their offspring.

Inducer: In molecular biology, an inducer is a molecule that regulates gene expression. An inducer can bind to protein repressors or activators.

Lethal mutation: A mutant trait that leads to a phenotype incapable of effective reproduction. Lethal mutations will often result in the death of the organism concerned.

Linkage: Genetic linkage is the tendency of alleles that are close together on a chromosome to be inherited together during the meiosis phase of sexual reproduction.

Locus: A locus (loci), in genetics, is the specific location or position of a gene's DNA sequence, on a chromosome.

Meiosis: Meiosis is a process where a single cell divides twice to produce four cells containing half the original amount of genetic information.

Monocistronic mRNA: An mRNA molecule is said to be monocistronic when it contains the genetic information to translate only a single protein chain.

Mutation: the changing of the structure of a gene, resulting in a variant form which may be transmitted to subsequent generations

Muton: In genetics, the smallest unit of a chromosome in which alteration can be effective in causing a mutation (a single nucleotide change).

Nucleotide: a compound consisting of a nucleoside linked to a phosphate group. Nucleotides form the basic structural unit of nucleic acids such as DNA

Operator: An operator is a segment of DNA to which a transcription factor binds to regulate gene expression

Operon: A segment of a chromosome comprising an operator gene and closely linked structural genes having related functions, the activity of the latter being controlled by the operator gene through its interaction with a regulator gene.

Palindromic sequence : A palindromic sequence is a sequence made up of nucleic acids within double helix of DNA and/or RNA that is the same when read from 5' to 3' on one strand and 3' to 5' on the other, complementary, strand. It is also known as a palindrome sequence.

Phenotype: the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.

Polycistronic mRNA: Polycistronic mRNA is a mRNA that encodes several proteins and is characteristic of many bacterial and chloroplast mRNAs.

Polygenic: Pertaining to or determined by several different genes.

Promoter: a promoter is a region of DNA that initiates transcription of a particular gene. Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA

Recombination: the rearrangement of genetic material, especially by crossing over in chromosomes or by the artificial joining of segments of DNA from different organisms.

Recons: They are locations within a gene which participate in recombination. There is a minimum distance between recons within a gene, and recombination cannot occur within a recon.

Regulon: A regulon is a group of genes that are regulated as a unit, generally controlled by the same regulatory gene that expresses a protein acting as a repressor or activator.

Repressor: substance which acts on an operon to inhibit enzyme synthesis.

RNA polymerase: Also known as DNA-dependent RNA polymerase is an enzyme that produces primary transcript RNA. In cells, RNA is necessary for constructing RNA chains using DNA genes as templates, a process called transcription.

Split gene: A eukaryotic gene in which the coding sequence is divided into two or more exons that are interrupted by a number of non-coding intervening sequences (introns).

Transposable element: A DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genetic identity and genome size.

7.7 SELF ASSESSMENT QUESTIONS

7.7.1 Multiple choice questions:

1-Degeneracy of genetic code is due to:

(a) First member of codons
(b) Second member of codons
(c) Third member of codons
(d) Entire codons

2-Which one is not applicable in respect of genetic code?
(a) Redundancy (b) Overlapping
(c) Degeneracy (d) Universality

3-The known number of amino acids used in protein synthesis is?
(a) 20 (b) 35
(c) More than 50 (d) More than 100

4-Ribosomes are the sites of?
(a) Respiration (b) Fat metabolism
(c) Protein Synthesis (d) Photosynthesis

5-The process by which mRNA is made by DNA and protein by mRNA are respectively called?
(a) Translation and Transcription (b) Transcription and Translation
(c) Synthesis of mRNA and protein (d) Replication of mRNA and protein

6-Amino acid sequence in protein synthesis is decided by:
(a) tRNA (b) sRNA
(c) mRNA (d) None of these

7-The codon causing chain termination is?
(a) UAG, UGA, UAA (b) GAT, AAT, AGT
(c) AGT, TAG, VGA (d) TAG, TAA, TGA

8-Tryptophan operon is regulated by
(a) Repressor protein only (b) Attenuator only
(c) Repressor protein and attenuator both (d) None of the above

9-The polypeptide chain is initiated by:
(a) Lysine (b) Glycine
(c) Methionine (d) Leucine

10-Work done by Mendel belongs to:
(a) Neoclassical genetics (b) Modern genetics
(c) Ultra modern genetics (d) Classical genetics

11-The genetic code translates the language of:
(a) RNA into proteins (b) Protein into DNA
(c) RNA into DNA (d) Amino acid into RNA

12-Which of the following is not produced by *E.coli* in the lac operon?
(a) Beta galactosidase (b) Transacetylase
(c) Lactose dehydrogenase (d) Lactose permease

13-The lac operon is an example of:
(a) Arabinose operon (b) Inducible operon
(c) Repressible operon (d) Overlapping genes

14-In the inducible operon, regulator gene release-
(a) Interferon (b) Co-repressor
(c) Repressor (d) Apo-repressor

15- Which of the following conditions leads to maximal expression of the lac operon?
(a) Lactose present, glucose absent (b) Lactose present, glucose present
(c) Lactose absent, glucose absent (d) Lactose absent, glucose present

16- Which of the following is a type of regulation of gene expression unique to eukaryotes?
(a) Attenuation (b) Use of alternate σ factor
(c) Alarmones (d) Chemical modification of histones

17- Lactose operon of Escherichia coli is-
(a) Monocistronic (b) Inducible
(c) Repressible (d) Both B and C

18- There are 64 types of codons in genetic code dictionary because?
(a) There are 64 types of tRNAs found in cell
(b) There are 64 amino acids for coding
(c) Genetic code is triplet
(d) There are 44 meaningless and 20 codons for amino acids

19- Genetic code consists of
(a) 4 codons, each with two nucleotides (b) 16 codons, each with four nucleotides
(c) 64 codons, each with two nucleotides (d) 64 codons, each with three nucleotides

20 -Who first discovered genetic code
(a) Khorana (b) Nirenberg and Mathaei
(c) Kornberg (d) Phil Lader and Barnett

21- Operon model for gene regulation in bacteria was proposed by
(a) Jacob and Monad (b) Barry Commoner

(c) Crick (d) Watson and Crick

22-Which is not true for Operon Model?
(a) Regulator gene (b) Promoter gene
(c) Repressor gene (d) Operator gene

23-Which of the following bases is absent in the coding dictionary
(a) Thymine (b) Uracil
(c) Cytosine (d) Adenine

24-When more than one codon code for the same amino acid, this is called as?
(a) Universal nature of genetic code (b) Redundancy of genetic code
(c) Punctuation in genetic code (d) Continuous nature of genetic code

25-In Operon concept, regulator gene functions as-
(a) Repressor (b) Regulator
(c) Inhibitor (d) All of these

26-Genes that are involved in turning on or off the transcription of a set of structural genes are called?
(a) Polymorphic genes (b) Operator genes
(c) Redundant genes (d) Regulatory genes

27-Functioning of structural genes is controlled by-
(a) Operator (b) Promoter
(c) Ligase (d) Regulatory gene

28-Wobble hypothesis was given by-
(a) R. W. Holley (b) H. G. Khorana
(c) M. Nirenberg (d) F. H. C. Crick

29-Which one of the following codons codes for the same information as UGC?
(a) UGU (b) UGA
(c) UAG (d) UGG

30- In the lactose operon of Escherichia coli, what is the function of promoter?
(a) Binding of Gyrase enzyme (b) Binding of RNA polymerase
(c) Codes for RNA polymerase (d) Processing of messenger RNA

7.7.2 Fill up the blanks:

1. The term gene was coined by _____.
2. There are a total of _____codons out of which _____ codes for amino acid
3. _____, _____ and _____ are stop codons.
4. Each codon comprises of three_____.
5. GGA codes for _____ and _____
6. Codons which specify same amino acid are called as _____.
7. UUU codes for _____ amino acid.
8. The smallest unit of mutation is called_____.
9. _____ of genetic code can be partial or incomplete.
10. _____operon is an example of positive gene regulation.
11. Concept of operon was given by _____.
12. The trp-operon is switched off in presence of _____level of tryptophan.
13. Enzyme galactosidase splits lactose into _____and_____.
14. The DNA sequence, to which repressors may bind, that lies between the promoter and the first structural gene is called the _____.
15. The prevention of expression of operons encoding substrate use pathways for substrates other than glucose when glucose is present is called _____.

7.7.3 True or False:

1. All genetic codes are universal except UUU.
2. Wobble hypothesis and degeneracy of genetic code is same thing.
3. Genetic code is comma less.
4. For all the codons there exists an anticodon.
5. Lac operon is only found in prokaryotes.
6. Tryptophan operon is found in prokaryotes and eukaryotes.
7. Introns are non coding sequences present in prokaryotic DNA.
8. Each codon is made up of three nitrogenous bases.
9. Promoter is a part of operon to which RNA polymerase binds.
10. Cyclic AMP exerts a positive effect on lac operon.
11. Promoter is located downstream to operator.
12. Each codon corresponds to single amino acid but a single amino acid can be coded by many codons.
13. Different organisms have different genetic codes for same amino acid.
14. Genetic code is bidirectional.
15. In the presence of glucose lac operon is switched on.
16. An epigenetic change in gene expression is an inherited change that does not involve any change in the nucleotide sequence of the gene.

7.7.4 Very short answer questions:

1. What is a gene?
2. What is promoter?
3. Why are Synonymous codons?
4. What is pleiotropic gene?
5. Define operon?
6. What are nonsense codons? Why are they called so?
7. What is recon?
8. Name the amino acids coded by only one codon?
9. Which codon acts as initiation codon for the process of protein synthesis?
10. Define split gene and nested gene.
11. Mention the function of regulator gene in an operon?
12. Differentiate between intron and exon?
13. What is genetic code?
14. What are inducers?
15. What are palindromic sequences?

7.7.1 Answer key: 1-(c), 2-(b), 3-(a), 4-(c), 5-(b), 6-(c), 7-(a), 8-(d), 9-(b), 10-(d), 11-(a), 12-(c), 13-(b), 14-(c), 15-(a), 16-(d), 17-(b), 18-(c), 19-(d), 20-(b), 21-(a), 22-(c), 23-(a), 24-(b), 25-(a), 26-(b), 27-(a), 28-(d), 29-(a), 30-(b)

7.7.2 Answer Key: 1-W. Johannsen, 2-64, 61, 3-UAA, UGA, UAG, 4-Nucleotide, 5- Glycine, Glutamic acid, 6-Synonomous codon, 7- Proline, 8-Muton, 9-Degeneracy, 10-Arabinose, 11-Jacob and Monad, 12-High, 13-Glucose, Galactose 14-Operator, 15-catabolite repression

7.7.3 Answer key: 1-False, 2-Fase, 3-True, 4-False, 5-True, 6-False, 7-False, 8-True, 9-True, 10-True, 11-False, 12-True, 13-False, 14-False, 15-False, 16-True

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7.10 TERMINAL QUESTIONS

7.10.1 Short answer questions:

1. Explain about different types of codons?
2. Differentiate between monocistronic and polycistronic mRNA?
3. What is a promoter? Mention about its essential elements?
4. What do you understand by differential gene expression?
5. How does mode of action of repressor protein differ from Co-repressor?
6. What is catabolite repression? Explain with reference to lac operon?
7. What do you mean by universal nature of genetic code?

8. Explain how presence or absence of inducer affects rate of transcription?
9. Compare and contrast repressible and inducible operons.
10. What is catabolite repression and how does it work?
11. What elements make up the lac operon and what roles do they play?
12. Describe the process of repression in the trp operon.

7.10.2 Long answer question:

1. Write an essay note on classical concept and modern concept of gene? Also discuss various forms of genes identifies as a part of modern genetics.
2. Define the concept of operon. Also mention the effect of cyclic AMP on lac operon?
3. What is genetic code? Explain about characteristic feature of genetic code?
4. How does positive and negative induction system differ from one another? Explain it with suitable examples?
5. Discuss the organization of tryptophan operon. Also explain the regulation of tryptophan operon.
6. What are the various components of operon? Write in detail about it.
7. Describe the organization of lac operon. With the help of detailed diagram explain the action of repressor protein on lac operon in presence and absence of Lactose.
8. Write a short note on the followings:
   (a) Constitutive genes
   (b) Neoclassical concept of gene
   (c) Control mechanism for the synthesis of protein.
9. Write about the contribution of H.G. Khorana in the field of molecular biology? What are the different types of codon?
10. Differentiate between partial and complete degeneracy of genetic code. What is the significance of degeneracy of genetic code

UNIT-8 PROTEIN SYNTHESIS AND GENE REGULATION OF PROTEIN SYNTHESIS

8.1 OBJECTIVES

Main objective of this unit is to make student understand about:

- What are proteins and their cellular significance
- Cellular machinery required for protein synthesis
- Process of protein synthesis
- How does protein synthesis in prokaryotes differ from eukaryotes
- Regulation of protein synthesis

8.2 INTRODUCTION

During the 1950s and 1960s, it became apparent that DNA is essential in the synthesis of proteins. Among many functions, proteins can serve as enzymes and as structural materials in cells. Many specialized proteins function in cellular activities. For example, in humans, the hormone insulin and the muscle cell filaments are composed of protein. The hair, skin, and nails of humans are composed of proteins, as are all the hundreds of thousands of enzymes in the body. Protein is a nutrient, needed by living organisms for growth and development. Proteins are the most abundant kind of molecules in the body. Proteins can be found in all cells of living organisms and comprise the major structural component of all cells in the body, especially muscle. Proteins are also used in membranes, such as glycoprotein. When protein broken down into amino acids, they used as precursors in nucleic acid, coenzymes, hormones, immune response, cellular repair, and other molecules essential for life. Additionally, protein is needed to form blood cells.

Proteins are one of the most important macromolecules of living cells. All the proteins are made up of 20 different types of amino acids; however, number of amino acid in each protein may vary from few hundred to thousands of amino acid. Amino acids are linked to one another by peptide bond which leads to formation of polypeptide chain. An amino acid consists of a basic amino group ($-NH_2$) and an acidic carboxyl group (-COOH). Different arrangement of amino acids in a polypeptide chain makes each protein unique. The key to a protein molecule is how the amino acids are linked.

Every protein has a specific sequence of amino acid with specific number of amino acids. For e.g., Protein **Insulin** is made up of 51 amino acids, Protein **Myoglobin** is made up of 153 amino acids, enzyme **DNA polymerase-I** is made up of 928 amino acids. The sequence of amino acids in a protein is a type of code that specifies the protein and distinguishes one protein from another. A genetic code in the DNA determines this amino acid code. The genetic code consists of the sequence of nitrogenous bases in the DNA. How the nitrogenous base code is translated to an amino acid sequence in a protein, is the basis for protein synthesis.

Proteins are synthesized in living cells by a process called as **translation** (protein synthesis). Protein synthesis is an extremely important process for life to exist. All the physiological functions occurring inside living cell are catalyzed or regulated by proteins. Several proteins are needed for growth and development (**Structural proteins**). Many other proteins function as enzyme in vital physiological processes such as digestion, excretion and many more. All living organisms require hormones as signaling molecules in different processes. Most of the hormones are themselves proteins (except steroids), and these proteins hormones regulate and control all the metabolic reactions occurring inside living organisms.

For protein synthesis to occur, several essential materials must be present, such as a supply of the amino acids, which comprise most proteins. Another essential component is a series of enzymes that will function in the process. DNA and another form of nucleic acid called ribonucleic acid (RNA) are essential. RNA is the nucleic acid that carries instructions from the nuclear DNA into the cytoplasm, where protein is synthesized. RNA is similar to DNA, with two exceptions. First, the carbohydrate in RNA is ribose rather than deoxyribose, and second, RNA nucleotides contain the pyrimidine Uracil rather than thymine.

8.3 CELLULAR MACHINERY REQUIRED FOR PROTEIN SYNTHESIS

1. Ribonucleic acid (RNA):

It is a polymeric molecule essential in various biological roles in coding, decoding, regulation, and expression of genes. RNA and DNA are nucleic acids, and, along with lipids, proteins and carbohydrates constitute the four major macromolecules essential for all known forms of life. In the synthesis of protein, three types of RNA function.

(a) R**ibosomal RNA** (rRNA): This form of RNA is used to manufacture ribosomes. Ribosomes are ultramicroscopic particles of rRNA and protein. They are the places (the chemical "workbenches") where amino acids are linked to one another to synthesize proteins. Ribosomes are found in large numbers along the membranes of the endoplasmic reticulum and in the cytoplasm of the cell.

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

(c) Messenger RNA (mRNA): The mRNA carries the genetic information copied from DNA in the form of a series of three-base code word (codons), each of which specifies a particular amino acid. In the nucleus, messenger RNA is constructed from DNA's code of base pairs and carries

the code into the cytoplasm or to the rough endoplasmic reticulum where protein synthesis takes place. Messenger RNA is synthesized in the nucleus using the DNA molecules. During the synthesis, the genetic information is transferred from the DNA molecule to the mRNA molecule. In this way, a genetic code can be used to synthesize a protein in a distant location. RNA polymerase, an enzyme, accomplishes mRNA, tRNA, and rRNA synthesis. There are also non-coding RNA molecules (ncRNA), which are not directly involved in protein synthesis.

Comparison of the structure of prokaryotic and eukaryotic mRNA

Eukaryotic mRNA is mostly monocistronic. It has a 5' cap, which is recognized by small ribosomal subunit. Protein synthesis, therefore, begins at an initiation codon near the 5' end of the mRNA. Upstream of the initiation codon contains a non-translatable sequences called 5' UTR (5'-untranslated region) or leader sequence. Similarly non translatable sequence at 3' end after stop codon is termed as 3' UTR (3'-untranslated region) or trailer sequence, which varies in length and sequence.

In prokaryotes, most of the mRNA is polycistronic. In contrast to eukaryotic mRNA, the 5' end has no cap-like structure, and there are multiple ribosome-binding sites (called shine-Dalgarno sequence) within the polycistronic mRNA chain, each resulting in the synthesis of different protein. Just like eukaryotic mRNA, prokaryotic mRNA also contains 5' UTR and 3'UTR. All mRNA (monocistronic and polycistronic) contain two types of region- the coding region (which starts with initiation codon and ends with a stop codon) and untranslated region (5'- and 3'-UTR). A polycistronic mRNA also contain intercistronic regions **(Fig 8.1)**.

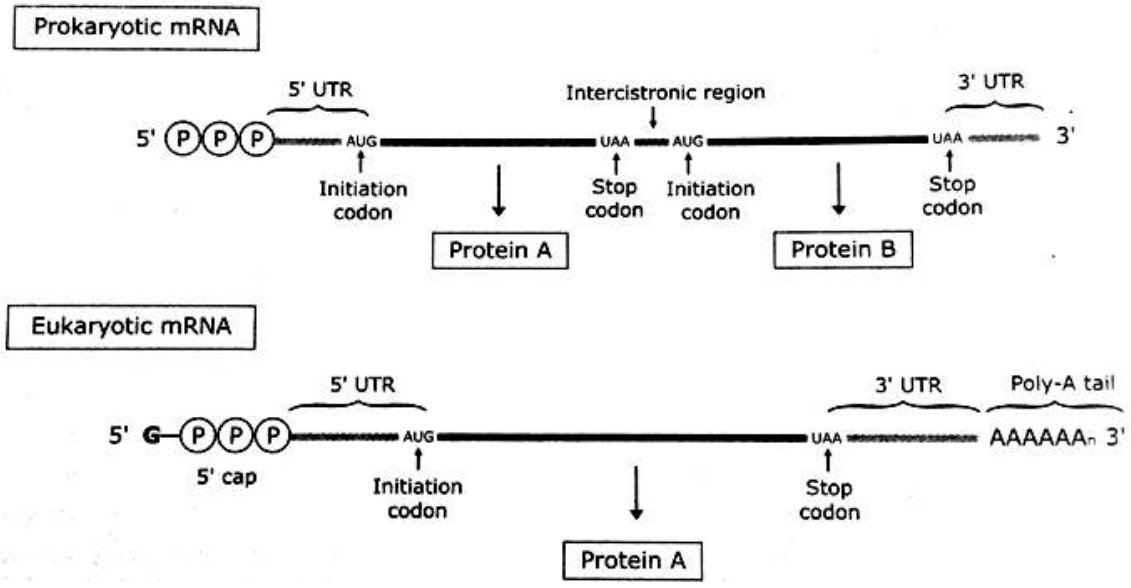


Fig. 8.2- Prokaryotic and eukaryotic mRNA having coding and untranslated region

2. Ribosome

Ribosomes are the sites of protein synthesis in both prokaryotic and eukaryotic cells. Ribosomes are designated according to their rates of sedimentation. The **70S** for bacterial ribosome (have two subunits) and **80S** for eukaryotic ribosome (have two subunits). The **'S'** refers to the **Svedberg unit**. This is a sedimentation coefficient which shows how fast cell organelle sediment in an ultra centrifuge.

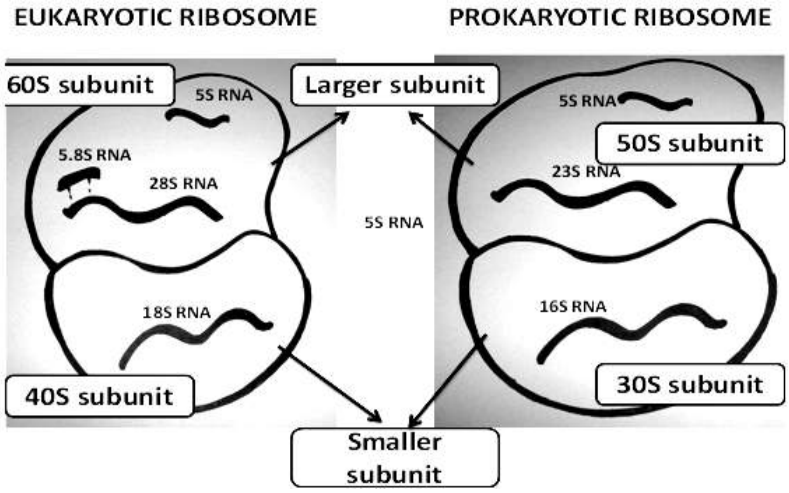


Fig. 8.2 - Eukaryotic and Prokaryotic organization of ribosomes

Each ribosome consists of larger and smaller subunits. Each subunit is composed of one or more ribosomal RNA (rRNA) molecules and a variety of ribosomal proteins. In prokaryotic ribosomes (70S), the smaller subunit (30S) consists of **16S rRNA** and 21 proteins, the larger subunit (50S) is composed of the **23S** and **5S rRNA** and 34 proteins. In eukaryotic ribosomes (80S), the smaller subunit (40S) of eukaryotic ribosomes is composed of **18S rRNA** and ~30 proteins, and larger subunit (60S) contains the **5.8S**, **28S**, and **5S rRNAs** and about ~45 proteins. Prokaryotic as well as in eukaryotic ribosomes, the small ribosomal subunit read the RNA and large subunit joins amino acids to form a polypeptide chain. Even though, ribosomes have slightly different structures in different species, their functional areas are all very similar. For example, prokaryotes have ribosomes that are slightly smaller than eukaryotes.

The 70S ribosome has three tRNA-binding sites:
P-site: P-site, also called the ***Peptidyl-tRNA-binding*** site, holds the tRNA molecule that is linked to the growing end of the polypeptide chain.
A-site: A-site, also called the ***aminoacyl-tRNA-binding*** site, holds the incoming tRNA molecule charged with an amino acid.
E-site: Deacylated tRNA (lacking any amino acid) exits via the E site, also called the ***exit site***.

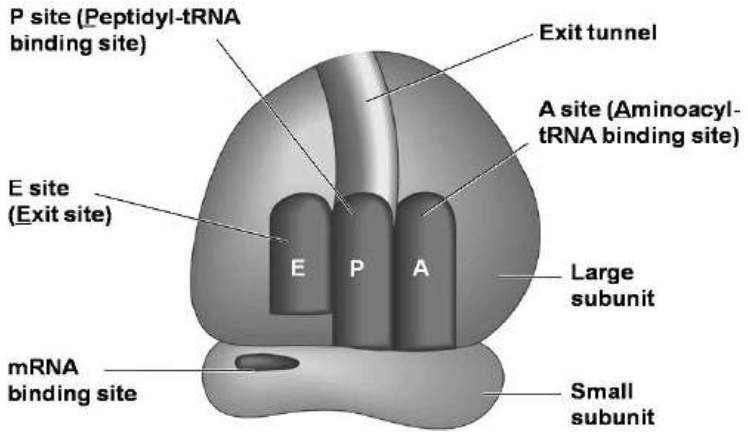


Fig. 8.3 - Schematic model showing RNA binding site in the ribosome

3. Amino Acids

Amino acids, initially considered simply substrates for protein synthesis, have been recently shown to act as modulators of intracellular signal transduction pathways typically associated with growth-promoting hormones such as insulin and insulin-like growth factor-1. Many of the end points of the signaling pathways regulated by amino acids are proteins involved in mRNA translation. Thus, particular amino acids not only serve as substrates for protein synthesis but are also modulators of the process. Hundreds of different types of proteins may be manufactured in a single cell. All types of proteins are formed from the same amino acids. It is the arrangement of amino acids in the polypeptides and the number of the latter which provide specificity to the proteins.

4. Aminoacyl tRNA Synthetases

It is the enzyme that helps in combining amino acid to its particular tRNA. The enzyme is specific for each amino acid. It is also called **aa-activating enzyme**. Through the process of tRNA "charging," each tRNA molecule is linked to its correct amino acid by a group of enzymes called aminoacyl tRNA synthetases. When an amino acid is covalently linked to a tRNA, the resulting complex is known as **aminoacyl-tRNA**. At least one type of aminoacyl tRNA synthetase exists for each of the 21 amino acids; the exact number of aminoacyl tRNA synthetases varies by species.

These enzymes first bind and hydrolyze ATP to catalyze the formation of a covalent bond between an amino acid and **adenosine monophosphate (AMP)**; a pyrophosphate molecule is expelled in this reaction. This is called "activating" the amino acid. The same enzyme then catalyzes the attachment of the activated amino acid to the tRNA and the simultaneous release of AMP. After the correct amino acid covalently attached to the tRNA, it is released by the enzyme. The tRNA is said to be charged with its cognate amino acid.

8.4 PROCESS OF PROTEIN SYNTHESIS

Biosynthesis of protein is under direct control of DNA in most cases or else under the control of genetic RNA where DNA is absent. Information for structure, of a polypeptide is stored in a polynucleotide chain. In 1958 **Crick** proposed that the information present in DNA (in the form of base sequence) is transferred to RNA and then from RNA it is transferred to protein (in the form of amino acid sequence), and that this information does not flow in the reverse direction, that is, from protein to RNA to DNA.

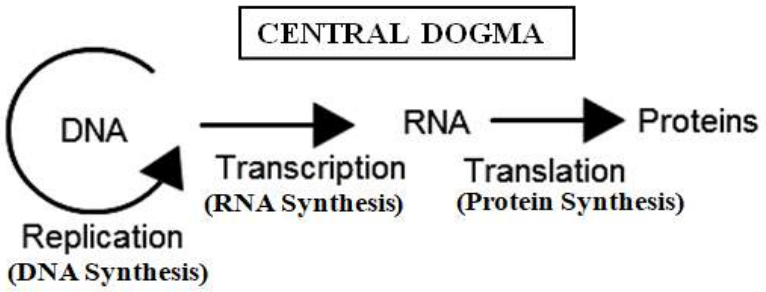


Fig. 8.3 - Central Dogma of living cell

DNA molecules provide the information for their own replication. This relationship between DNA, RNA and protein molecules is known as **Central Dogma**. Temin (1970) reported that retroviruses operate a central dogma reverse (inverse flow of information) or feminism inside host cells. Genomic RNA of these viruses first synthesizes DNA through reverse transcription; this process is catalyzed by the enzyme reverse transcriptase, DNA then transfers information to messenger RNA which takes part in translation of the coded information to form polypeptide. Every living cell follows a process of central dogma, which ultimately leads to synthesis of protein.

8.4.1 Mechanism of Protein Synthesis

The synthesis of proteins takes two major steps; **(1) transcription**, involving transfer of genetic information from DNA to mRNA, which heads out of the cell's nucleus and into the cytoplasm, and **(2) translation**, involving translation of the language of nucleic acid into that of proteins. During translation, the mRNA works with a ribosome and tRNA to synthesize proteins.

(1) Transcription

It is one of the first processes in the mechanism of protein synthesis. In transcription, a complementary strand of mRNA is synthesized according to the nitrogenous base code of DNA. The first step in transcription is the partial unwinding of the DNA molecule so that the portion of DNA that codes for the needed protein can be transcribed. Once the DNA molecule is unwound at the correct location, an enzyme called **RNA polymerase** helps line up nucleotides to create

a **complementary strand** of mRNA. Since mRNA is a single-stranded molecule, only one of the two strands of DNA is used as a template for the new RNA strand, and the other DNA strand remains dormant.

The enzyme RNA polymerase binds to an area of one of the DNA molecules in the double helix. The enzyme moves along the DNA strand and "reads" the nucleotides one by one. Similar to the process of DNA replication, the new nucleic acid strand elongates in a 5'-3' direction, as shown in Figure 8.5. The enzyme selects complementary bases from available nucleotides and positions them in an mRNA molecule according to the principle of complementary base pairing. The chain of mRNA lengthens until a "stop" message is received. The new strand of RNA is made according to the rules of base pairing:

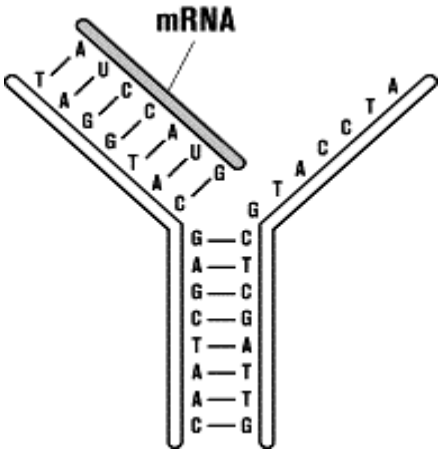


Fig. 8.4-Base pairing of mRNA strand to DNA strand

- DNA cytosine pairs with RNA guanine
- DNA guanine pairs with RNA cytosine
- DNA thymine pairs with RNA adenine
- DNA adenine pairs with RNA uracil

For example, the mRNA complement to the DNA sequence TTGCAC is AACGUG. The RNA uses uracil in place of thymine.

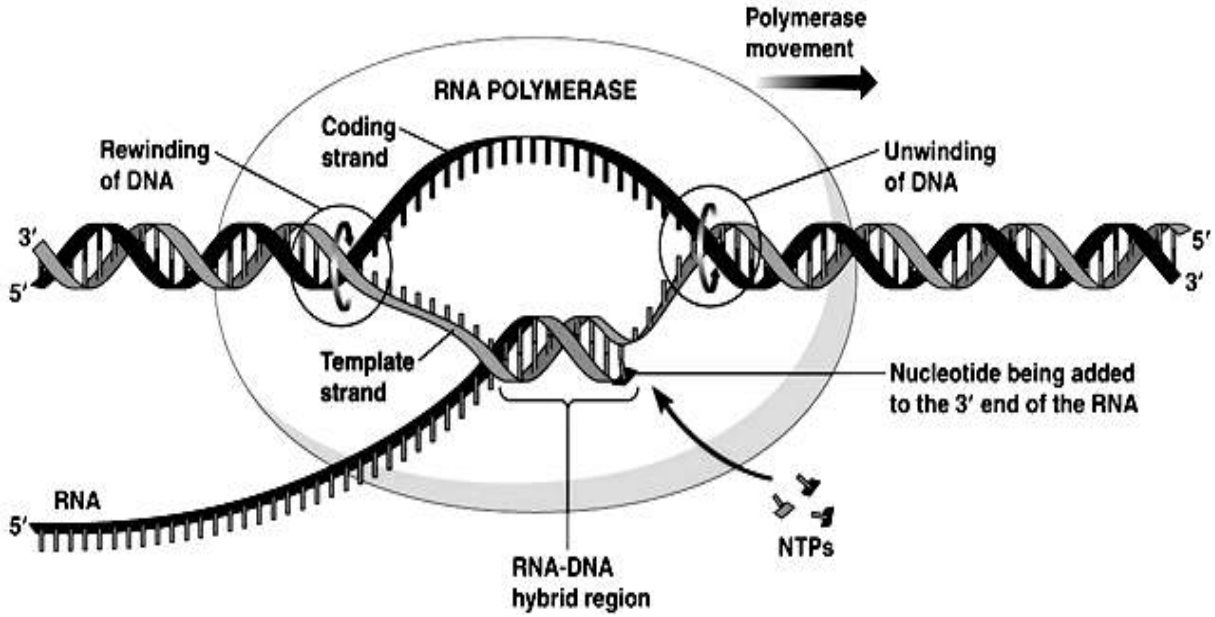


Fig. 8.5- Process of transcription, DNA double helix opens, and the enzyme RNA polymerase synthesizes a molecule of mRNA according to the base sequence of the DNA template

The nucleotides of the DNA strands are read in groups of three. Each group is a *codon.* Thus, a codon may be CGA, or TTA, or GCT, or any other combination of the four bases, depending on the codons complementary sequence in the DNA strand. Each codon will later serve as a "code word" for an amino acid. First, however, the codons are transcribed to the mRNA molecule. Thus, the mRNA molecule consists of nothing more than a series of codons received from the genetic message in the DNA.

After the "stop" codon is reached, the synthesis of the mRNA comes to an end, the new RNA strand is released and the two unzipped DNA strands bind together again to form the double helix. Meanwhile, the mRNA molecule passes through a pore in the nucleus and proceeds into the cellular cytoplasm, where it moves toward the ribosomes located in the cytoplasm or on the rough endoplasmic reticulum. Because the DNA template remains unchanged after transcription, it is possible to transcribe another identical molecule of RNA immediately after the first one is complete. A single gene on a DNA strand can produce enough RNA to make thousands of copies of the same protein in a very short time.

(2) Translation

The genetic code is transferred to an amino acid sequence in a protein through the process of **translation**, which begins with the arrival of the mRNA molecule into the cytoplasm where it binds with ribosomes, the sites of protein synthesis. Ribosomes have three important binding sites: one for mRNA and two for tRNA. The two tRNA sites are labeled as 'A' site and 'P' site. Once the mRNA is in place, tRNA molecules, each associated with specific amino acids, bind to the ribosome in a sequence defined by the mRNA code.

The tRNA molecules then began transporting their amino acids to the ribosomes to meet the mRNA molecule. Transfer RNA (tRNA) molecules can perform this function because of their special structure. It is made up of many nucleotides that bend into the shape of a cloverleaf. At the tail end, tRNA has an acceptor stem that attaches to a specific amino acid. At its head, tRNA has three nucleotides that make up an **anticodon**.

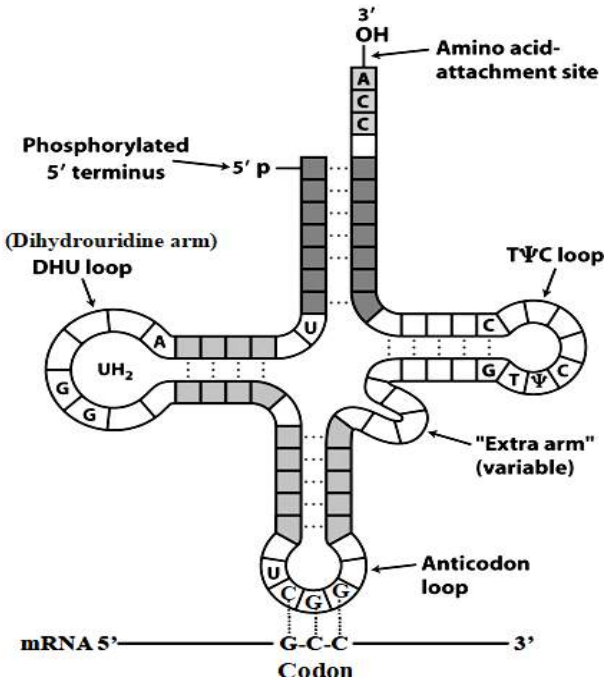


Fig. 8.6- General tRNA structure

An anticodon pairs complementary nitrogenous bases with mRNA. For example, if mRNA has a codon AUC, it will pair with anticodon sequence UAG of tRNA. The tRNA molecules

with same anticodon sequence will always carry the same amino acids, ensuring the consistency of the proteins coded for in DNA.

The Process of Translation

Translation begins with the binding of the mRNA chain to the ribosome. The first codon, which is always the start codon methionine, fills the P site and the second codon fills the A site. The tRNA molecule whose anticodon is complementary to the mRNA forms a temporary base pair with the mRNA in the A site. A peptide bond is formed between the amino acid attached to the tRNA in the A site and the methionine in the P site.

The ribosome now slides down the mRNA, so that the tRNA in the A site moves over to the P site, and a new codon fills the A site. (One way to remember this is that the A site brings new amino acids to the growing polypeptide at the P site.) The appropriate tRNA carrying the appropriate amino acid pairs bases with this new codon in the A site. A peptide bond is formed between the two adjacent amino acids held by tRNA molecules, forming the first two links of a chain.

The ribosome slides again. The tRNA that was in the P site is let go into the cytoplasm, where it will eventually bind with another amino acid. Another tRNA comes to bind with the new codon in the A site, and a peptide bond is formed between the new amino acid to the growing peptide chain.

The process continues, one by one, amino acids are added to the growing chain until one of the three stop codons enters the A site. At that point, the protein chain connected to the tRNA in the P site is released. Because of the specificity of tRNA molecules for their individual amino acids, and because of the base pairing between codons and anticodons, the sequence of codons on the mRNA molecule determines the sequence of amino acids in the protein being constructed. And because the codon sequence of the mRNA complements the codon sequence of the DNA, the DNA molecule ultimately directs the amino acid sequencing in proteins. The primary "start" codon on an mRNA molecule is AUG, which codes for the amino

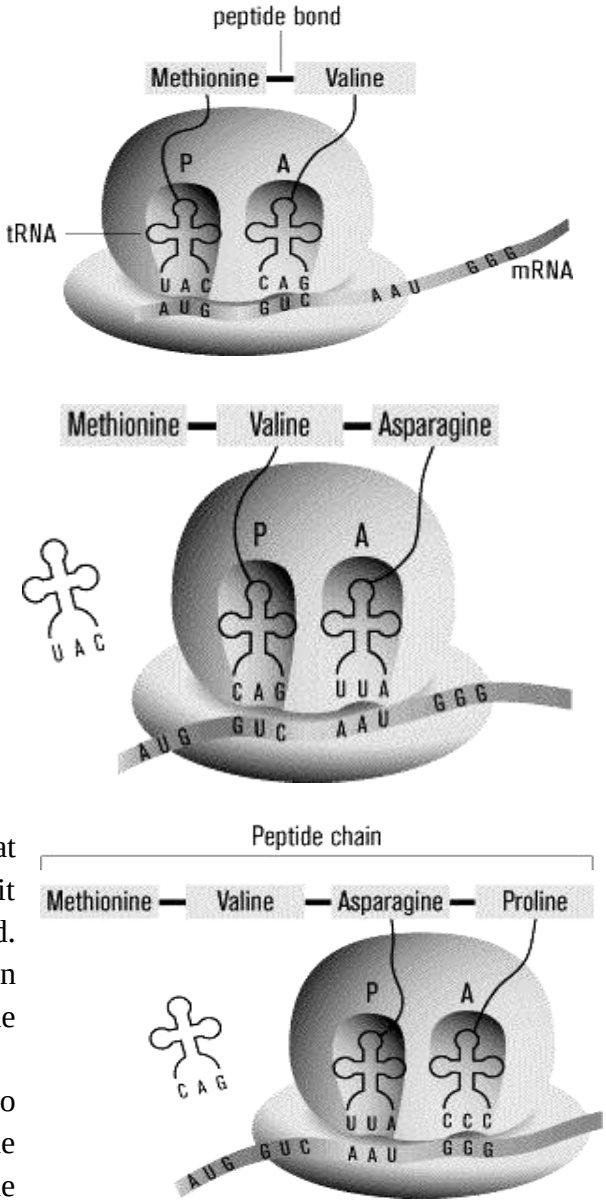


Fig. 8.7.Process of translation

acid methionine. Therefore, each mRNA transcript begins with the AUG codon, and the resulting polypeptide begins with methionine.

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

Steps involved in the process of protein synthesis

The process of protein synthesis translates the codons (nucleotide triplets) of the messenger RNA (mRNA) into the 20-symbol code of amino acids that build the polypeptide chain of the proteins. The process of mRNA translation begins from its 5′-end towards its 3′-end as the polypeptide chain is synthesized from its amino-terminal (N-end) to its carboxyl-terminal (C-end). There are almost no significant differences in the protein synthesis steps in prokaryotes and eukaryotes, however there is one major distinction between the structure of the mRNAs – prokaryotes often have several coding regions (polycistronic mRNA), while the eukaryotic mRNA has only one coding region (monocistronic mRNA). Proteins are synthesized by ribosomes on mRNA strand. The process of protein synthesis occurs in following steps

1. Activation of amino acids
2. Transfer of amino acid to tRNA
3. Initiation of synthesis
4. Elongation of polypeptide chain
5. Chain termination

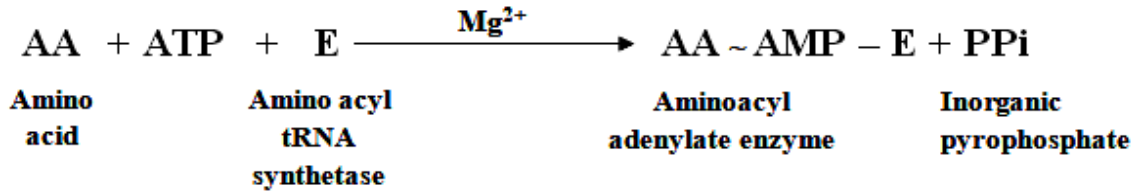
1. Activation of amino acids

Amino acids found to be present in cytoplasm are present in inactive or dormant state. They cannot undergo the process of protein synthesis in this inactive state. Hence, the process synthesis begins with activation of amino acids. Each amino acid is catalyzed by enzyme, known as **aminoacyl tRNA Synthetase** which results in formation of aminoacyl adenylate-enzyme complex.

Energy in the form of ATP is required for the process. In the presence of ATP, an amino acid combines with its specific aminoacyl-tRNA synthetase, and Mg^{2+} is also required. These enzymes first bind and hydrolyze ATP to catalyze a high-energy bond between a carboxyl group of amino acid and α- phosphate of ATP i.e. adenosine monophosphate (AMP).

The other two phosphates (β and γ) molecules of ATP are removed as inorganic pyrophosphates (PPi) in this reaction. Hydrolysis of pyrophosphate with the help of enzyme

pyrophosphatase provides energy for driving the initial reactions. The activated amino acid is then transferred to the tRNA, and AMP is released. The energy made available to amino acid during its activation is later used in formation of peptide bonds.



2. Transfer of amino acid to tRNA

Once the amino acids get activated the next step in protein synthesis is transfer of activated amino acids to charging of tRNA. It is also known as **charging of tRNA**. There are about 100 different types of tRNA found in cytoplasm. Each activated amino acid is transferred to its specific tRNA by formation of an ester bond between carboxylic group (-COOH) of amino acid and the –OH group attached to either the 2' or 3' carbon on the sugar of the terminal adenine nucleotide of tRNA.

As we already discussed that the enzymes synthetase play a key role in activation as well as transfer of activated amino acid. The enzymes have two active sites. One site is utilized for identifying specific amino acid and other to recognize specific tRNA molecule. The overall function of enzymes is to bring amino acid and its specific tRNA molecule together. Different tRNA is given names corresponding to their specific amino acids. For example, tRNA for amino acid tryptophan and arginine, are named as $tRNA^{Try}$ and $tRNA^{Arg}$ respectively.

3. Initiation of protein synthesis

Two mechanisms are involved in the recognition of nucleotide sequence (AUG) by the ribosome, which actually initiates translation:

(a) Shine-Dalgarno (SD) sequence

In Escherichia coli, the observed sequence with high percentage of purine nucleotide bases, known as the *Shine-Dalgarno sequence*. It is a ribosomal binding site in bacterial and archaeal mRNA, generally located close to 5' end of the mRNA molecule, around 6-10 bases upstream of the initiating or start codon (AUG). The RNA sequence helps in recruitment of smaller ribosomal subunit to the messenger RNA (mRNA) to initiate protein synthesis by aligning the ribosome with the start codon. The Shine Dalgarno sequence was first of all described by

scientist of Australian origin **John Shine and Lynn Dalgarno** and the sequence has been named so. Although multiple types of Shine Dalgarno sequences have been reported to exist, all of them have been found to possess a common sequence of six nucleotides "**A**GGAG**G"**.

The 16S rRNA component of the small ribosomal subunit possess a complementary to the SD sequence near its 3'-end. Thus the two complementary sequences can couple, which facilitates the positioning of the 30S ribosomal subunit on the mRNA in proximity to the initiation codon. The mechanism is slightly different in eukaryotes because they do not have SD sequences.

In eukaryotes, with the assistance of the **eIF-4** initiation factors, the 40S ribosomal subunit binds close to a structure called "**cap structure**" at the 5-end of the mRNA and then moves down the mRNA sequence till it finds the initiating codon. However, this process requires energy from ATP. Selection of the initiating AUG is facilitated by specific surrounding nucleotides called the ***Kozak sequence***, 5'**A**CCAUG**G**3'. Once the small ribosomal subunit with its bound Met-tRNAi is correctly positioned at the start codon, union with the 60S ribosomal subunit completes formation of an 80S ribosome.

Initiation Factor

For initiation of protein synthesis different **initiation factors** (IF) are required. In Prokaryotes are three known initiation factors namely **IF-1**, **IF-2**, and **IF-3** with molecular weight 9200, 80000 and 30000 Daltons respectively. IF-1 and IF-2 are required for binding of initiation tRNA to 30S ribosomal subunit. Beside this IF-2 is also needed for binding of guanosine triphosphate (GTP). IF-3 is the initiation factor which is rich in lysine and arginine residues and helps in binding of 30S ribosomes subunit to mRNA stand all these factors are found to be located in 30S subunit of ribosome. Eukaryotic translation initiation factors include **eIF1**, **eIF2**, **eIF3**, **eIF4**, and **eIF5**. The eIF4E subunit of eIF4 binds to the 5′ cap structure on eukaryotic mRNAs.

(b) Initiating amino acid and Initiating codon

The synthesis of all proteins starts with the same amino acid, methionine. In E.coli and in other eubacteria, the first amino acid in any newly synthesized polypeptide is N-formylmethionine because the starting methionine amino acid carries a formyl group. However, formylation is not necessary, because non-formylated methionine can function as an initiator amino acid. In prokaryotic initiation of protein synthesis, tRNA binds to N- formyl methionine to form N-formylmethionyl-$tRNA^{met}$, whereas in eukaryotic protein synthesis initiation, tRNA binds to methionine to form methionyl-$tRNA^{met}$.

The initiating AUG triplet is recognized by a special initiator tRNA. In prokaryotes this event is facilitated by **IF-2-GTP**, while in eukaryotes by **eIF-2-GTP** and additional eukaryotic initiating factors (eIFs). The charged initiator tRNA approaches the **P site** on the small ribosomal subunit. In bacteria (and in mitochondria), a methionine is attached to the initiator tRNA and subsequently a formyl group is added by the enzyme transformylase, which uses N10-

formyltetrahydrofolate as the carbon donor – finally an N-formylated methionine is attached to the initiator tRNA.

In eukaryotes, the initiator tRNA attaches a non-formylated methionine. In both types of cells, this N-terminal methionine attached to the 5'-end is removed before the end of the translation. In the last step of the initiation, the large ribosomal subunit joins the complex formed by now, and thus a fully functional ribosome is formed. This complex has a charged initiating tRNA in the P site, and the A site empty. During this, protein synthesis step is used the energy within the GTP on eIF-2, which gets hydrolyzed to GDP. The reactivation of eIF-2-GDP is facilitated by guanine nucleotide exchange factor.

Formation of 30S initiation Complex

The next step in the process of protein synthesis is formation of 30S initiation complex. mRNA, 30S subunit of ribosome, initiator tRNA containing attached N- formylmethionine, GTP and initiation factors (IFa-1, IF-2 and IF-3) combine to form a complex called as 30S initiation complex. The mRNA gets attached to 30S ribosomal subunit to form mRNA -30S complex. IF-3 is required for this process to occur. Besides its role in binding of mRNA with 30S ribosomal subunit, IF-3 has other functions to such as it affects the conformation of 30S subunit and also it presents re-association of 30S and 50S subunits of ribosome. Once they are dissociated. Now, to the above formed mRNA-30S complex, fmet–tRNA is added. IF-2 and IF-1 along with GTP are required for this process to occur. Among these IF-2 is the most crucial Factor required for binding of fmet-tRNA to 30S complex.

The mRNA contains AUG and the tRNA which is attached first of all contains UAC anticodon. In eukaryotes initially Met-tRNA binds to 40S ribosomal subunit and then mRNA attaches with its AUG codon. Now as we have seen that AUG codon present in mRNA works as initiation codon. A specific nucleotide sequence is present near initiator AUG codon, or the conformation of tRNA is such that most of the AUG codons are presented to act as initiator codon. After tRNA gets attached to mRNA-30S subunit complex, 50S subunit of ribosome is added, which contains two binding sites (A and P) in which two tRNA molecules can bind. **A site** is called Aminoacyl or Acceptor site and **P site** is called Peptidyl or Polymerization site or Donor site. The initiator tRNA, fmet-tRNA always directly binds to p site. However, all other tRNA (other than initiator tRNA), first binds to A site and then they are translocated to P site. During attachment of 50S subunit, GTP is converted to GDP + Pi and initiation factors IF-1 and IF-2 are released for recycling.

The initiation of translation is in fact a complex reaction **(Fig. 8.3.1-e)**. First, initiation factors (IFs) dissociate the large and small subunits, and the small subunit is bound to Met-tRNA (fmet-tRNA in prokaryotes) with mRNA and IFs attached. The large subunit then binds to it, forming a complex consisting of a ribosome, mRNA and Met-tRNA. This is known as an **initiation complex**.

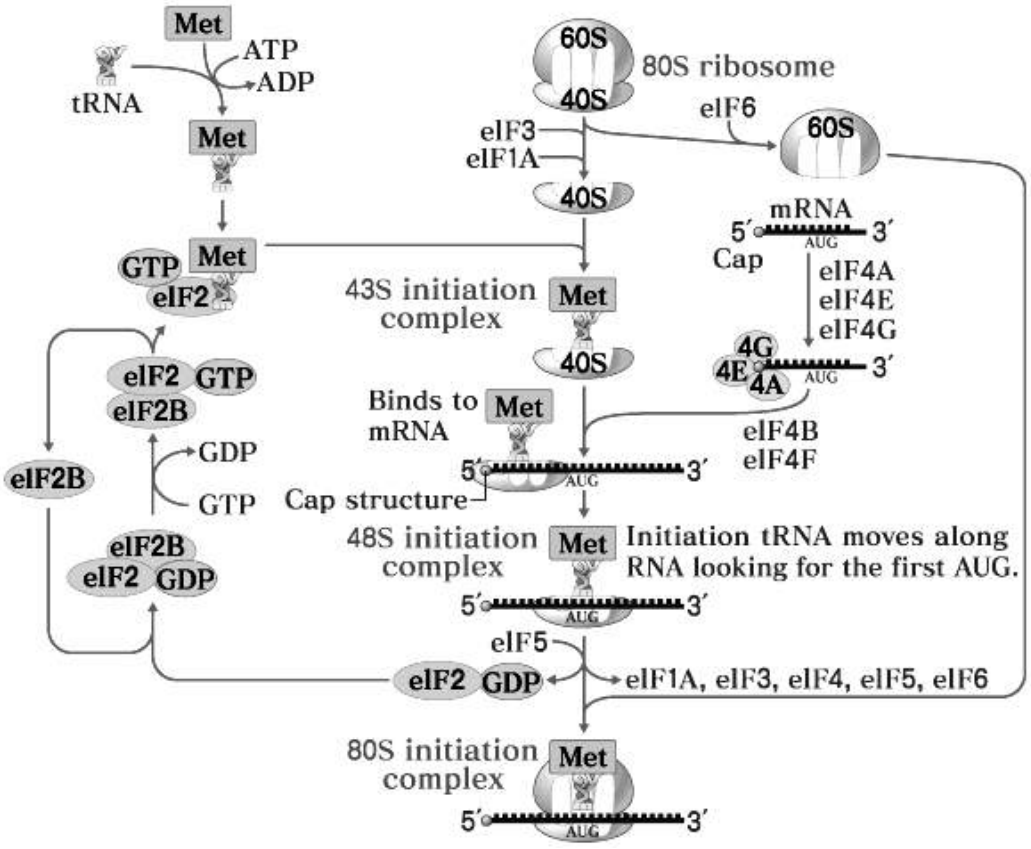


Fig. 8.8 - Formation of initiation complexes in eukaryotic organisms

4. Elongation of polypeptide chain

Translation elongation is second in protein synthesis steps. During the elongation step the polypeptide chain adds amino acids to the carboxyl end. The protein chain grows as the ribosome moves from the 5' -end to the 3'-end of the mRNA. Factors for the elongation of polypeptide chain are called as elongation factors (EF). In prokaryotes, the delivery of the aminoacyl-tRNA to ribosomal **A**-site is facilitated by elongation factors **EF-Tu-GTP** and **EF-Ts**, and requires GTP hydrolysis. In eukaryotes, the analogous elongation factors are EF-1α–GTP and EF-1βγ. Both EF-Ts (in prokaryotes) and EF-1βγ (in eukaryotes) function as nucleotide exchange factors. EF-2 in eukaryotes and EF-G in prokaryotes bring about translocation of aminoacyl–tRNA from **'A'** site to **'P'** site. Elongation factors EF-T4 and EF-T5 in prokaryotes are required for binding of amino acyl tRNA ribosome. EF-T4 also forms a complex with aa-tRNA and GTP.

Now the initiator tRNA containing initiation amino acid binds to P-site and another tRNA with second amino acid binds to A-site. Now a peptide bond will be formed between initiation amino acid (at P-site) and second amino acid (at A-site). The Peptidyl-transferase is an important enzyme which catalyzes the formation of the peptide bonds. The enzymatic activity is found to be intrinsic to the 23S rRNA found in the large ribosomal subunit. Because this rRNA catalyzes the polypeptide bound formation reaction, it is named as a ribozyme.

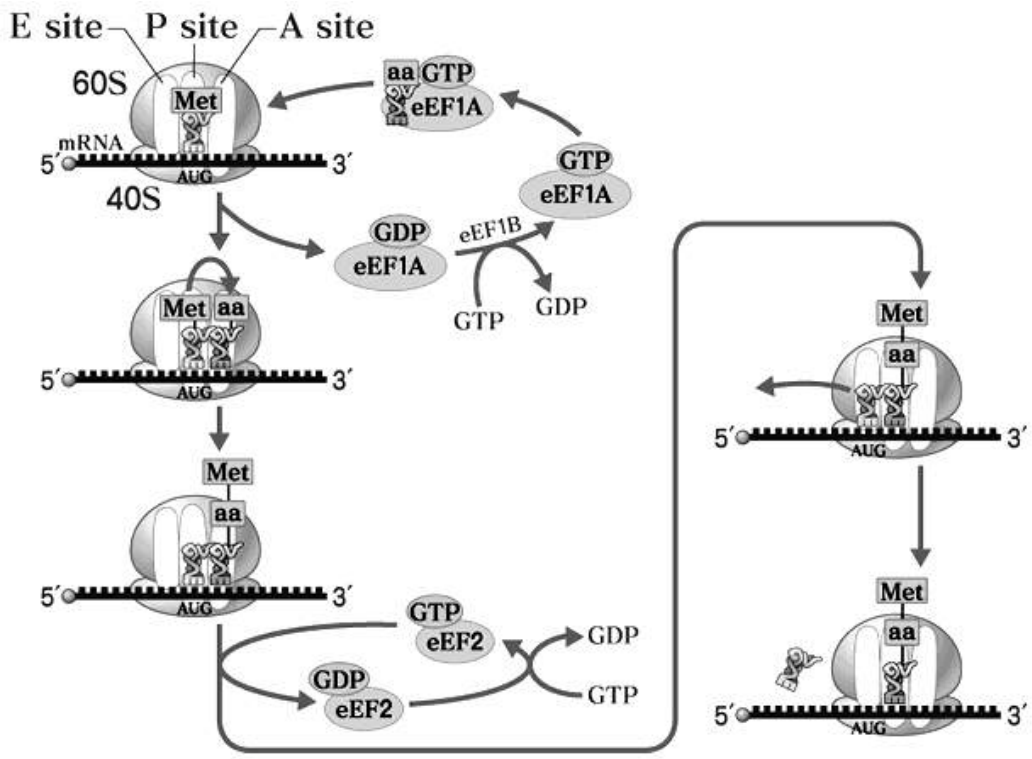


Fig. 8.9: Elongation Reaction of Peptide Chains

After peptide bond formation, the ribosome moves 3 nucleotides toward the 3'-end of the mRNA by which tRNA molecule of fmet–tRNA is released from P site. This process is known as translocation - in prokaryotes, it requires the participation of **EF-G-GTP** and GTP hydrolysis, while the eukaryotic cells use **EF-2-GTP** and GTP hydrolysis again. During the translocation, the uncharged tRNA moves from the P to the E site and Peptidyl-tRNA (to which the growing polypeptide chain is attached) leaves the A site and go to the P site and a third tRNA comes into A-site. Now a second peptide bond will be formed between second amino acid and third amino acid. Again second tRNA from P-site is released and third tRNA from A-site will be translocated to P-site and A-site will become free for entry of next tRNA molecule. By this process large number of amino acids will be linked to one another by formation of peptide bond leading to formation of polypeptide (protein) chain. This is an iterative process that is repeated until the ribosome reaches the termination codon.

The elongation reaction of peptide chains is also complex (**Fig.8.9)**. At the onset of this reaction, Met-tRNA is situated at the P site, and aminoacyl-tRNA bound with an elongation factor (EF) binds to the A site. The ester bonds between the first amino acid (methionine) and tRNA are then cut, and the methionine and an amino acid at the A site form peptide bonds. During this process, two GTP molecules are hydrolyzed for each amino acid added. The first amino acid of proteins synthesized is always methionine, which is synthesized from the side of the free amino group (N-terminal) to the carboxyl group (C-terminal).

5. Chain termination

Termination happens when the A site of the ribosome reaches one of the three termination or **stop codons** (UAA, UAG or UGA). The process of termination of protein synthesis can be divided into three parts starting with recognition of termination signal (Stop codons). Followed by release of polypeptide chain and finally processing of polypeptide chain. Elongation of polypeptide chain continuous until a stop codon is read on mRNA. As we already know that these three codons are regarded as non-coding since they do not code for any amino acid, hence when the moving ribosome will reach a point where any of these codons is present the process of protein synthesis will terminate.

Since the synthesis of polypeptide chain stops, the polypeptide chain still remains attached to tRNA molecule. There are several release factors which brings about hydrolysis of polypeptide chain at P-site. As a result of action of release factors tRNA is released from P site, ribosome dissociates into 30S and 50S subunit in prokaryotes. In prokaryotes there are three known **release factors RF1**, **RF2** and **RF3**. Release factors RF1 and RF2 recognize stop codons which ultimately results in termination of protein synthesis. RF3 does not recognize any stop codon but it helps in stimulating binding and release of RF1 and RF2 from the ribosome. For this reason RF3 is also called as **stimulatory factor** or S factor.

As compared to prokaryotes in eukaryotes there is only one release factor (RF) and this single release factor recognizes all the three stop codons. This eukaryote release factors have been found to be a dimer formed of two subunits. In termination of eukaryotic translation release factor binds to A-site (Where termination codon is present). Termination of protein synthesis in eukaryotes requires GTP as energy source. With which hydrolysis of Peptidyl-tRNA at P site occurs and polypeptide (protein) chain is released from ribosome. GTP hydrolysis results in dissociation of RF from ribosomes.

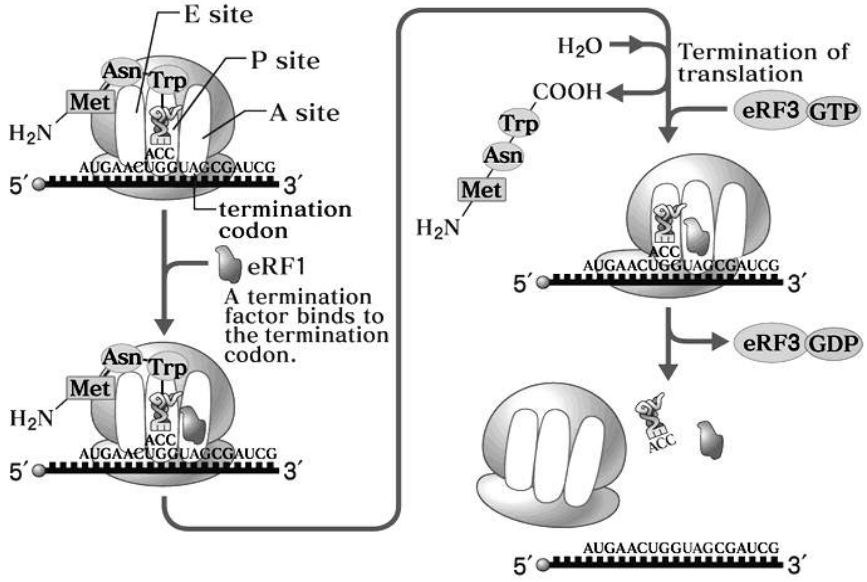


Fig. 8.10 - Termination reaction of translation

8.5 REGULATION OF PROTEIN SYNTHESIS

All the processes occurring inside living cells (prokaryotic or eukaryotic) are strictly controlled and regulated. Translation, the process of protein synthesis is also under cellular control or regulation. Depending upon species to species an organism can contain from few hundred to several thousand genes. It means that a living cell is capable of synthesizing large number of proteins. All these proteins are required by the organism at its various stages of growth and development. Now all the cell types in an organism have the same genotype (genetic makeup/ genes) but their requirement of protein may greatly vary from one another depending upon their structure and function. A protein required by one type of cell may not be needed by another cell type and vice versa. Similarly, some proteins are required by organisms during embryonic development only and these proteins are not synthesized during adulthood. On the other hand there are many proteins which are synthesized only in adulthood.

Hence, it can now be understood that depending upon the cellular need some genes are selectively expressed to synthesize proteins and several genes may be ***switched off*** at a particular time when their protein is not required by the cell. This is done by regulation of gene expression to control which gene to be expressed by which cell type and at what time. The control of protein synthesis can mainly be studied at three different stages, at the stage of transcription, post transcriptional modification and post translational modification.

Regulation of protein synthesis (gene expression) in prokaryotes

1. Some structures are formed by regions of mRNA which obscure ribosomes binding sites and hence, specifically inhibit translation of certain genes.
2. In prokaryotes, formation of stem and loop occurs that inhibits activity of enzyme ***Exonucleases***. As a result there is no activity of Exonucleases and hence regions of polycistronic mRNA gets enhanced half life leading to possibly enhanced rate of translation.
3. Regulation of protein synthesis also occurs through operon in prokaryotes. We have already studies in previous unit, how ***operon*** can control expression of specific genes depending upon the physiological requirement of the cell. Genes are expressed if needed and proteins are synthesized. On the other hand expression of a gene can be blocked if protein formed from the gene is not required by the cell. The control of operon over protein synthesis (translation) is exerted as transcriptional level.
4. Some prokaryotes sometimes also utilises antisense mRNA to restrict translation of respective genes. ***Antisense mRNA*** is a short RNA sequence transcribed from segment of DNA and is complementary to an mRNA sequence, sense mRNA transcribed from another DNA segment. Antisense mRNA binds to sense mRNA and from a duplex. As a result, translation of sense mRNA is blocked.

Regulation of protein synthesis (gene expression) in eukaryotes

The genes in eukaryotes are also regulated in more or less the same manner as that of prokaryotes, but the regulation is mostly positive and very rarely negative regulation is seen. In higher eukaryotes the regulation of gene expression is solely by positive modulation and negative inhibition of the genes/operon is totally absent. However, in yeast some genes are regulated by negative regulation. Process of protein synthesis in eukaryotes is regulated at four levels:-

1. **Transcriptional level of regulation:** At this level, regulation is done by controlling which genes to be transcribed and which genes should not be transcribed.
2. **Post transcriptional regulation:** In eukaryotes after formation of mRNA (from DNA), it undergoes post transcriptional modification. It includes splicing (removal of introns), capping and tailing of mRNA formed. Regulation of gene expression can also be achieved at this part. If proper modification of mRNA does not occurs, such mRNA is not translated into proteins.
3. **Translational control:** Translation controls how often and how rapidly mRNA transcripts will be translated into proteins.
4. **Post translational control:** Different mechanisms which can affect rate of activity of a protein and the time at which it remains functional**.**

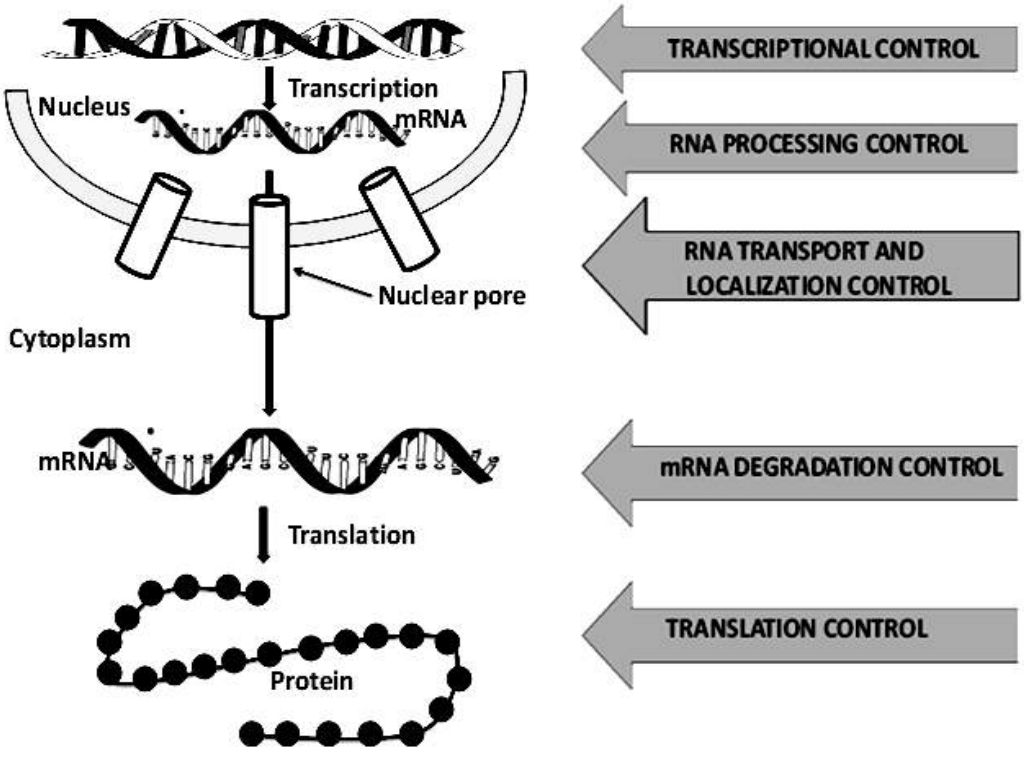


Fig. 8.11- Different points where synthesis of proteins (gene expression) can be regulated

Regulation of transcription is the main process by which the process of translation is also regulated. As you have already seen the concept of central dogma in which mRNA is from DNA by transcription and this mRNA undergoes process of translation to form protein. Now it is obvious if transcription (mRNA synthesis) is blocked, protein synthesis of the respective gene will automatically block. Hence regulation of protein synthesis at transcriptional level is most effective, obvious and suitable method of regulating translation.

Besides this, RNA processing or splicing is another method by which protein synthesis is regulated in eukaryotes. Control of eukaryotic translation also lies in cytoplasm. There are many copies of sequence 5'-AUUUA-3' (usually in 3' non-coding region), they are known to mark mRNA for degradation and hence, the process of translation is inhibited. Since there is no mRNA, protein synthesis can't occur.

There are many proteins present in cytoplasm which directly bind to mRNA and hence results in inhibition of translation. Such blocked mRNA is called as **masked mRNA**. We already seen that different types of proteins called as **translation factors** (initiation factor, elongation factor and release factor) are required for protein synthesis. Gene expression or translation is also regulated by modification of these translation factors.

Processing of primary transcript in eukaryotes

Eukaryotic DNA contains two types of sequences. **Exons**, which are coding sequence, code for a particular protein and **introns**, which are non-coding sequences. During the process of transcription (synthesis of mRNA from DNA), initially a primary transcript of mRNA is formed which contains introns as well as exons. This primary transcript mRNA undergoes the process of splicing in which all the introns (non coding sequences) are removed and the final mRNA contains only coding sequence exons. Now at this point of time the expression of gene can be controlled **Fig. 8.4 (b)**. The exons can be spliced together in different ways which permits different types of proteins can be formed from same sets of exons. Such type of alternate splicing is common in insects and vertebrates where two or more proteins are produced from one gene.

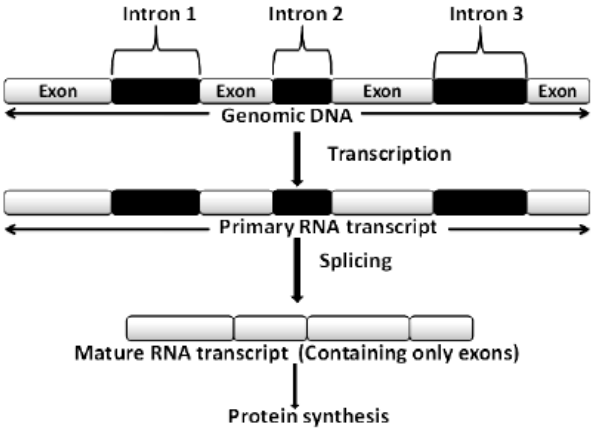


Fig. 8.12 - Splicing of mRNA in eukaryotes

8.6 SUMMARY

Protein synthesis is one of the most fundamental biological processes by which individual cells build their specific proteins. Within the process are involved both DNA (deoxyribonucleic acid) and different in their function ribonucleic acids (RNA). The process is initiated in the cell's nucleus, where specific enzymes unwind the needed section of DNA, which makes the DNA in this region accessible and a RNA copy can be made.

This RNA molecule then moves from the nucleus to the cell cytoplasm, where the actual the process of protein synthesis take place. All cells function through their proteins. Protein function is defined by their molecular function, localization within cell and involvement in a particular biological process. All components of protein function are defined by the exact composition, structure and conformation of the proteins, which is encrypted within the DNA region (called locus) encoding that protein. With the process of protein synthesis biological cells generate new proteins, which on the other hand are balanced by the loss of cellular proteins via degradation or export.

Transcription is the first of overall two protein synthesis steps. During transcription, the information encoded in the DNA is copied to a RNA molecule as one strand of the DNA double helix is used as a template. The RNA molecule is sent to the cytoplasm, which helps to bring all components required for the actual protein synthesis together – amino acids, transport RNAs, ribosomes, etc. In the cytoplasm the protein polymers are actually "synthesized" through chemical reactions – that is why the process is known as "protein synthesis" or even more precisely – "protein biosynthesis".

The RNA copy of the protein genetic information encoded in DNA molecule is produced in the nucleus and it is called messenger RNA (mRNA). Each mRNA encodes the information for a single protein and is much smaller in size compared to the DNA molecule. This makes possible for mRNA molecules to exit the nucleus through tiny openings called nuclear pores. Once it exits the nucleus and enters the cytoplasm, the mRNA could interact with a cellular structure known as a ribosome, which serves as the cell's assembler within the process of protein synthesis. The ribosome consists of proteins and ribosome RNA molecules (rRNA), which are organized in two subunits.

The mRNA initially binds to just one of the ribosome sub-units. When the mRNA interacts with the big ribosome sub-unit, this triggers the approach of another RNA molecule, called transfer RNA (tRNA). The tRNA molecule possess a specific sequence of 3-bases (anti-codon), which hast to complement a corresponding sequence (codon) within the mRNA sequence. When it finds it, it attaches to the mRNA, as the other end of the tRNA is "loaded" with an amino acid. At this point arrives the other sub-unit of the ribosome and a complete structure is formed. The first tRNA binds to a so called "**start codon**", which is one and the same for all proteins. As the complete ribosome structure is formed, another tRNA molecule approaches. The next tRNA differs from the first one and is carrying another amino acid. Again,

the tRNA must have an anti-codon that matches complementary the second codon of the mRNA. The two amino acids carried by the first two tRNAs are bind together with help from the ribosome and using cellular energy in the form of adenosine triphosphate (ATP).

The above steps repeats until there are uncoupled codon sequences on the mRNA, and thus the chain of amino acids grows longer. Once the sequence of amino acids are successfully assembled in a protein, the two ribosome sub-units separate from each other to be joined again for later use. The actual sequence of amino acids forms the so called primary structure of the proteins. Depending on the exact composition and order of the amino acids in the protein sequence, the chain folds into a three-dimensional shape. When this happens the protein is complete. The process of protein synthesis takes place in multiple ribosomes simultaneous and all throughout the cell cytoplasm. A living cell can synthesize hundreds of different proteins every single second.

A brief summary of steps involved in the protein synthesis:

1. The protein synthesis takes place in following steps including Transcription, Activation of amino acids, Attachment of activated amino acids with t-RNA, Translation.
2. Amino acids in the cytoplasm are inactive. They cannot take part directly in protein synthesis.
3. The **activation of amino acids** is done by ATP resulting in the formation of aminoacyl adenylate and pyrophosphate.
4. Activated amino acids (aminoacyl adenylate) get attached with their specific t-RNA molecules with the help of the enzyme aminoacyl transfer RNA synthetase.
5. The product formed is known as **aminoacyl transfer RNA complex**.
6. A small ribosomal subunit attaches to the m-RNA near initiation codon.
7. The first or initiator tRNA (with f-met) pairs with this codon, a large ribosomal subunit joins to the small unit and translation begins.
8. Ribosome contains two sites, the **P-site** (for polypeptide) and the **A**-site (for amino acid). A tRNA with attached polypeptide is at the P-site and a t-RNA amino acid complex just arrives at the A-site.
9. The polypeptide is transferred and attached by a peptide bond to the newly arrived amino acid.
10. An enzyme **Peptidyl transferase** is needed to bring about the transfer. Now the t-RNA molecule at the P-site leaves.
11. **Translocation** occurs when the m-RNA along with peptide-bearing t-RNA moves from the A-site to the empty P-site. Since the ribosome has moved three nucleotides, there is a new codon now located at empty A-site.
12. Chain termination of polypeptide synthesis occurs at **stop codons** (UAA UAG and UGA) which do not code for an amino acid.
13. The arrival of a stop codon in A-site results in the completion of protein synthesis and enzymatic cleavage of last t-RNA from polypeptide.

14. Release factor attaches to A-site and protein synthesis assembly disassociated.
15. The mRNA, smaller and larger subunit of ribosomes, last tRNA, release factor and synthesized protein chain gets separated.
16. Process of protein synthesis in prokaryotes as well as eukaryotes can be regulated at different stages.
17. **Regulation** at transcription level is the most important and significant control of gene expression in prokaryotes and eukaryotes.
18. **Gene expression** can also be regulated at the level of post transcription processing of mRNA and also during post translational modifications.
19. Control of gene expression (protein synthesis) is also achieved through **operons** in prokaryotes.

8.7 GLOSSARY

Active site: A region on an enzyme that binds to a protein or other substance during a reaction.
Chromosome: A thread-like structure of nucleic acids and protein found in the nucleus of most living cells, carrying genetic information in the form of genes.
Cistron: a section of a DNA or RNA molecule that codes for a specific polypeptide in protein synthesis
Codon: A triplet of adjacent nucleotides in the messenger RNA chain, those codes for a specific amino acid in the synthesis of a protein molecule.
DNA: (Deoxyribonucleic acid), the chemical molecule that is the basic genetic material found in all cells.
Enzymes: A protein that accelerates the rate of chemical reactions. Enzymes are catalysts that promote reactions repeatedly, without being damaged by the reactions.
Eukaryotes: A eukaryote is any organism whose cells contain a nucleus and other organelles enclosed within membranes.
Exon: a segment of a DNA or RNA molecule containing information coding for a protein or peptide sequence.
Exonucleases: Exonucleases are enzymes that work by cleaving nucleotides one at a time from the end (exo) of a polynucleotide chain. A hydrolyzing reaction that breaks phosphodiester bonds at either the 3' or the 5' end occurs.
Gene: A unit of heredity which is transferred from a parent to offspring.
Genome: A full set of chromosomes; all the inheritable traits of an organism.
GTP: Guanosine-5'-triphosphate (GTP) is a purine nucleoside triphosphate. It also has the role of a source of energy or an activator of substrates in metabolic reactions.
Introns: Introns are non-coding sections of an RNA transcript, or the DNA encoding it, that are spliced out before the RNA molecule is translated into a protein.

Monocistronic: Describing a type of messenger RNA that can encode only one polypeptide per RNA molecule. In eukaryotic cells virtually all messenger RNAs are monocistronic.

M-RNA: A single-stranded molecule of RNA that is synthesized in the nucleus from a DNA template and then enters the cytoplasm, where its genetic code specifies the amino acid sequence for protein synthesis.

Nucleic acid: a complex organic substance present in living cells, especially DNA or RNA, whose molecules consist of many nucleotides linked in a long chain.

Nucleolus: A conspicuous, rounded body within the nucleus of a cell.

Nucleoprotein: Any of the class of conjugated proteins occurring in cells and consisting of a protein combined with a nucleic acid, essential for cell division and reproduction.

Nucleotide: Nucleotides are organic molecules that serve as the monomers, or subunits, of nucleic acids like DNA (deoxyribonucleic acid) and RNA (ribonucleic acid).

Nucleotides: Nucleotides are the building blocks of nucleic acids; they are composed of three subunit molecules: a nitrogenous base, a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group.

Operon: An operon is a functioning unit of genomic DNA containing a cluster of genes under the control of a single promoter.

Peptide bond: A peptide bond is a chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule, releasing a molecule of water.

Polycistronic: Describing a type of messenger RNA that can encode more than one polypeptide separately within the same RNA molecule. Bacterial messenger RNA is generally polycistronic.

Polypeptide: a linear organic polymer consisting of a large number of amino-acid residues bonded together in a chain, forming part of (or the whole of) a protein molecule.

Prokaryotes: A prokaryote is a unicellular organism that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle.

Ribonuclease: Any of the class of enzymes that catalyze the hydrolysis of RNA.

Ribozymes: Ribozymes (ribonucleic acid enzymes) are RNA molecules that are capable of catalyzing specific biochemical reactions, similar to the action of protein enzymes

RNA: Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation, and expression of genes.

Splicing: Splicing is the editing of the nascent precursor messenger RNA (pre-mRNA) transcript. After splicing, introns are removed and exons are joined together

Svedberg: A Svedberg unit is a non-SI unit for sedimentation rate.

Transcription: Transcription is the first step of gene expression, in which a particular segment of DNA is copied into RNA.

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

T-RNA: A small RNA molecule, consisting of a strand of nucleotides folded into a clover-leaf shape that picks up an unattached amino acid within the cell cytoplasm and conveys it to the ribosome for protein synthesis.

8.8 SELF ASSESSMENT QUESTIONS

8.8.1 Multiple choice questions:

1. Ribosomes are the sites of?

(a) Protein Synthesis (b) Respiration
(c) Fat metabolism (d) Photosynthesis

2. Which site of a tRNA molecule binds to an mRNA molecule?

(a) Codon (b) 5'end of the tRNA molecule
(c) Anticodon (d) 3'end of the tRNA molecule

3. The polypeptide chain is initiated by?

(a) Glycine (b) Methionine
(c) Lysine (d) Leucine

4. All the terminator codon begins with the nucleotide of-

(a) Uracil (b) Adenine
(c) Guanine (d) Cytosine

5. Which of these is **incorrect** for translation?

(a) Protein are synthesized from it (b) It is under operon regulation
(c) It occurs inside the cytoplasm (d) It occurs inside the nucleus

6. During translation initiation in prokaryotes, a GTP molecule is needed in-

(a) Formation of formyl-met-t-RNA
(b) Binding of 30S subunit of ribosome with m-RNA
(c) Association of 30S - mRNA with formyl-met-t-RNA
(d) Association of 50S subunit of ribosome with initiation complex

7. UGA, UAG and UAA are called termination codons because they-

(a) Terminate anticodon (b) Do not specify any amino acid
(c) Present at the beginning of m-RNA (d) Indicate initiation of polypeptide chain

8. The first step in the biosynthesis of polypeptide is catalysed by?

(a) Terminal transferase (b) Peptidyl transferase

(c) Initiation factors (IFs) (d) Aminoacyl-t-RNA synthetase

9. An amino-acyl synthetase is responsible for-
(a) Joining an amino acid to a t-RNA
(b) Attaching an amino acid group to an organic acid
(c) Formation of a peptide bond
(d) Non of the above

10. What will be the correct gene expression pathway?
(a) Gene – mRNA – transcription - translation - protein
(b) Transcription – gene – translation – mRNA - protein
(c) Gene – transcription – mRNA – translation - protein
(d) Gene – translation – mRNA – transcription – protein

11. Sequence of amino acids in a polypeptide is determined by?
(a) m-RNA (b) t-RNA
(c) r-RNA (d) sn-RNA

12. Operon control is a method of gene regulation in-
(a) Both eukaryotes and prokaryotes (b) Eukaryotes only
(c) Prokaryotes only (d) Plants

13. Which of the following inactivates the 60S subunit of eukaryotic ribosomes?
(a) Chloramphenicol (b) Cycloheximide
(c) Diphtheria toxin (d) Ricin

14. The role of mRNA in protein synthesis is-
(a) It translates genetic code to a specific amino acid
(b) It modifies mRNA molecules prior to protein synthesis
(c) It provides genetic blueprint for the protein
(d) It catalyzes the process

15. The role of small nuclear RNAs in protein synthesis is-
(a) It translates genetic code to a specific amino acid
(b) It modifies mRNA molecules prior to protein synthesis
(c) It provides genetic blueprint for the protein
(d) It catalyzes the process

16. The eukaryotic mRNA binding to the ribosomes is facilitated by?
(a) T-RNA (b) Poly-A tail
(c) Shine Dalgarno sequence (d) 7-methyl guanosine cap

17. Which of the following is **not** a necessary component of translation?
(a) Anticodon (b) mRNA
(c) Ligase (d) Amino acid

18. Amino acids are joined together into a protein chain by which of the following?
(a) Transfer RNA (b) DNA polymerase
(c) Hydrogen bonds (d) Messenger RNA

19. Transcription is initiated when RNA polymerase binds to-
(a) An initiator (b) A promoter
(c) A transcriptor (d) A codon

20. The genetic code consists of groups of three nucleotides called?
(a) Codons (b) Introns
(c) Anticodons (d) Reading frames

21. Initiation codon of protein synthesis in eukaryotes is-
(a) GUA (b) GCA
(c) CCA (d) AUG

22. The pathway of a tRNA during polypeptide elongation on the ribosome is-
(a) A site → P site → E site (b) P site → entry site → exit site
(c) A site → P site → entry site (d) P site → A site → E site

23. Which of the following amino acid starts all proteins synthesis?
(a) Glycine (b) Proline
(c) Thymine (d) Methionine

24. Peptide bond formation between amino acids of growing polypeptide chain is catalysed by?
(a) Peptide polymerase (b) Amino acyl-tRNA synthetase
(c) Peptidyl transferase (d) Peptidyl synthetase

25. Which of the following elongation factor is called as translocase?
(a) EF2 (b) EFG
(c) EF-Tu and EF-Ts (d) Both a and b

8.8.2 Fill in the blanks:

1. Ribosomal subunits of prokaryotic translation are _____ and _____.
2. _____ is the enzyme required for activation of amino acids.
3. Peptide bond is formed between _____.
4. _____ are recognised as the site of protein synthesis.

5. A sequence of three bases along the DNA molecule is called _____
6. A codon consists of _____ nitrogenous bases
7. The triplet **UUU** codes for _____
8. Usually _____ is the initiation codon of translation process
9. The non-sense codons help in _____of protein synthesis.
10. Terminating codons are also called _____
11. Translation of genetic information results in the synthesis of _____
12. IF3 is rich in _____and _____amino acids.
13. _____is a process to remove introns from mRNA.
14. _____DNA contains only exons whereas _____DNA contains both introns and exons.
15. Initiation amino acid in prokaryotes is_____.
16. In the formation of an initiation complex, a _____ is positioned first.
17. In a process called _____, the initial tRNA is ejected from the ribosome.
18. Proteins contain _____ different amino acids, whereas DNA and RNA are composed of _____ different nucleotides
19. The process of _____ cuts introns from the primary transcript and the final "processed" mRNA is produced.
20. As polypeptides are formed at the ribosome, elongation continues until _____is exposed.

8.8.3 True and false

1-Prokaryotic and eukaryotic ribosomes have similar functions.
2-Genes are composed of segments of "extra," nonessential materials called exons.
3-EF-1 of prokaryote is similar to EF-T of eukaryotic translation.
4-Initiator tRNA first of all binds to A-site.
5-Activation of amino acids for protein synthesis requires energy.
6-Transcription takes place in the nucleus using the DNA in the nucleus as a template for the formation of proteins.
7-Ribosomal subunits of eukaryotic translation are 40s and 60s.
8- Aminoacyl-tRNA synthetase directs the synthesis of tRNA molecules from a DNA template.
9- The main gene regulation mechanism in prokaryote is through operon regulation.
10-Shine Dalgarno (SD) sequence is found only in eukaryotes.
11-Release factor-3 (RF-3) does not possess its own release activity but stimulates binding are release of RF1 and RF2 from ribosome.
12-Single mRNA can act as template for synthesis of many proteins in eukaryotes.
13-Post translational modification occurs only in eukaryotes and not in prokaryotes.
14-The products of protein synthesis are exclusively enzymes and enzyme products.
15-Just as one amino acid may be specified by more than one codon, one codon may specify more than one amino acid.

8.8.1 Answer keys- 1-(a), 2-(b), 3-(c), 4-(a), 5-(d), 6-(c), 7-(b), 8-(d), 9-(a), 10-(c), 11-(a), 12-(c), 13-(d), 14-(c), 15-(b), 16-(d), 17-(c), 18-(a), 19-(b), 20-(a), 21-(d), 22-(a), 23-(d), 24-(c), 25-(d)

8.8.2 Answer Keys: (1) 50S, 30S, (2) Amino acyl tRNA, (3) Amino acid, (4) Ribosomes, (5) Codon, (6) Three, (7) Phenyl alanine, (8) AUG, (9) Termination, (10) Stop codons, (11) Proteins, (12) Lysine and arginine, (13) Splicing, (14) Prokaryotic, eukaryotic, (15) N-formyl methionine, (16) met-tRNA, (17) Translocation, (18) 20 & 4, (19) RNA splicing, (20) Nonsense codon

8.8.3 Answer Keys: 1-True, 2-False, 3-True, 4-False, 5-True, 6-False, 7- True, 8-False 9- True, 10- False, 11-True, 12-False, 13-False, 14-True and 15-False

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8.11 TERMINAL QUESTIONS

8.11.1 Very short answer type questions

1. What is the function of tRNA?
2. What is the function of mRNA?
3. What is promoter?
4. What are release factors?
5. Define transcription and translation?
6. What are termination codons?
7. Differentiate between codes and anticodes.
8. Define splicing?
9. What is initiation factor?
10. How does intron differ from exons?
11. Name initiation amino acids in prokaryotes and eukaryotes?
12. If sequence of codon on mRNA is UGA, what will be the sequence of anticodon on tRNA?
13. What does 'S' means in **70S** ribosomes?
14. What is Shine Dalgarno sequence?
15. What is cap and tail of mRNA?

8.11.2 Short answer type question

1. Briefly describe about structure of amino acids?
2. What is central dogma?
3. Mention the role of splicing and antisense RNA in gene regulation?
4. Point out differences between eukaryotic and prokaryotic ribosomes?
5. What is peptide bond? How its formation does occur?
6. Explain translocation with respect to elongation of polypeptide chain?
7. Briefly mention about initiation factors utilized in prokaryotic protein synthesis?
8. Mention the function of different elongation factors involved in eukaryotic elongation?
9. How termination of protein synthesis does occur?
10. Briefly describe about initiator tRNA molecule in prokaryotic and eukaryotic translation?
11. How does activation of amino acids occur?
12. What is ternary complex?
13. Differentiate between prokaryotic and eukaryotic ribosome?
14. How does a eukaryotic and prokaryotic release factor differ?

8.11.3 Long answer type question

1. Describe protein synthesis (transcription and translation). Explain the roles of chromosomal DNA, messenger RNA, transfer RNA, and ribosomal RNA in the process as well as how complementary base pairing is involved.
2. Elaborate the differences and similarities between prokaryotic and eukaryotic translation?
3. What is the difference between transcription and translation?
4. What is the role of messenger RNA and ribosomes in protein synthesis?
5. With the help of well labelled diagrams explain the process of protein synthesis in prokaryotes.
6. How do the nucleotides of mRNA chains encode information for the formation of the amino acids sequences of a protein?
7. Explain in detail how regulation of gene expression occurs in prokaryotes and eukaryotes.
8. Describing about their structure mention the role of ribosome and tRNA in protein synthesis.
9. How are amino acids brought to the sites of the cell where translation takes place? What is an anticodon?
10. Why do ribosomes move along mRNA during translation? Does an mRNA molecule codify only one type of protein?

BLOCK-3 BIOTECHNOLOGY

UNIT-9 RECOMBINANT DNA

9.1 OBJECTIVES

After reading this student will be able to know:

- Meaning of recombinant DNA and Technology
- What are cloning vectors and how do they work
- Creation and introduction of recombinant DNA construct into the host cell
- Isolation of plasmid DNA
- Analysis of plasmid DNA
- Polymerase chain Reaction (PCR)
- DNA sequencing

9.2 INTRODUCTION

All organisms on Earth evolved from a common ancestor, so all organisms use DNA as their molecule of heredity. At the chemical level, DNA is the same whether it is taken from a microscopic bacterium or a blue whale. As a result, DNA from different organisms can be "cut and pasted" together, resulting in "recombinant DNA". The first recombinant DNA molecule was produced in 1972 by Stanford researcher **Paul Berg**. Berg joined together DNA fragments from two different viruses with the help of particular enzymes; restriction enzymes and ligase. Restriction enzymes (such as EcoR1) are like "molecular scissors" that cut DNA at specific sequences. If the DNA from the different sources is cut with the same restriction enzyme, the cut ends can be joined together and then sealed into a continuous DNA strand by the enzyme ligase.

In 1973, the first organism to contain recombinant DNA was engineered by **Herbert W. Boyer** (UCSF) and **Stanley N. Cohen** (Stanford University). Together they introduced an antibiotic resistance gene into E.coli bacteria. Notably, they also produced bacteria that contained genes from the toad, ***Xenopus laevis***, which showed that, DNA from different species could be spliced together. Paul Berg was awarded the 1980 Nobel Prize in Chemistry "for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA". The ability to cut, paste, and copy molecules of DNA was not only a watershed moment for scientific research but spawned an entire industry built on genetic engineering. **Genentech**, the first biotechnology company, was founded by Herbert Boyer in 1976. By 1982, the FDA approved Genentech's first successful product, a synthetic form of human insulin produced by bacteria that were engineered to contain the insulin gene.

Today, recombinant DNA technology is used extensively in research laboratories worldwide to explore myriad questions about gene structure, function, expression pattern, regulation, and much more. One widely used application involves, genetically engineering "knock-out" animals (typically mice) to contain a non-functional form of a particular gene of interest. The goal of such experiments is to determine gene function by analyzing the consequences of the missing gene. While, knockout mice are generated to answer questions in

many different fields, they are particularly useful in developmental biology and have led to an understanding of some of the essential genes involved in the development of an organism from a single fertilized egg.

Recombinant DNA techniques are also a cornerstone of the biotechnology industry. One example is the generation of genetically engineered plants to produce an insect toxin called **Bt toxin**. The Bt gene is derived from a bacterium called ***Bacillus thuringiensis*** and produces a toxin that disrupts gut function in the larvae (caterpillars) of certain insects that are crop pests. The gene that produces Bt toxin is introduced into such plants by recombinant DNA technology, and results in the selective killing of crop-feeding insects. This development has had a major economic impact and reduced the expenses of pesticides used per year and has increased the longevity and success of several crops.

9.3 RECOMBINANT DNA TECHNOLOGY

9.3.1 What Is Recombinant DNA?

Recombinant DNA technology (also known as genetic engineering) is the set of techniques that enable the DNA from different sources to be identified, isolated and combined so that new characteristics can be introduced into an organism. Recombinant DNA is the general name for a piece of DNA that has been created by the combination of at least two strands. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, and differ only in the nucleotide sequence within that identical overall structure. Recombinant DNA molecules are sometimes called **chimeric DNA**, because they can be made of material from two different species, like the mythical chimera. R-DNA technology uses **palindromic sequences** and leads to the production of sticky and blunt ends.

The DNA sequences used in the construction of recombinant DNA molecules, can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms. Proteins that can result from the expression of recombinant DNA within living cells are termed **recombinant proteins**. When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced. Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences.

Recombinant DNA differs from genetic recombination in that the former results from artificial methods in the test tube, while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms. Recombinant DNA technology, joining together of DNA molecules from two different species that are inserted into

a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry. Since, the focus of all genetics is the gene, the fundamental goal of laboratory geneticists is to isolate, characterize, and manipulate genes.

Although, it is relatively easy to isolate a sample of DNA from a collection of cells, finding a specific gene within this DNA sample can be compared to finding a needle in a haystack. Consider the fact that each human cell contains approximately 2 meters (6 ft.) of DNA. Therefore, a small tissue sample will contain many kilometers of DNA. However, recombinant DNA technology has made it possible to isolate one gene or any other segment of DNA, enabling researchers to determine its nucleotide sequence, study its transcripts, mutate it in highly specific ways, and reinsert the modified sequence into a living organism.

Basic Principle of Recombinant DNA Technology

Although there are many diverse and complex techniques involved, the basic principles of genetic manipulation are reasonably simple. The premise on which the technology is based is that genetic information, encoded by DNA and arranged in the form of genes, is a resource that can be manipulated in various ways to achieve certain goals in both pure and applied science and medicine. Every living entity consists of one or more cells with DNA-molecules that contain the blueprint for thousands of cellular proteins. A piece of DNA with the code for a particular protein (called gene of interest), together with appropriate control codes (like promoter, operator and regulator), can be inserted into a host cell, where it becomes integrated into the latter's genome.

The recombinant cell is then grown in large quantities to produce the protein of interest. This recombinant protein, which is stored inside the cell or secreted into the culture medium, can be recovered, purified and formulated into a product used in healthcare, industry or agriculture. The host cell can be a bacterium, fungus, yeast or animal derived cell. In some cases we just want to make multiple copies of the inserted gene of interest and don't want it be translated into proteins. In this case we collect these replicated copies of the gene, purify it and store it.

One important aspect in recombinant DNA technology is DNA cloning. It is a set of techniques that are used to assemble recombinant DNA molecules and to direct their replication within host organisms. The use of the word cloning refers to the fact that the method involves the replication of single DNA molecule starting from a single living cell to generate a large population of cells containing identical DNA molecules.

DNA Cloning

In biology, a clone is a group of individual cells or organisms descended from one progenitor. This means that the members of a clone are genetically identical, because cell replication produces identical daughter cells each time. The use of the word clone has been extended to recombinant DNA technology, which has provided scientists with the ability to produce many copies of a single fragment of DNA, such as a gene, creating identical copies that constitute a

DNA clone. In practice the procedure is carried out by inserting a DNA fragment into a small DNA molecule and then allowing this molecule to replicate inside a simple living cell such as a bacterium. The small replicating molecule is called a DNA vector (carrier). The most commonly used vectors are plasmids (circular DNA molecules that originated from bacteria), viruses, and yeast cells. Plasmids are not a part of the main cellular genome, but they can carry genes that provide the host cell with useful properties, such as drug resistance, mating ability, and toxin production. They are small enough to be conveniently manipulated experimentally, and, furthermore, they will carry extra DNA that is spliced into them.

Molecular cloning is the laboratory process used to create recombinant DNA. It is one of two most widely used methods, along with polymerase chain reaction (PCR), used to direct the replication of any specific DNA sequence chosen by the experimentalist. There are two fundamental differences between the methods. One is that molecular cloning involves replication of the DNA within a living cell, while PCR replicates DNA in the test tube, free of living cells. The other difference is that cloning involves cutting and pasting DNA sequences, while PCR amplifies by copying an existing sequence.

Formation of recombinant DNA requires a cloning vector, a DNA molecule that replicates within a living cell. Vectors are generally derived from plasmids or viruses, and represent relatively small segments of DNA that contain necessary genetic signals for replication, as well as additional elements for convenience in inserting foreign DNA, identifying cells that contain recombinant DNA, and, where appropriate, expressing the foreign DNA. The choice of vector for molecular cloning depends on the choice of host organism, the size of the DNA to be cloned, and whether and how the foreign DNA is to be expressed.[8] The DNA segments can be combined by using a variety of methods, such as restriction enzyme/ligase cloning or Gibson assembly.

In standard cloning protocols, the cloning of any DNA fragment essentially involves seven steps:

(1) Choice of host organism and cloning vector,
(2) Preparation of vector DNA,
(3) Preparation of DNA to be cloned,
(4) Creation of recombinant DNA,
(5) Introduction of recombinant DNA into the host organism,
(6) Selection of organisms containing recombinant DNA, and
(7) Screening for clones with desired DNA inserts and biological properties.

Tools of Recombinant DNA technology

The enzymes which include the restriction enzymes – help to cut, the polymerases (help to synthesize) and the ligase (help to bind). The restriction enzymes used in recombinant DNA technology play a major role in determining the location at which the desired gene is inserted into the vector genome. They are two types, namely Endonucleases and Exonucleases. The Endonucleases cut within the DNA strand whereas the Exonucleases remove the nucleotides from the ends of the strands. The restriction endonucleases are sequence-specific which are

usually palindrome sequences and cut the DNA at specific points. They scrutinize the length of DNA and make the cut at the specific site called the restriction site. This gives rise to sticky ends in the sequence. The desired genes and the vectors are cut by the same restriction enzymes to obtain the complementary sticky notes, thus making the work of the ligase easy to bind the desired gene to the vector.

The vectors – help in carrying and integrating the desired gene. These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism. Plasmids and Bacteriophages are the most common vectors in recombinant DNA technology that are used as they have very high copy number. The vectors are made up of an origin of replication- This is a sequence of nucleotide from where the replication starts, a **selectable marker** – constitute genes which show resistance to certain antibiotics like ampicillin; and **cloning sites** – the sites recognized by the restriction enzymes where desired DNAs are inserted.

Host organism – into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes.

9.3.2 Formation of Recombinant DNA

In most cases, rDNA is created in a laboratory setting using a process of molecular cloning. This method allows *in vivo* DNA replication, in the living cells of the subject. A **cloning vector** is a DNA molecule that replicates inside a living cell and is used to form rDNA. The cloning vector is usually a small part of a DNA strand that holds the genetic information that is needed for the replication of cells. Polymerase chain reaction (PCR) is another method that can be used to replicate a specific DNA sequence and create rDNA, which is used to replicate DNA in a laboratory test tube.

The standard method of making recombinant DNA involves:

- Choosing the appropriate host organism and cloning vector.
- Preparation of vector DNA and DNA to be cloned.
- Creation of recombinant DNA.
- Introduction of rDNA to host organism.
- Screening for rDNA with specific properties sought from host organisms.

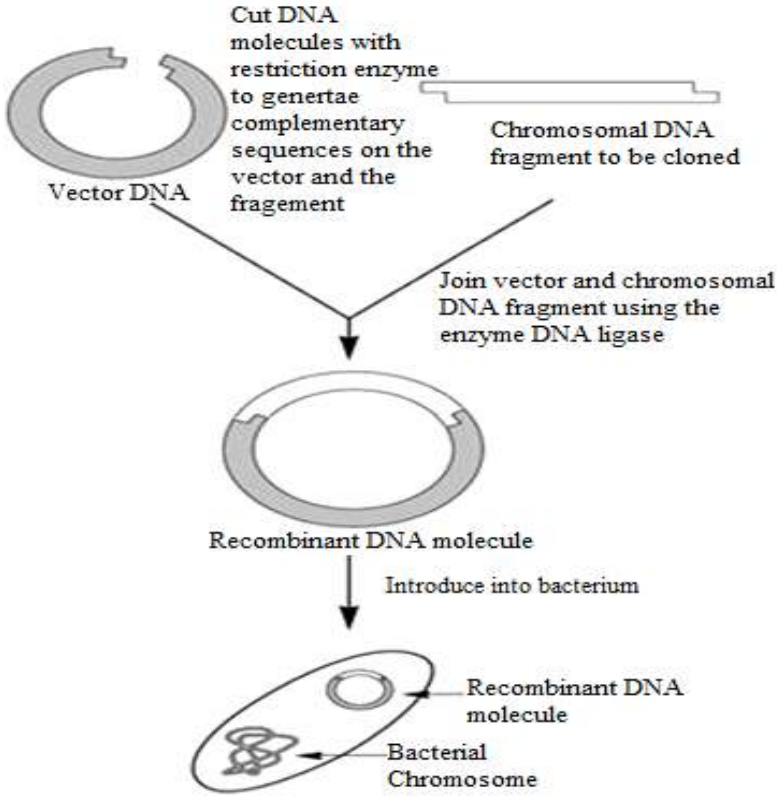

Fig. 9.1: Flow chart showing process of formation of recombinant DNA

Cloning Vectors

Major biological tool in rDNA technology is the vector which is used for delivery of desired foreign DNA into a host cell. It acts as a vehicle or carrier. The vector must possess following features when acting as cloning vector.

1. It should be easily be isolated from the organisms.
2. It should be small in size because lager vector DNA molecules often get broken during purification.
3. It must have an 'origin of replication' (ori), so that it may multiply within the host cell along with the foreign DNA.
4. Many vectors used commonly contain several recognition sites for several restriction enzymes in a small region. These are called multiple cloning sites (MCS) or polylinkers. Presence of MCS facilitates the use of restriction enzymes of choice.
5. It must have a selectable marker that is a gene which helps to select the host cell containing it from the population of the cells.
6. It must contain at least a unique recognition site for restriction enzyme, when the desired foreign DNA should be inserted.

The host cell copies the cloned DNA using its own replication mechanisms. A variety of cell types are used as hosts, including bacteria, yeast cells and mammalian cells.

Plasmid Vectors

Plasmids are defined as autonomous elements, whose genomes exist in the cell as extra chromosomal units. They are self replicating circular (rarely linear) duplex DNA molecules, which are maintained in a characteristic number of copies in a bacterial cell, yeast cell or even in the organelles found in eukaryotic cells. Although plasmids are not essential for normal cell growth and division, they often confer useful properties to the host such as resistance to antibiotics that can be selective advantage under certain conditions. Plasmids range in size from a few thousand base pairs to more than 100 kilo bases (kb). There are several plasmid cloning vectors such as PBR322, pSC102, ColE1, Ti- and Ri-DNA plasmid etc. Like the host-cell chromosomal DNA, plasmid DNA is duplicated before every cell division. During cell division, at least one copy of the plasmid DNA is segregated to each daughter cell, assuring continued propagation of the plasmid through successive generations of the host cell.

Circular plasmid DNA which is used as a vector can be cleaved at one site with the help of a **restriction enzyme** to give a linear molecule. A foreign DNA segment can now be inserted by joining the ends of broken circular DNA to the two ends of foreign DNA. Thus a bigger circular DNA molecule is generated which can now be separated by gel electrophoresis. Plasmids are the most common vectors used in plant transformation. They are used to multiply the gene of interest and to transfer foreign genes into the recipient organism. Plasmids are small circular pieces of DNA found in almost all bacteria and in some fungi, protozoa, plants and animals. Plasmids are separate from the chromosome. Chromosomes are the primary structures containing DNA in cells (9.3.2-b). Plasmids replicate independently of the chromosome.

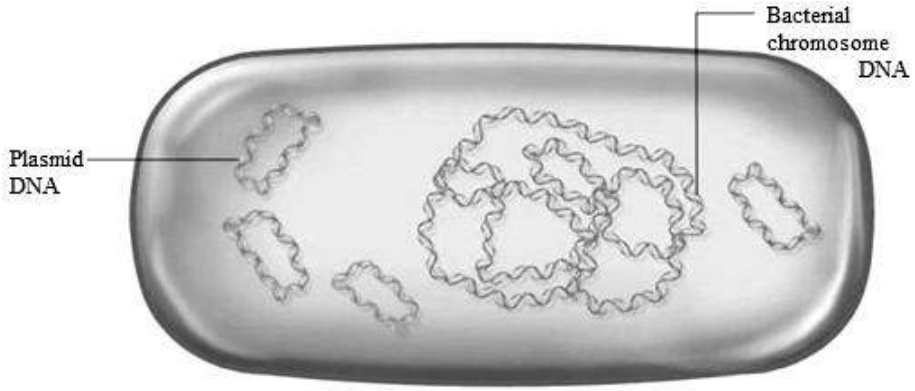

Fig. 9.2: Bacterial chromosome DNA and plasmid DNA

Shuttle Vector

The prokaryotic vectors cannot exist and work in eukaryotic cells. Therefore several vectors have been constructed which exist both in prokaryote (E coli) and eukaryotic cell. Such vectors having two origin for replication i.e. oriE and oriEuk are called shuttle vectors. The oriE, function in E coli and oriEuk, function in eukaryotic cells. These vectors also contain the antibiotic resistance gene (e.g. amp^R) that acts as selectable marker. Example: – Yeast Episomal Plasmid (YEP).

Ti Plasmid

In a case of plant a naturally occurring plasmid of the bacterium *Agrobacterium tumefaciens* by using Ti-DNA based plasmids several transgenic plants have been constructed.

Expression Vector

The goal of the cloning experiment may be to produce a foreign protein in the host. Expression of cloned genes is carried out by inserting a **'promoter sequence'** (signal for initiation of transcription), and a terminator sequence). Near cloning site a translation initiation sequence (a ribosome binding site and short codon) is also incorporated into the vector. The cloning vectors which contain these signals for protein synthesis are called expression vectors. For example: - pSOMI and pUC19

Vectors Based On Bacteriophages

Bacteriophages are viruses that infect bacterial cells by injecting their DNA into them and consequently take over the machinery of the bacterial cells to multiply themselves. The injected DNA hence is selectively replicated and expressed in the host bacterial cells resulting in a no. of phages eventually extrude out of the cells and infect neighboring cells. The ability of transferring the viral DNA from phage capsid specific bacterial cell gave insight to the scientist to exploit Bacteriophages and design them as cloning vectors.

(i) Phage Lambda: Bacteriophage lambda has a double stranded, linear DNA genome contain 48,154 bp, in which 12 bases on each end are unpaired but complementary. These ends therefore are sticky and are called **cohesive** or **cos sites** and are important for packaging DNA into phage heads. Phage genome has a large non essential region which is not involved in cell lysis. Taking advantage of it two types of cloning vectors can be produced, either by inserting foreign DNA (insertion vectors) or replacing by a foreign DNA (replacement vectors). These phage vectors allow cloning of DNA fragments up to 23 Kb in size.

(ii) Phage M-13: M-13 is a filamentous phage of E coli which infects only such cells which contain sex pilli. M-13 consists of single stranded circular DNA molecule having 6,407 bases. In the life cycle of the phage following infection of the host E coli cell the single stranded DNA is converted to a double stranded molecule which is referred to as the replicative form (RF). The RF replicates until there about 100 copies in the cell.

The major advantages of developing vectors based on M-13 are that its genome is less than 10Kb in size; the RF can be purified and manipulated exactly like a plasmid. In addition, genes cloned into M13 based vectors can be obtained in the form of single stranded DNA. Single stranded forms of cloned DNA are useful for use in various techniques including DNA sequencing and site-directed mutagenesis technique.

Restriction Enzyme

The restriction enzyme is called as molecular scissors. These act as foundation of rDNA technology. Restriction enzymes are present in bacteria and provide a type of defense mechanism called the restriction-modification system. Molecular basis of these systems was elucidated first by Werner Arber in 1965. This system consists of two components:

1. A restriction enzyme that selectively recognize a specific DNA sequence and digest any DNA fragment containing that sequence.
2. A modification enzyme that adds a methyl groups to one or two bases within the sequence recognized by the enzyme.

Types of Restriction Enzyme

There are three main types of restriction endonucleases. These are designated as Type I, Type II, and Type III.

Type I: this enzyme cuts DNA at random sites that can be more than 1000 base pairs from the recognition sequence. These move along the DNA in a reaction and require MG++, S-adenosyl methionine ATP as co-factor.

Type II: this type of restriction enzyme was first isolated by Hamilton Smith. These are simple and require no ATP for degradation of DNA. They cut DNA within the recognition sequence.

Type III: this group of endonuclease cut the DNA about 25 base pairs from the recognition sequence. In a reaction, it moves along the DNA and requires ATP as source of energy.

Naming Of Restriction Enzyme

The first letter of the name comes from the genus and the next two letters from the name of the species of the prokaryotic cell from which they are isolated. The next letter comes from the strain of the prokaryote. The roman numbers following these four letters indicates the order in which the enzymes were is isolated from the strain of bacterium. For example:

- **ECOR-I** is isolated from *Escherichia coli*, RY 13.
- **Hind-II** is isolated from *Haemophilus influenza*.
- **Bam H-I** is from *Bacillus amyloliquefaciens*.

Restriction enzyme were first discovered and studied by the molecular biologists W**. Arber, H. Smith** and **D. Nathans** for which they were awarded the Nobel Prize in 1978

9.3.3 Creation of Recombinant DNA Constructs

Host Cells

For the multiplication of foreign DNA efficient host cells are required. Different types of host cells such as E.coli, Yeast, plant and animal cells are available for gene cloning. These cells are used according to the aim of experiment. E.coli has become the most widely used organism in rDNA technology because its genetic makeup has been extensively studied. It is easy to handle and grow, can accept a range of vectors and has been extensively studied for safety. It doubles its

cell number in each 20 min. Within hour thousand of bacteria cells and that much number of foreign genes are produced and its recombinant proteins are expressed. For expression of eukaryotic gene, the eukaryotic hosts like Yeast cells are exploited, like E.coli, Yeast also has several advantages like easy to grow and manipulate, simplest unicellular eukaryotes, well characterized, requirement of no complex medium.

Construction of Recombinant DNA (rDNA)

The first step in the construction of an rDNA molecule is to isolate the vector and the fragments containing the gene to be cloned. The vector and target DNA fragments are separately digested with the same restriction enzyme such as ECOR I which generate sticky ends. The vector is then treated with alkaline phosphatase enzyme so that later in the ligation step the vector does not self ligate. The cut vector and DNA fragments are mixed in a suitable ratio and ligated with the enzyme DNA ligase to yield a recombinant vector containing insert.

The principal element in creating recombinant DNA constructs is the incorporation of the foreign DNA segment to be cloned (the so-called insert) into the vector DNA. The insert to be cloned is often a segment of the genomic DNA of an organism to be investigated, or a cDNA (complementary DNA) segment synthesized via reverse transcription from RNA molecules of the organism of interest. The genomic DNA fragments to be cloned are often produced by the fragmentation of the genome of the investigated organism by using restriction endonuclease enzymes, and the subsequent isolation of these restriction fragments. Restriction endonuclease-catalyzed fragmentation is also often necessary during the cloning of cDNA products.

The vector DNA enables the replication of the recombinant DNA construct within the host cell. (It should be noted that, in addition to the plasmid vectors, vectors of other origin (e.g. viral vectors, artificial chromosomes) are also in use. These vector types will not be dealt with within the confines of the current chapter. The appropriate linking of the vector and insert molecules takes place most often by applying restriction endonuclease digestion followed by DNA ligase-catalyzed ligation (Fig.9.3).

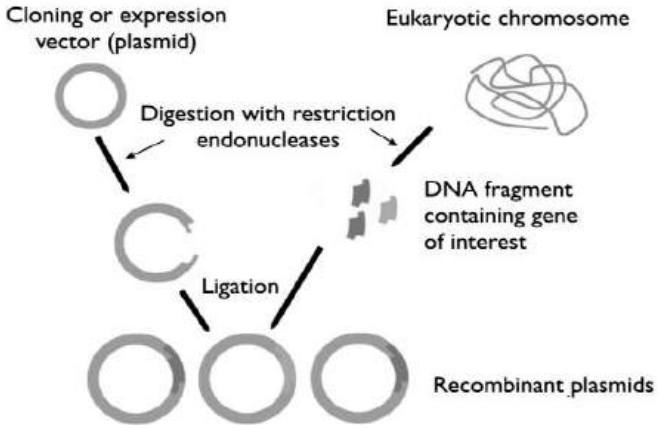

Fig.9.3: Incorporation of the insert into a plasmid vector

Among the known types of restriction endonuclease enzymes, type II enzymes are the most suitable for cloning procedures. These enzymes generally have short (4-8 base-pair (bp)) and specific recognition sequences, and cut the double-stranded DNA at specific sites within or in the vicinity of the recognition site. Other enzymes, which cut the DNA strands just opposite each other, produce blunt DNA ends with no overhangs. Bam HI, EcoRI, HaeIII, HhaI, XhoI are some of the most commonly used endonuclease enzymes.

The ends of two DNA fragments containing complementary DNA overhangs (so-called, sticky ends") can form base pairs with each other. Such overhangs are called compatible ends. Aligned compatible ends can be joined by using DNA ligase enzymes (Fig.9.3.3). The DNA ligase enzyme forms a covalent linkage between the 3'-OH group of the deoxyribose moiety of one of the DNA ends and the terminal 5'-phosphate group of the adjacent DNA molecule. Similarly, to sticky ends, two blunt DNA ends can be ligated too. Thus, two blunt ends are also considered as compatible ends.

As described above, recombinant DNA constructs are formed via the joining of the insert and vector fragments containing compatible ends. If the two ends of the vector fragment are compatible with each other (because they were cut by the same restriction endonuclease), this condition will also allow for the formation of empty (non-insert-containing) original circular vector molecules. The undesired formation of such molecules (i.e. the auto-ligation) can be prevented by treatment of the cut and isolated vector molecules with a phosphatase enzyme, which will lead to the elimination of their terminal 5'-phosphate groups. In the absence of these groups, DNA ligase will be unable to auto-ligate the two ends of the vector DNA molecule.

9.3.4 Introducing of Recombinant DNA (rDNA) Into Host Cell

The recombinant vector carrying foreign DNA needs to be transferred into the suitable host cell. These are several methods to introduce recombinant vectors and these are dependent on several factors such as the vector type and host cell. Some commonly used procedures are as follows:

(a) Transformation: In rDNA technology the most common method to introduce rDNA into living cell are called transformation. However, in nature the frequency of transformation of many cells (example- yeast and mammalian cells) is very less. Secondly, all the time host cells do not undergo transformation, because they are not prepared for it. When they develop competence factors in the cells, transformation phenomenon occurs. There are some factors which affect transformation such as concentration of foreign DNA molecule, host's cell density, temperature etc. In this procedure, bacterial cells take up DNA from the surrounding environment. In 1970, Mendel and Hugo found that E.coli cells become markabely competent to take up external DNA when suspended briefly in cold calcium chloride ($CaCl_2$) solution.

(b) Transfection: Transfection is the transfer of foreign DNA into cultured host cells mediated through chemicals. The charged chemical substances such as cationic liposome, calcium phosphate of DEAE dextran are taken and mixed with DNA molecules. The recipient host cells are overlayed by this mixture.

(c) Electroporation: An electric current is used to create transient microscopic pores in the recipient host cell membrane allowing recombinant DNA to enter.

(d) Microinjection: Exogenous DNA can also be introduced directly into animal and plant cells without the use of eukaryotic vectors. In the procedure of microinjection, foreign DNA is directly injected into recipient cells using a fine micro syringe under a phase contrast microscope to aid vision.

(e) Biolistics: Microscopic particles of gold or tungsten are coated with the DNA of interest and bombarded onto cells with a device much like a particle gun. Hence the term biolistics is used. Another method of introducing foreign genes is by natural genetic engineer *Agrobacterium tumefaciens*.

Under laboratory conditions, the isolated plasmids are introduced into bacterial host cells via a process called transformation. One of two principal means is generally applied to achieve an experimentally feasible efficiency of transformation:

- Prior to the transformation procedure, bacterial cells can be made "competent" via treatment with solutions containing bivalent cations. Plasmids are then introduced into host cells via a transformation procedure involving co-incubation and a subsequent heat shock step.
- Plasmids can also be introduced into the host cell via electroporation. During this procedure, cells co-incubated with plasmids are exposed to short pulses of electric shock.

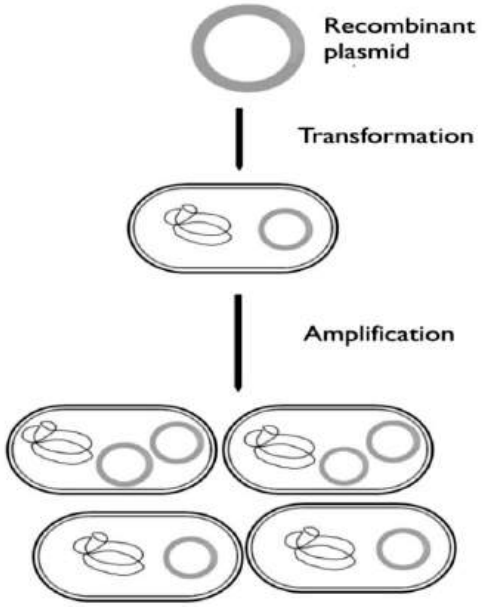

Fig.9.4: Introduction of plasmids into the host cell and their amplification

The extent of competence, which can be defined for a given set of experimental conditions, is specified as the number of plasmid-containing colonies grown after extrapolated to units of mass of plasmids used for transformation.

Even with the maintenance of the above conditions facilitating the introduction of plasmids into the host cells, only a very small fraction of bacterial cells will stably take up a

plasmid. The identification of transformed, i.e. plasmid-containing—cells is made possible by the utilization of selection marker genes. The most commonly used selection markers confer resistance to certain antibiotics. The most commonly used antibiotics and the enzymes inactivating these antibiotics are listed below.

a) **Ampicillin**, a penicillin derivative, inhibits one of the enzymes involved in the synthesis if the bacterial cell wall. Ampicillin resistance (amp^R) is conferred by an enzyme called β-lactamase, which is located in the periplasm of *E. coli*. The enzyme hydrolyses the lactam ring of penicillin. The hydrolyzed penicillin molecule is ineffective.
b) **Tetracycline** binds to one of the proteins of the 30S ribosomal subunit. This molecule inhibits the translocation of the ribosome during translation. The tetracycline resistance gene (tet^R) encodes a membrane protein that prevents the entry of the antibiotic into the host cell.
c) **Chloramphenicol** binds to the 50S ribosomal subunit, thus inhibiting protein synthesis. The chloramphenicol resistance gene (cm^R) encodes the enzyme chloramphenicol acetyl transferase (*cat*). This enzyme is localized in the cytoplasm, and acetylates chloramphenicol by using acetyl coenzyme A. The modified chloramphenicol molecule is unable to bind to the ribosome.

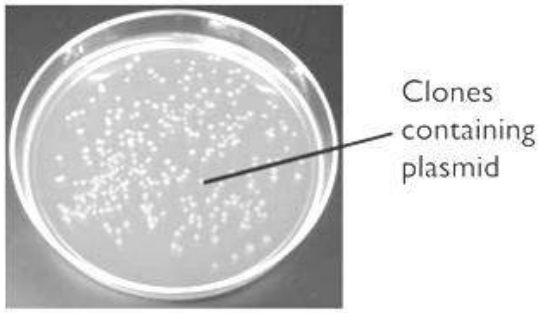

Fig.9.5: Appearance of plasmid-containing bacterial colonies on an antibiotic containing agar nutrient plate

Following transformation, bacterial cells are spread onto a nutrient agar plate containing antibiotics according to the resistance gene contained in the plasmid. Only the plasmid-containing resistant bacteria will be able to grow on the antibiotic-containing agar plate. Ideally, the appearance of separate bacterial colonies can be observed on the agar nutrient plate (Fig. 9.3.4-b). Theoretically, one colony contains the descendants (clones) of a single bacterial cell that had taken up the plasmid. Following the selection of plasmid-containing bacterial cells, the next step is the identification of cells harboring recombinant plasmids, i.e. the plasmids containing the insert of interest.

Identification of Recombinants & Insertional Selection Inactivation Method

(i)Identification of recombinants: After the introduction of rDNA into suitable host cells, it is essential to identify those cells which have received the rDNA molecules. This process is called screening or selection. Generally, the selection methods are based on the expression or

non-expression of certain traits such as antibiotic resistance, expression of an enzyme such as β-galactosidase or protein such as GFP (Green Fluorescent Protein) and dependence or independence of a nutritional requirement such as the amino acid leucine. The most widely applied methodologies for the identification of recombinant colonies are the following.

(ii) Direct selection of recombinants: If the host E.coli cells have taken up the plasmid pBR322, then these cells will grow in media containing the antibiotic ampicillin or tetracycline, whereas, normal E.coli cells will be killed by the antibiotics. Thus, only transformed cells, however few, will be selected for growth and division.

(iii) Insertional selection inactivation method: This is more efficient method than the direct selection. In this approach, one of the genetic traits is disrupted by inserting foreign DNA. If the foreign DNA segment (the insert) is inserted within an antibiotic resistance gene, the successful insertion will result in the loss of the corresponding antibiotic resistance. Antibiotic resistance genes act as a good insertion inactivation system. Plasmid pBR322 contains two antibiotic resistance genes, one for ampicillin (ampR gene), and the other for tetracycline (tetR gene).

If the target DNA is inserted into tetR gene using Bam HI, the property of resistance to tetracycline will be lost. Such recombinants would be test sensitive. When such recombinants (containing target DNA in tetR gene) are grown into medium containing tetracycline, they will not grow because their tetR gene has been inactivated. But they are resistant to ampicillin because ampR gene is functional. On the other hand, the self-ligated recombinants will show resistance to ampicillin and tetracycline. Therefore, they will grow on medium containing both the antibiotics.

(iv) Blue-white selection method: Another powerful method of screening for the presence of recombinant plasmid is referred to as blue-white selection. This method is based upon the insertional inactivation of the lac-Z gene present on the vector (e.g. PUC19). This gene expresses the enzyme β-galactosidase whose activity can cleave a colorless substrate called X-gal into blue colored product.

If the lac-Z gene is inactivated due to the presence of the insert, then the enzymes is not expresses. Hence, if after a transformation experiment the E.coli host cells are plated on an ampicillin and X-gal containing solid media plate then colonies which appears blue are those which have transformed cells (antibiotic resistant) but do not have the insert (express active enzyme). Colonies which appear white are both ampicillin resistant and have the insert rDNA and thus are the cells to be used for future experiment.

9.3.5 Analysis of Plasmid DNA by Gel Electrophoresis

Agarose gel electrophoresis is the most commonly used method for the size- and shape-based separation of DNA molecules comprising several hundred or more base pairs, including plasmid DNA molecules. Agarose is one of the main components of agar extracted from the cell wall of red algae. Agarose is a linear polysaccharide composed of galactose and anhydrogalactose units.

The Agarose gel possesses a number of features that make it especially advantageous for the purposes of gel electrophoresis. The gel is hydrophilic, chemically inert and stable. It does not bind the dye molecules used to visualize DNA molecules that are separated in the gel. The three-dimensional matrix of the Agarose gel is brought about by non-covalent bonds formed between polysaccharide units.

As the structure of the Agarose gel is held together by non-covalent bonds, the gel undergoes a phase transition at elevated temperature, and forms a sol state. The gel is prepared by mixing Agarose powder into a running buffer, with subsequent formation of the sol state at high temperature, casting and subsequent cooling. The pore size of the resulting gel depends on Agarose concentration. The pore size determines the size range of DNA molecules that can be efficiently separated in the gel. For Agarose gel electrophoresis, gels with Agarose concentrations of 0.5-3 w/v % are generally used. The lower and higher ends of this concentration range are applicable in the case of larger and smaller DNA molecules, respectively.

DNA is a negatively charged molecule. Therefore, the electric field applied during electrophoresis will cause migration of DNA molecules towards the positive pole, i.e. the anode. Therefore, the gel is placed into the electrophoresis tank in an orientation that the sample loading wells will be towards the negative pole (i.e. the cathode).

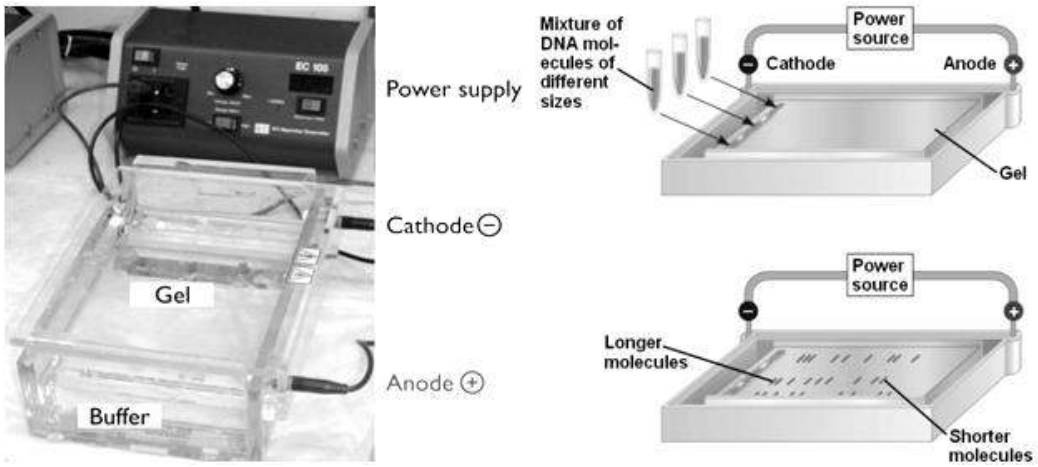

Fig.9.6: Gel electrophoresis apparatus

9.3.6 Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) can be used for the selective amplification of a specific segment (target sequence or amplicon) of a DNA molecule. The DNA to be amplified can theoretically be present in a very small amount-even as a single molecule. The PCR reaction is carried out *in vitro* and, as such, it does not require a host organism. The size of the DNA region amplified during the PCR reaction typically falls within the range of 100 bp to 10 kbp. In 1983, **Kary Mullis** (American biochemist) invented the polymerase chain reaction (PCR). The basic

principle under laying this technique is that when a double stranded DNA molecule is heated to a high temperature, the two DNA strands separate giving rise to single stranded DNA molecules which can be made to hybridize with small oligonucleotides primer (single stranded) by bringing down the temperature. If to this an enzyme called DNA polymerase and nucleotide tri phosphate is added, much like what happen during replication i.e. primer extension occurs. This event is repeated several times; therefore original DNA strand is produced in multiple copies. Due to reaction in several cycles, it is called polymerase chain reaction (PCR).The basic requirements of PCR reactions are:

1. DNA template to be amplified.
2. Primers which are oligonucleotides, usually 10-18 nucleotides long, that hybridizes to the target DNA, one to each strand of DNA.
3. DNA polymerase enzyme which is stable at temperature above 80˚C. Taq polymerase which has been isolated from a thermostable bacterial species, *Thermus aquaticus*, is used.
4. Deoxynucleotide triphosphate and PCR buffer.

Working Mechanism of PCR

The action of PCR includes several cycles. A single PCR amplification cycle involves these basic steps:

1. Denaturation: The two strands of DNA are separated by applying a high temperature (95˚C). After separation each strand acts as template for DNA synthesis.

2. Primer annealing: Each single strand anneals with a primer at a lower temperature between 50˚ – 60˚in such a way that extension can occur from it in a 5'-3' direction. The annealing temperature (in ˚C) can be calculated using the formula- T= 2(AT) + 4(G+C).

3. Extension (Polymerization): In this step, the enzyme Taq Polymerase extends each primer using dNTPs and the DNA strand as template. The temperature for extension is around 70˚C. The procedure is repeated and each set of steps is considered as one cycle (i.e. Denaturation, annealing and extension). At the end of one cycle two DNA molecules becomes four and this geometric progression occurs with each cycle. Always after n number of cycles, 2n molecules of DNA are generated using single stranded DNA as template.

Applications of PCR

The invention of PCR technique has revolutionized every aspect of modern biology. To detect pathogens, microbiologists in the past used techniques based on culturing and detecting antibodies against enzymes or protein specific to the pathogen. They are grown slowly so their cells are found less in number in the infected cell or tissue. It is difficult to culture them on artificial medium. Apart from taking time, many of these procedures were not specific. Hence, for their diagnosis, PCR-based assays have been developed. These detect the presence of certain specific sequences of the pathogen present in the infected cells or tissues. Some of the characteristics of PCR are as follow:

1. PCR diagnosis is faster, safer and more specific because it does not use live pathogen.

2. PCR is also valuable tool in forensic science as large amounts of DNA can be amplified from the small amounts present at the crime site, for DNA fingerprinting analysis.
3. In recent years, PCR has also found use in detecting specific microorganisms from environmental samples of soil, sediments and water.
4. In humans, there are thousands of generic diseases. Mutations are also related to genetic diseases. Presence of faulty DNA sequence can be detected before establishment of diseases.
5. It is useful in parental diagnosis of genetic diseases.
6. Polymerase chain reaction (PCR) is a common laboratory technique used to make many copies (millions or billions!) of a particular region of DNA. This DNA region can be anything the experimenter is interested in. For example, it might be a gene whose function a researcher wants to understand, or a genetic marker used by forensic scientists to match crime scene DNA with suspects.
7. Typically, the goal of PCR is to make enough of the target DNA region that it can be analyzed or used in some other way. For instance, DNA amplified by PCR may be sent for sequencing, visualized by gel electrophoresis, or cloned into a plasmid for further experiments.
8. PCR is used in many areas of biology and medicine, including molecular biology research, medical diagnostics, and even some branches of ecology.

Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the thermophilic bacterium *Thermus aquaticus*. If heat-susceptible DNA polymerase is used, it will denature every cycle at the Denaturation step. Before the use of Taq polymerase, DNA polymerase had to be manually added every cycle, which was a tedious and costly process.[9] This DNA polymerase enzymatically assembles a new DNA strand from free nucleotides, the building blocks of DNA, by using single-stranded DNA as a template and DNA oligonucleotides (the primers mentioned above) to initiate DNA synthesis.

The PCR, like recombinant DNA technology, has had an enormous impact in both basic and diagnostic aspects of molecular biology because it can produce large amounts of a specific DNA fragment from small amounts of a complex template. Recombinant DNA techniques create molecular clones by conferring on a specific sequence the ability to replicate by inserting it into a vector and introducing the vector into a host cell. PCR represents a form of "in vitro cloning" that can generate, as well as modify, DNA fragments of defined length and sequence in a simple automated reaction. In addition to its many applications in basic molecular biological research, PCR promises to play a critical role in the identification of medically important sequences as well as an important diagnostic one in their detection.

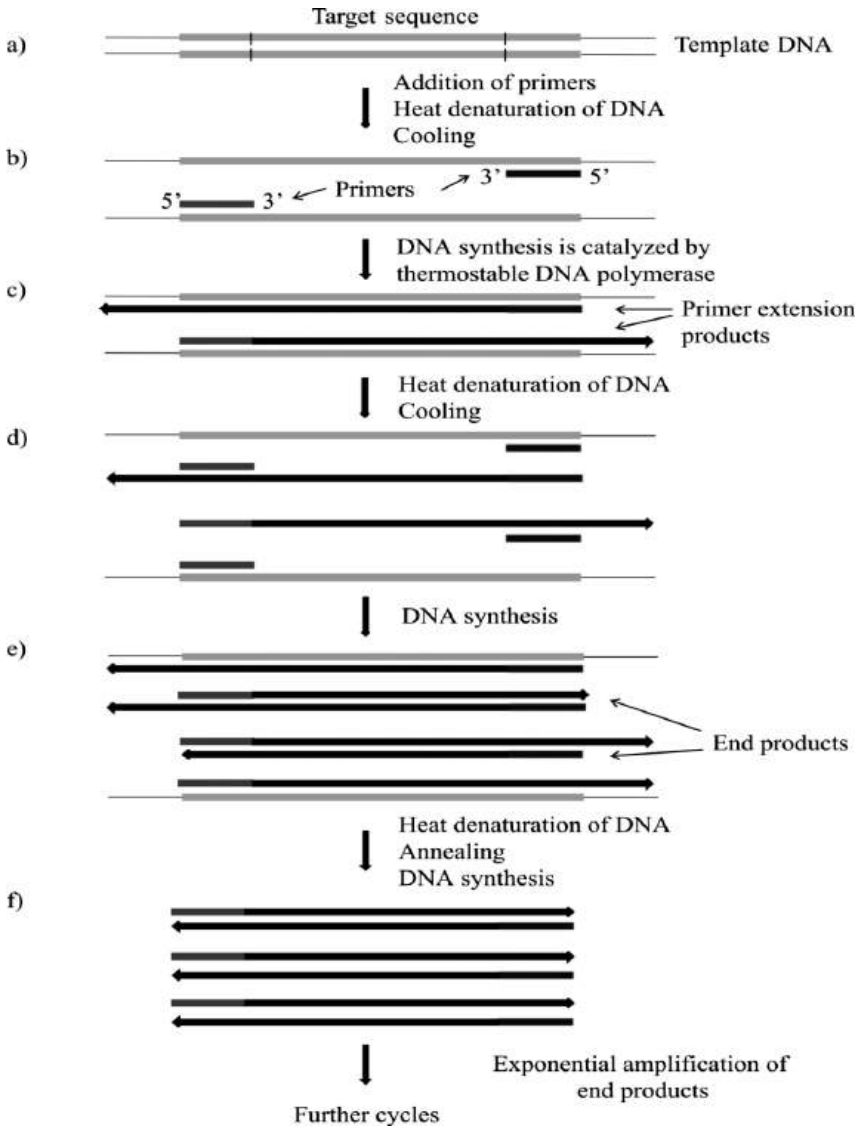

Fig.9.7: Cycles of the polymerase chain reaction (PCR)

In order to successfully perform the reaction described above, the following solution components are necessary:

a) DNA molecules that serve as template for the reaction. The amount of the template can be very low—in principle, the reaction can start even from a single template molecule. Another advantage of PCR is that the selective amplification of the desired DNA segment can be accomplished even using a heterogeneous DNA sample as template.

b) A pair of oligonucleotides serving as primers. The 3' ends of the oligonucleotides must be able to anneal to the corresponding strands of the template. A further advantage of PCR is that the 5' end of the applied primers may contain segments that do not anneal to the original template. These regions of the primers may be specific engineered sequences or even contain labeling or other modifications, which will be present in the end product and thus facilitate its

further analysis and/or processing. (For instance, recognition sites of restriction endonucleases can be incorporated in order to facilitate the subsequent cloning of the PCR product.)

c) The DNA polymerase enzyme catalyzing DNA synthesis. As the heat-induced denaturation of the template is required during each cycle, heat stable polymerases are usually applied that originate from thermophilic organisms (e.g. *Thermus aquaticus* (Taq) or *Pyrococcus furiosus* (Pfu) DNA polymerase).

d) Deoxyribonucleoside triphosphate (dNTP) molecules that serve as building blocks for the DNA strands to be synthesized. These include dATP (deoxyadenosine triphosphate), dGTP (deoxyguanosine triphosphate), dTTP (deoxythymidine triphosphate), and dCTP (deoxycytidine triphosphate).

e) A buffer providing optimal reaction conditions for the activity of DNA polymerase. Among other components, PCR buffers contain bivalent cations (e.g. Mg^{2+} or Mn^{2+}).

For an effective polymerase chain reaction, it is necessary to change the temperature of the solution rapidly, cyclically and in a wide range. This can be achieved by using a programmable instrument containing a thermoblock equipped with a **Peltier cell**. To achieve effective heat exchange, PCR reactions are performed in thin-walled plastic tubes in small reaction volumes (typically, in the order of 10-200 μl). The caps of the PCR tubes are constantly held at high temperature by heating the lid of the thermoblock, in order to prevent condensation of the reaction mixture in the upper part of the tubes. In the absence of a heated lid, oil or wax can be layered on top of the aqueous PCR samples in order to prevent evaporation.

The programmed heat profile of a PCR reaction generally consists of the following steps:

a. Initial Denaturation of the template, performed at high temperature (typically, around 95°C).
b. **Denaturation:** Heat-induced separation of the strands of double-stranded DNA molecules at high temperature (typically, around 95°C).
c. **Annealing:** Cooling of the reaction mixture to a temperature around 45-65°C in order to facilitate the annealing of the oligonucleotide primers to complementary stretches on template DNA molecules.
d. **DNA synthesis:** This step takes place at a temperature around the optimum of the heat-stable DNA polymerase (typically, 72°C), for a time period dictated by the length of the DNA segment to be amplified (typically, 1 minute per kilo-base pair).
    - Steps (b)-(d) are repeated typically 20-35 times, depending on the application.
    - Final DNA synthesis step: After completion of the cycles consisting of steps (b)-(d), this step is performed at a temperature identical to that during step (d) (72^{o}C), in order to produce complementary strands for all remaining single-stranded DNA molecules.

9.3.7 DNA Sequencing

Determination of the base sequence of DNA molecules (DNA sequencing) is necessary in various cases. Below we provide an overview of DNA sequencing methodologies.

Principle of chain-termination DNA sequencing

The principles of the Sanger-Coulton DNA sequencing method were laid down in the late 1970s in order to provide a readily executable, rapid, and relatively cheap method to determine the nucleotide sequence of DNA molecules. During the procedure, the two strands of the DNA to be sequenced are separated via high-temperature treatment, and then a short single-stranded DNA molecule (a primer oligonucleotide), is hybridised upstream (5') of the region of interest within the strand to be sequenced. Four different sequencing reaction mixtures are assembled. All reaction mixtures contain the DNA template, the sequencing primer, the DNA polymerase enzyme, and equal amounts of the four deoxynucleoside triphosphate (dNTP) building blocks. In addition, each of the four parallel reactions contains one of four dideoxynucleoside triphosphate molecules (ddNTPs). In the ribose ring of ddNTPs, the 3' carbon atom has an attached H atom instead of the -OH group present in "normal" dNTPs.

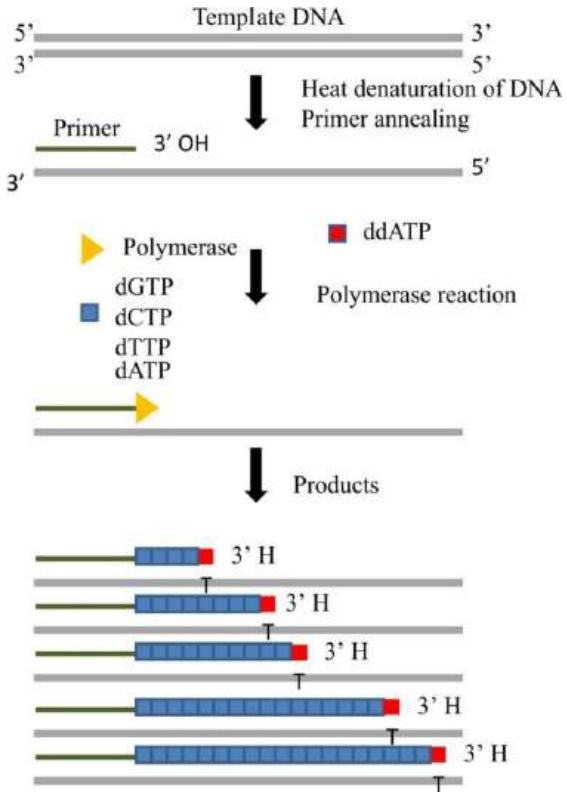

Fig.9.8: DNA sequencing with the chain termination method

Size-based separation of DNA strands in a Polyacrylamide gel

The knowledge of the sizes of the DNA strands synthesized in the four different sequencing reactions enables the determination of the base sequence of the template DNA molecule. Agarose gel electrophoresis is suitable only for the separation of large (mostly double-stranded) DNA chains. This technique can detect a difference of at least 100 base pairs. High-resolution separation of shorter DNA strands can be achieved via Polyacrylamide gel electrophoresis combined with urea Denaturation. Similarly to the situation during Agarose gel electrophoresis, in PAGE the negatively charged DNA molecules are separated in an electric field while they are moving towards the anode. However, in the Polyacrylamide gel that has a much smaller pore size than the Agarose gel, it is possible to resolve much smaller size differences (down to a

single nucleotide unit). Polyacrylamide gels are created using synthetic acrylamide and bisacrylamide mixtures.

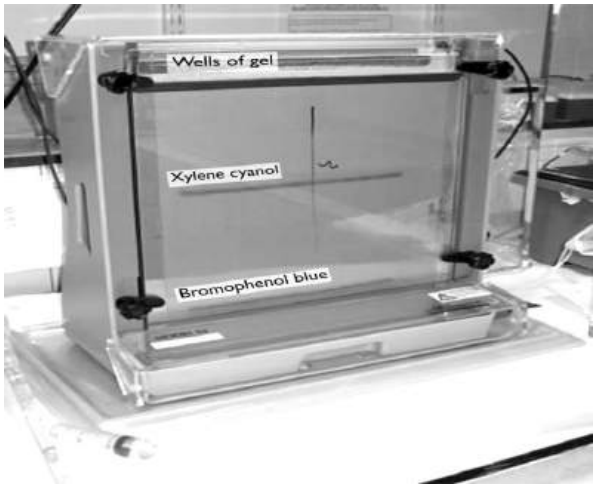

Fig.9.9: Polyacrylamide gel electrophoresis

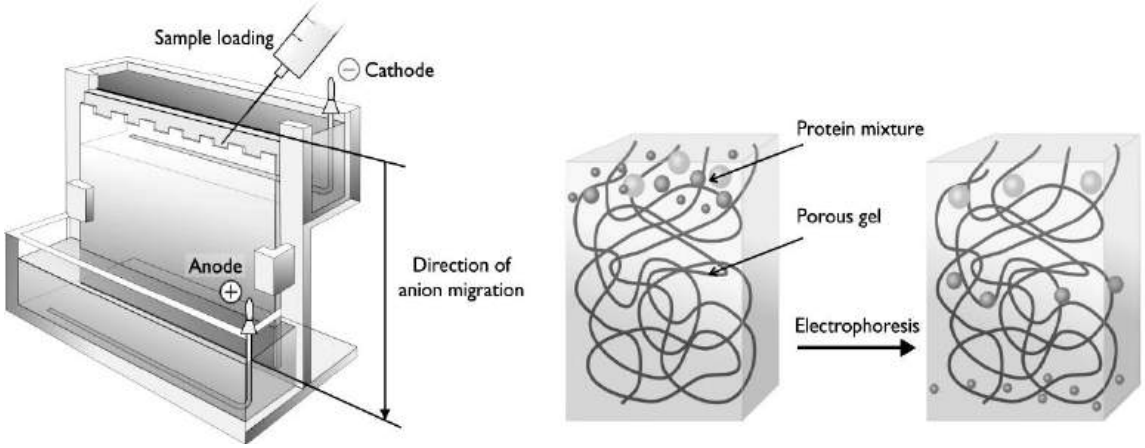

Fig. 9.10: Separation of proteins in a Polyacrylamide gel

Detection of DNA and determination of the base sequence

In the sequencing gel, the detection of DNA is achieved by applying a sensitive radiometric method. During the sequencing reaction, the DNA strands are labeled by using α-^{32}P-dATP (α-^{32}P-deoxyadenosine triphosphate). Following the run, the gel is dried and placed onto a radiosensitive photo paper. ^{32}P is a β-emitting radioactive isotope. During β-decay, an electron leaves the nucleus of the isotope. As the electrons interact with the photo paper, the film will darken, thus, the "fingerprint" of DNA bands will become visible. Recently, photo papers have mostly been replaced by the usage of Phosphorimager equipment, which is reusable and offers enhanced sensitivity. Radioisotope techniques have the advantages of low cost and very high sensitivity. However, their application presents a considerable health hazard (due to carcinogenicity), and thus requires special care and attention.

Automated DNA sequencing

In the currently most widely applied method, the fluorescent dyes are covalently linked to the ddNTP molecules. The fluorophores are attached to ddNTPs in a way that they do not interfere with the enzymatic incorporation of the given ddNTP molecule into the nascent DNA strand. The four different ddNTP molecules have four different fluorophores attached, characterized by different absorption and emission wavelengths. This setup does not need four different sequencing reactions: the four different labeled ddNTP molecules are added to a single sample at the same time. After performing the sequencing reaction using the DNA polymerase enzyme, samples are loaded in a single well of the gel. Following gel electrophoresis, the gel is placed into a reader that is capable of separately detecting the signals of all four fluorescent ddNTP conjugates. By reading in the direction towards the top of the gel, the base sequence of the template DNA strand can be determined. A key advantage of the method is that all four (differently labelled) ddNTPs are present in a single reaction mixture, enabling the determination of the base sequence of DNA.

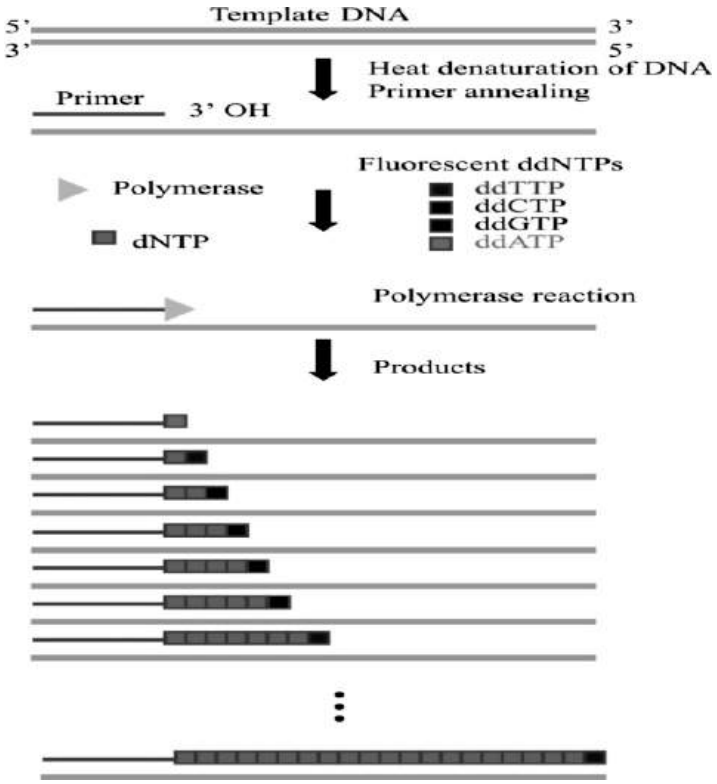

Fig. 9.11: Automated sequencing of DNA using fluorescently-labeled ddNTP Deoxyribonucleoside analogues

The use of fluorescent dNTPs has enabled the full automation of the sequencing procedure. Instead of slab gel electrophoresis, the DNA strands synthesized during the sequencing reaction are separated via capillary gel electrophoresis. During capillary gel electrophoresis, the gel matrix is contained in a capillary with a length of 50-70 cm and an inner diameter of 50-100 µm.

The sample is loaded into the capillary, and then an electric voltage is applied between the ends of the capillary. Thus, the DNA molecules will start to migrate towards the positively charged anode.

During migration, the gel matrix will exert a molecular sieving effect, and thus the molecules of smaller sizes will migrate faster. At the anode-oriented end of the capillary, the fluorescent signals linked to the four different ddNTP molecules are continuously monitored and recorded via a computer. The result of the continuous detection will be a chromatogram on which the time-dependent passage of different fluorophores is tracked. This way, the sequence of the template DNA molecule can be determined.

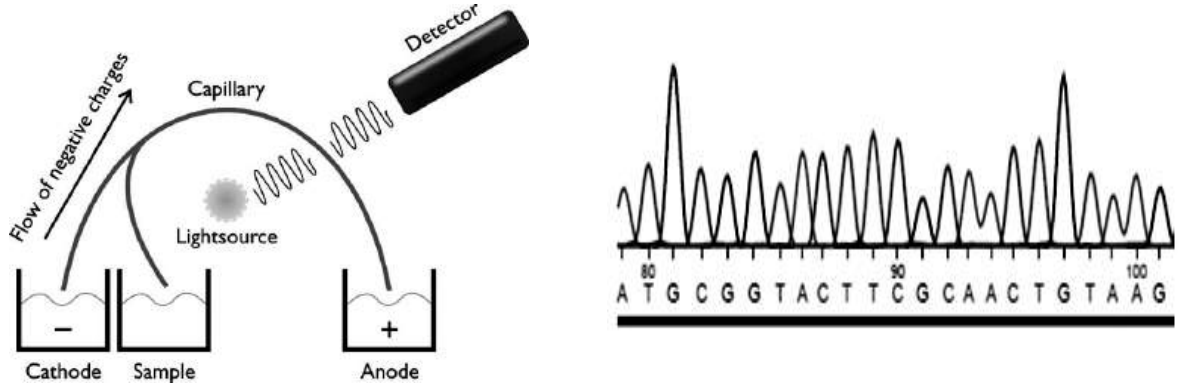

Fig.9.12: Capillary gel electrophoresis and Chromatogram produced via a capillary gel electrophoretic method

Second-generation sequencing methods

With the help of a fully automated procedure, a DNA stretch of about 1000 bases can be read. With an automated apparatus working at full capacity (which can typically run 96 samples at a time), ca. 5 million bases can be read in one day. The size of the human genome is ca. 3.2 billion base pairs. In an ideal case, considering the operating time of one sequencing apparatus, it would take around 3 years to read the entire human genome. In recent years, so-called second-generation sequencing techniques have emerged. These techniques represented a major breakthrough in the field of DNA sequencing by enabling the parallel reading of as many as 10^5-10^6 different DNA samples in a single experiment. These procedures do not require the laborious and time-consuming size-based separation of DNA molecules. Production and development of second-generation DNA sequencing apparatuses has become a remarkably big line of industry. Many different biotechnological companies provide second-generation DNA sequencers applying various methodological principles.

9.3.8 Genomic and cDNA library

Genomic library

A genomic library is a collection of independently isolated vector linked DNA fragments derived from a single organism. It contains at least one copy of every DNA sequence in the genome. An

ideal library is one that represents all of the sequences with smallest possible number of clones. The genomic DNA libraries can be prepared by the complete digestion of the total genomic DNA with a restriction enzyme and the fragments are inserted into a suitable vector like X phages (Fig.9.13).

The drawback of this method is that sometimes the sequence of interest may contain multiple restriction sites, so digestion with RE results into two or more pieces, in this method the eukaryotic DNA is broken up into smaller fragments, thus an entire library would nece-ssarily contain a large number of phages, and screening of which is very laborious.

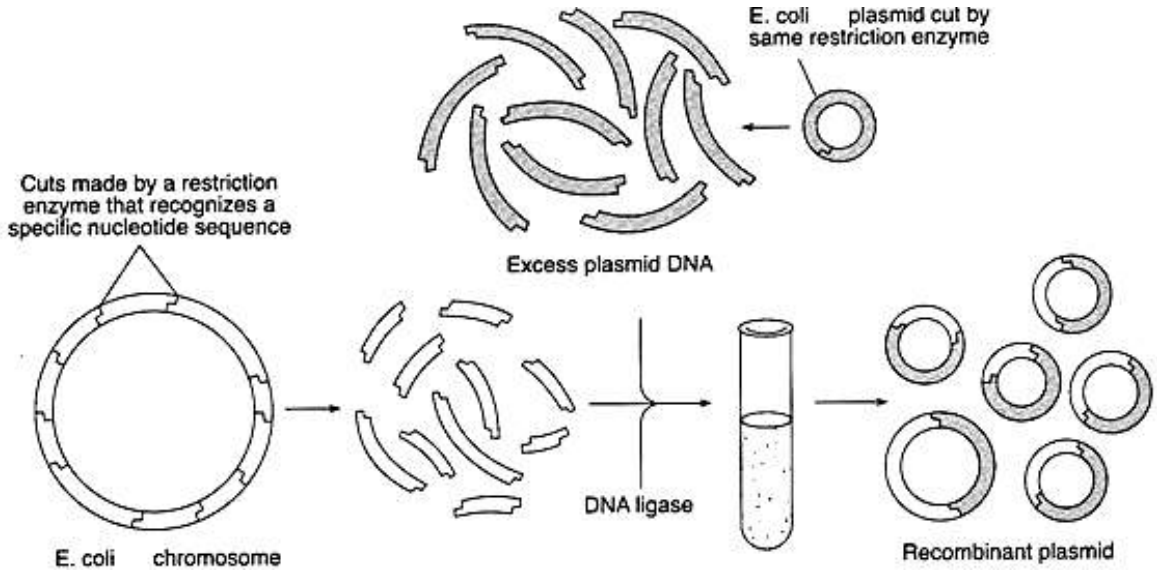

Fig. 9.13: Construction of a genomic library using recombinant DNA technology

The problems of this method can be avoided by random shearing of total DNA and cloning of large fragments. This method ensures that sequences are not excluded from the cloned library simply because of the distribution of restriction sites. In this procedure the randomly fragmented DNA is partially digested with RE which has short recognition sites. The fragments of desired size are collected through agarose gel electrophoresis, so the population of overlapping fragments that are close to random can be cloned directly.

cDNA Library:

Complementary DNA (cDNA) libraries can also be prepared by isolating mRNAs from tissues which are actively synthesizing proteins, like roots and leaves in plants, ovaries or reticulocytes in mammals, etc. The mRNAs are used for copying them into cDNAs through the use of reverse transcriptase. Then the cDNA molecule can be made double stranded and cloned (Fig.9.14).

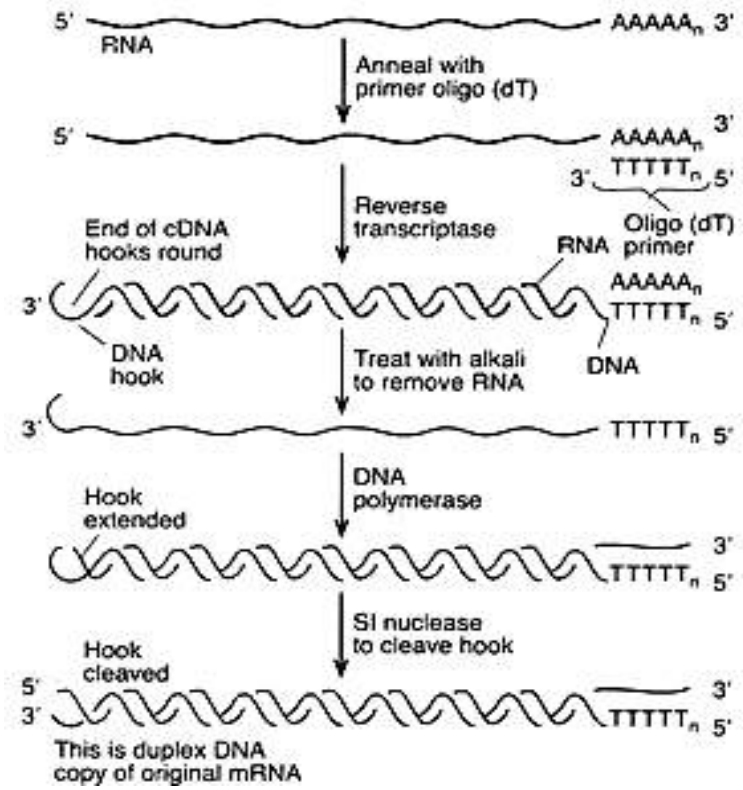

Fig. 9.14: Synthesis of cDNA from mRNA using reverse transcriptase enzyme

However, cDNA clones will differ from genomic clones in lacking the introns present in split genes, and have the advantage of being capable to be expressed in bacteria, which do not have the machinery to process the eukaryotic mRNA. There are far less number of cDNA clones in a bank than in a genomic library, which makes easier to look for a desired gene. Screening of cDNA bank also provides fairly unambiguous results.

9.3.9 Applications of Recombinant DNA Technology

Genetic engineering or rDNA technology has enormous and wide-spread applications in all the fields of biological sciences. Some important applications of rDNA technology are enlisted below:

(1) Production of Transgenic Plants: By utilizing the tools and techniques of genetic engineering it is possible to produce transgenic plants or the genetically modified plants. Many transgenic plants have been developed with better qualities like resistance to herbicides, insects or viruses or with expression of male sterility, etc.

Also they allow the production of commercially important biochemical, pharmaceutical compounds, etc. Genetic engineering is capable of introducing the improved post-harvest characteristics in plants also. Transgenic plants also aid in the study of the functions of genes in plant species.

(2) Production of Transgenic Animals: By the use of rDNA technology, desired genes can be inserted into the animal so as to produce the transgenic animal. The method of rDNA technology aids the animal breeders to increase the speed and range of selective breeding in case of animals. It helps for the production of better farm animals so as to ensure more commercial benefits.

Another commercially important use of transgenic animals is the production of certain proteins and pharmaceutical compounds. Transgenic animals also contribute for studying the gene functions in different animal species. Biotechnologists have successfully produced transgenic pigs, sheep, rats and cattle.

(3) Production of Hormones: By the advent of techniques of rDNA technology, bacterial cells like E.coli are utilized for the production of different fine chemicals like insulin, somatostatin, somatotropin and p-endorphin. Human Insulin Hormone i.e., Humulin is the first therapeutic product which was produced by the application of rDNA technology.

The genes of interest are incorporated into the bacterial cells which are then cloned. Such clones are capable of producing a fair amount of hormones like insulin which have great commercial importance.

(4) Production of Vaccines: Vaccines are the chemical preparations containing a pathogen in attenuated (or weakened) or inactive state that may be given to human beings or animals to confer immunity to infection. A number of vaccines have been synthesized biologically through rDNA technology.

These vaccines are effective against numerous serious diseases caused by bacteria, viruses or protozoa. These include vaccines for polio, malaria, cholera, hepatitis, rabies, smallpox, etc. The generation of DNA vaccines has revolutionized the approach of treatment of infectious diseases. DNA-vaccine is the preparation that contains a gene encoding an immunogenic protein from the concerned pathogen.

(5) Biosynthesis of Interferon: Interferon's are the glycoprotein's which are produced in very minute amounts by the virus-infected cells. Interferon's have antiviral and even anti-cancerous properties. By rDNA technology method, the gene of human fibroblasts (which produce interferons in human beings) is inserted into the bacterial plasmid.

These genetically engineered bacteria are cloned and cultured so that the gene is expressed and the interferons are produced in fairly high quantities. This interferon, so produced, is then extracted and purified.

(6) Production of Antibiotics: Antibiotics produced by microorganisms are very effective against different bacterial or protozoan diseases. Some important antibiotics are tetracycline, penicillin, streptomycin, etc. Recombinant DNA technology helps in increasing the production of antibiotics by improving the microbial strains through modification of genetic characteristics.

(7) Production of Commercially Important Chemicals: Various commercially important chemicals can be produced more efficiently by utilizing the methods of rDNA technology. A few

of them are the alcohols and alcoholic beverages obtained through fermentation; organic acids like citric acid, acetic acid, etc. and vitamins produced by microorganisms.

(8) Application in Enzyme Engineering: As we know that the enzymes are encoded by genes, so if there are changes in a gene then definitely the enzyme structure also changes. Enzyme engineering utilizes the same fact and can be explained as the modification of an enzyme structure by inducing alterations in the genes which encode for that particular enzyme.

(9) Prevention and Diagnosis of Diseases: Genetic engineering methods and techniques have greatly solved the problem of conventional methods for diagnosis of diseases. It also provides methods for the prevention of a number of diseases like AIDS, cholera, etc. Monoclonal antibodies are useful tools for disease diagnosis. The monoclonal antibodies bear specificity against a specific antigen. These are used in the diagnosis of diseases due to their specificity.

(10) Gene Therapy: Gene therapy is undoubtedly the most beneficial area of genetic engineering for human beings. It involves delivery of specific genes into human body to correct the diseases. Thus it is the treatment of diseases by transfer and expression of a gene into the patients' cells so as to ensure the restoration of a normal cellular activity.

(11) Practical Applications of Genetic Engineering: Recombinant DNA technology has an immense scope in Research and Experimental studies. It is applied for:

a. Localizing specific genes.
b. Sequencing of DNA or genes.
c. Study of mechanism of gene regulation.
d. Molecular analysis of various diseases.
e. Study of" mutations in DNA, etc.

(12) Applications in forensic science: The applications of rDNA technology in forensic sciences largely depend on the technique called DNA profiling or DNA fingerprinting. It enables us to identify any person by analyzing his hair roots Wood stains, serum, etc. DNA fingerprinting also helps to solve the problems of parentage and to identify the criminals.

(13) Biofuel Production: Biofuels are derived from biomass and these are renewable and cost effective. Genetic engineering plays an essentially important role in a beneficial and large scale production of biofuels like biogas, bio hydrogen, biodiesel, bio-ethanol, etc. Genetic engineering helps to improve organisms for obtaining higher product yields and product tolerance. Genetically stable high producing microorganisms are being developed by using modern rDNA techniques, which aid in an efficient production of bioenergy.

(14) Environment Protection: Genetic engineering makes its contributions to the environment protection in various ways. Most important to mention are the new approaches utilized for waste treatments and bioremediation Environment protection means the conservation of resources and hence to limit the degradation of environment.

Major approach in environment protection is the use of rDNA technology for degradation of toxic pollutants which harm the environment. Different microbes used for sewage treatment,

waste water treatment, industrial effluent treatment and for bioremediation are greatly improved by genetic engineering practices and thus present better results.

Transgenic Plants

A genetically modified plant, consisting in its genome, one or more inserted genes of an unrelated plant is termed as a transgenic plant and those inserted genes are called as transgenic. The development of transgenic plant is possible by using rDNA technology, gene delivery strategies and the tissue culture techniques.

Production of transgenic plants involves two main steps that are; transformation of the target plant cells and then regeneration of transformed cells into whole plant. In the transformation step, foreign gene of interest is introduced into the target plant cells. This can be done by following any of the gene delivery systems available like AMGT (Agrobacterium mediated gene transfer), using plant viruses as vectors or by direct gene delivery system i.e., electroporation, microinjection, particle-gun method, etc. At present, the particle gun method and AMGT are the most favourable methods of gene delivery into plant cells.

9.4 SUMMARY

Techniques for manipulating Prokaryotic as well as Eukaryotic DNA have undergone a remarkable development in late 20^{th} century. These techniques include breakage of a DNA molecule at two desired places to isolate a specific DNA segment and insertion in another DNA molecule at a desired position. The product thus obtained is called Recombinant DNA and the technique as Recombinant DNA technology. Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning), to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome. Recombinant DNA in a living organism was first achieved in 1973 by **Herbert Boyer**, of the University of California at San Francisco, and **Stanley Cohen**, at Stanford University, who used E. coli restriction enzymes to insert foreign DNA into plasmids.

Using this technique we can isolate and clone single copy of a gene or a DNA segment into an identified number of copies, all similar. This is possible because vectors like plasmids and phages reproduce in the host (e.g. *E.coli*) in their usual manner even after insertion of foreign DNA. This technique is called **gene cloning** and the vectors used for this purpose are called **Cloning vectors**. With this technique, using a variety of cloning vectors, genes can be isolated, cloned and characterized. Thus, this technique has led to a great deal of progress in the area of molecular biology.

Recombinant DNA is the general name for a piece of DNA that has been created by the combination of at least two strands. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, and differ only in the nucleotide sequence within that identical overall structure. Recombinant DNA molecules are sometimes called

chimeric DNA, because they can be made of material from two different species, like the mythical chimera. R-DNA technology uses palindromic sequences and leads to the production of sticky and blunt ends.

The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms.

The basic technique of recombinant DNA involves digesting a vector DNA with a restriction enzyme, which is a molecular scissors that cuts DNA at specific sites. A DNA molecule from the organism of interest is also digested, in a separate tube, with the same restriction enzyme. The two DNAs are then mixed together and joined, this time using an enzyme called **DNA ligase**, to make an intact, double-stranded DNA molecule. This construct is then put into Escherichia coli cells, where the resulting DNA is copied billions of times. This novel DNA molecule is then isolated from the E. coli cells and analyzed to make sure that the correct construct was produced. This DNA can then be sequenced, used to generate protein from E. coli or another host, or for many other purposes.

There are many variations on this basic method of producing recombinant DNA molecules. For example, sometimes researchers are interested in isolating a whole collection of DNAs from an organism. In this case, they digest the whole genome with restriction enzyme, join many DNA fragments into many different vector molecules, and then transform those molecules into E. coli. The different E. coli cells that contain different DNA molecules are then pooled, resulting in a "**library**" of E. coli cells that contain, collectively, all of the genes present in the original organism.

Another variation is to make a library of all expressed genes (genes that are used to make proteins) from an organism or tissue. In this case, RNA is isolated. The isolated RNA is converted to DNA using the enzyme called reverse transcriptase. The resulting DNA copy, commonly abbreviated as cDNA, is then joined to vector molecules and put into E. coli. This collection of **recombinant cDNAs** (a cDNA library) allows researchers to study the expressed genes in an organism, independent from non-expressed DNA. Recombinant DNA technology has enabled scientists to provide cheap, pure, and readily available medicines for a variety of illnesses. By using restriction enzymes, plasmids, and ligase, we can cut and paste the genes for almost any protein into bacteria and express usable proteins. These proteins include human growth hormone, insulin and many others used to treat dwarfism, diabetes, stroke, and heart attacks, as well as cancer.

9.5 GLOSSARY

Antibiotic: A medicine (such as penicillin or its derivatives) that inhibits the growth of or destroys microorganisms.

Biotechnology: A collection of technologies that use living cells and/or biological molecules to solve problems and make useful products.

Cloning: A process of producing similar populations of genetically identical individuals that occurs in nature when organisms such as bacteria, insects and plants reproduce asexually.

DNA probe: Are stretches of single-stranded DNA used to detect the presence of complementary nucleic acid sequences (target sequences) by hybridization.

DNA: (Deoxyribonucleic acid), the chemical molecule that is the basic genetic material found in all cells.

E. coli (Escherichia coli): A bacterium found in the intestinal tracts of most vertebrates. It is used extensively in recombinant DNA research because it has been genetically well characterized.

Electrophoresis: A technique used to separate molecules such as DNA or proteins using an electric current. The mixture of molecules is added to one end of a gel-like medium. When a current is applied to it, the molecules will travel through the medium to the other end at different speeds depending on the charge and size of the molecule. Once the molecules are separated, the gel can be used in a blot (Southern, Northern and Western).

Enzymes: A protein that accelerates the rate of chemical reactions. Enzymes are catalysts that promote reactions repeatedly, without being damaged by the reactions.

Expression: The process of converting genetic information into RNA and protein for use in the cell. Every gene is not expressed at the same level and at the same time. Expression patterns, easily analyzed using microarray technology, can give a lot of information about the roles genes play in different situations, such as disease and health.

Gene gun: A gene gun or a biolistic particle delivery system, originally designed for plant transformation, is a device for delivering exogenous DNA to cells.

Gene therapy: Altering DNA within cells in a living organism to treat or cure a disease. It is one of the most promising areas of biotechnology research. New genetic therapies are being developed to treat diseases such as cystic fibrosis, AIDS and cancer.

Gene: A unit of hereditary information. A gene is a section of a DNA molecule that specifies the production of a particular protein.

Genetic disorder: A genetic problem caused by one or more abnormalities in the genome, especially a condition that is present from birth (congenital).

Genetic engineering: The technique of removing, modifying or adding genes to a DNA molecule in order to change the information it contains. By changing this information, genetic engineering changes the type or amount of proteins an organism is capable of producing.

Genome: All of an organism's genetic information, including the entire DNA that makes up the genes that are carried on the chromosomes.

Genome: The complete set of an organism’s genetic information. In humans this corresponds to twenty-three pairs of chromosomes.

Genomics: The study of the entire genome (chromosomes, genes and DNA) and how different genes interact with each other. Genomics and molecular biology form the basis for modern biotechnology and, more specifically, pharmacogenomics, or the application of genetic analysis to identify potential targets for therapeutic products (drugs, vaccines).

GMO: Genetically modified organism**, a**n organism that has been modified, or transformed, using modern techniques of genetic exchange is commonly referred to as a genetically-modified organism.

Insulin: A polypeptide hormone, produced by the beta cells of the islets of Langerhans of the pancreas that regulates the metabolism of glucose and other nutrients.

In-vitro: Made to occur in a laboratory vessel or other controlled experimental environment rather than within a living organism or natural setting.

Molecular biology: A branch of biology concerned with studying the chemical structures and processes of biological phenomena at the molecular level molecule.

Monoclonal: Pertaining to cells or cell products derived from a single clone.

Mutant: A new type of organism produced as the result of mutation.

Northern blotting: (RNA blot), is a technique used in molecular biology research to study gene expression by detection of RNA (or isolated mRNA) in a sample.

PAGE: Polyacrylamide gel electrophoresis (PAGE), describes a technique widely used in biochemistry, forensics, genetics, molecular biology and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility.

PCR: Polymerase chain reaction is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Plaque blotting: A technique used in Molecular biology for the identification of recombinant phages. The procedure can also be used for the detection of differentially represented repetitive DNA.

Plasmid: The circular DNA structure used by bacteria.

Recombinant DNA (rDNA) technology: The laboratory manipulation of DNA in which DNA, or fragments of DNA from different sources, is cut and recombined using enzymes. This recombinant DNA is then inserted into a living organism. Recombinant DNA technology is usually used synonymously with genetic engineering.

Recombinant DNA: DNA that is formed by combining DNA from two different sources. Humans direct formation of recombinant DNA through selective breeding and genetic engineering.

Restriction enzyme: An enzyme that "cuts" DNA when specific base pair sequences are present.

RNA: Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation, and expression of genes.

Southern blotting: A method used in molecular biology for detection of a specific DNA sequence in DNA samples.

Ti plasmid: A Ti or tumor inducing plasmid is a plasmid that often, but not always, is a part of the genetic equipment that *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* use to transduce their genetic material to plants.

Transgenic plant: Genetically engineered plant or offspring of genetically engineered plants. Transgenic plants result from the insertion of genetic material from another organism so that the plant will exhibit a desired trait. Recombinant DNA techniques are usually used.

Vector: In molecular cloning, a vector is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed (e.g.- Plasmid, Cosmid, Lambda phages). A vector containing foreign DNA is termed recombinant DNA.

9.6 SELF ASSESSMENT QUESTIONS

9.6.1 Multiple Choice Questions:

1. The first successful transformation of rDNA molecule into a bacterium was carried out by
(a) Nathan, Arber and Smith (b) Watson, Crick and Wilkins
(c) Boyer and Cohen (d) Paul Berg

2. The DNA molecule to which the gene of insert is integrated for cloning is called
(a) Carrier (b) Vector
(c) Transformer (d) None of these

3. The most important discovery that lead to the development of rDNA technology was
(a) Double helix model of Watson and Crick (b) Discovery of ligase enzyme
(c) Discovery of plasmids (d) Discovery of restriction enzymes

4. The mechanism of intake of DNA fragments from the surrounding medium by a cell is called
(a) Transformation (b) Transduction
(c) both a and b (d) conjugation

5. The DNA segment to be cloned is called
(a) gene segment (b) DNA fragment
(c) DNA insert (d) all of these

6. Which of the following enzyme is used to cut DNA molecule in rDNA technology
(a) Restriction enzymes (b) Ligase
(c) Phosphatase (d) Ribonuclease

7. Restriction enzymes
(a) Protect bacteria from viral infection (b) Cut DNA in a staggered fashion

(c) Cut DNAs producing a blunt end (d) All of the above

8. Which of these restriction enzymes produce blunt ends?
(a) SaII (b) EcoRV
(c) XhoI (d) HindIII

9. A recombinant DNA molecule is produced by
(a) Joining of two DNA fragments
(b) Joining of two or more DNA fragments
(c) Both a and b
(d) Joining of two or more DNA fragments originating from different organisms

10. Which of the technologies listed below is a valuable method for mass-producing drugs and other useful proteins?
(a) Recombinant DNA technology (b) Transgenic technology
(c) Biotechnology (d) Gene targeting

11. Which gene transfer technique involves a tiny needle which is used to inject DNA into a cell lacking that DNA sequence?
(a) Electroporation (b) Liposome transfer
(c) Microinjection (d) Particle bombardment

12. Palindromic sequences in DNA
(a) Reflect the same sequence on two sides
(b) Form "blunt" ends when cut by restriction enzymes
(c) Not useful in recombinant DNA experiments
(d) All of the above

13. A human cDNA library
(a) Contains DNA for specific human proteins
(b) Contains DNA for virtually all of the human proteins in vectors
(c) Cannot be used to obtain human genes because it would be radioactive
(d) None of the above

14. The steps involved during PCR includes
(a) Denaturation (b) Annealing
(c) Extension (d) All of above

15. For cloning, DNA samples are taken from
(a) Different individual (b) Different species

(c) Same individual (d) None of above

16. DNA sequencing is used in
(a) Forensic studies (b) Biotechnology
(c) Diagnostics (d) All of above

17. Both DNA gel electrophoresis and SDS-PAGE of proteins are similar because
(a) In both cases molecules migrate to the anode
(b) Both techniques rely on a constant charge to mass ratio
(c) Both techniques utilize the sieving properties of gels
(d) All of the above

18. In order to insert a foreign gene into a plasmid, both must _____
(a) Have identical DNA sequences (b) Originate from the same type of cell
(c) Be cut by the same restriction enzyme (d) Be of the same length

19. The gene formed by the joining of DNA segments from two different sources are called as
(a) Recombinant gene (b) Joined gene
(c) Both a and b (d) Chimeric gene

20. Paul Berg's gene splicing experiment created the first rDNA molecule which was
(a) A T4 phage fragment incorporated into SV40 vector
(b) A lambda phage fragment incorporated into SV40 vector
(c) A T4 phage fragment incorporated into pSC 101 vector
(d) A lambda phage fragment incorporated into pSC 101 vector

9.6.2 Fill in the blanks:

1. In gel electrophoresis, DNA molecules migrate from _____ to _____ ends of the gel.
2. The process of _____ involves the introduction of a gene into a cell where it exchanges places with its counterpart in the host cell.
3. Genetic engineering manipulates gene products at the level of the_____
4. _____ consist of recombinant cells containing different fragments of a foreign genome.
5. _____ are used to select genes of interest from a genomic library.
6. The first drug produced using recombinant DNA technology was_____
7. Genomic library is normally made by_____
8. The small piece of DNA can be amplified by_____
9. In gel electrophoresis the fragments are separated on basis of_____ and_____
10. A collection of total genomic DNA from a single organism is called_____

9.6.1 Answer Key: 1-(c), 2-(b), 3-(d), 4-(a), 5-(c), 6-(a), 7-(d), 8-(b), 9-(d), 10-(a), 11-(c), 12-(a), 13-(b), 14-(d), 15-(a), 16-(d), 17-(d), 18-(c), 19-(d), 20-(b)

9.6.2 Answer Key: 1-Negative to positive, 2- Gene targeting, 3- DNA, 4- Genomic libraries, 5- DNA probes, 6- Insulin, 7- λ phage vectors, 8- PCR, 9- Size and charge, 10- Gene library

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9.9 TERMINAL QUESTIONS

1. What do you understand by Recombinant DNA technology? Discuss the various uses of this technology in Biotechnology.
2. What is DNA cloning? Why it is considered as an important aspect in recombinant DNA technology?
3. Discuss the role of restriction enzyme used for rDNA technology. What are the different types of restriction enzyme?
4. Name various kinds of vectors used in Recombinant DNA Technology. Describe the plasmid vectors in detail.
5. What are the various procedure used for introducing recombinant DNA into host cell?
6. What are the tools used in recombination DNA technology? Describe them in detail?
7. Describe in details about PCR and their applications?
8. Write about the methodology of DNA sequencing?
9. How can plant and animal viruses be used as vectors? Discuss using suitable examples.
10. What is the difference between cDNA library and genome library?

UNIT-10 GENETIC ENGINEERING

10.1 OBJECTIVES

After reading this unit students will be able to know:

- What are genes
- Concept of genetic engineering
- Tools and techniques in genetic engineering
- Steps involved in genetic engineering
- Genetically modified organism
- Ethical issues of genetic engineering\

10.2 INTRODUCTION

Genes are at the essence of life. Together they constitute the blueprint of an organism. In computer terms they are the **master program of life**. They decide all the properties and all the capabilities of an organism. In biological terms this master program is called the hereditary substance, the **chromosomes**. It is constituted by chains of so called **DNA molecules** that carry the "code words" or instructions of the master program.

There is an identical set of this master program in every cell. For example a corn plant has about a billion cells, each with a set of this master program. In different parts of the plants different parts of the program are active, giving rise to different structures like the leaves, the seeds and the root. The cell is like a huge computer network, much larger than any man-made one. Science has a very incomplete understanding how this billion of master programs is able to cooperate in a very harmoniously and effectively coordinated way.

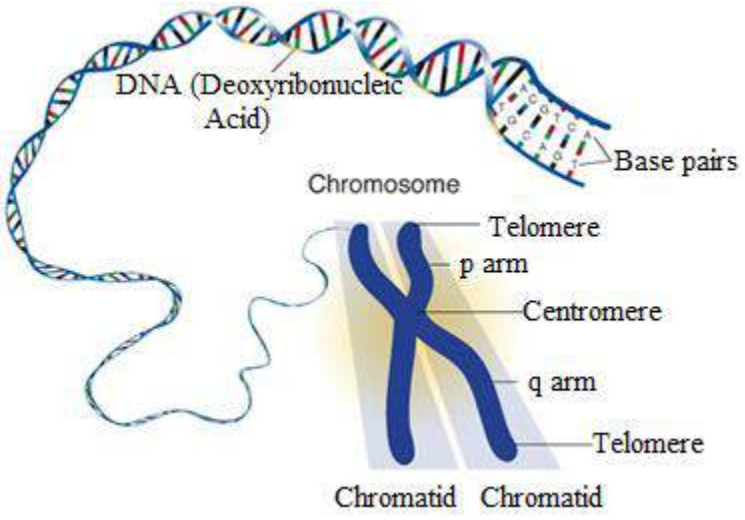

Fig.10.1 Chromosome structure (DNA chain)

Genetic engineering, also called genetic modification, is the direct manipulation of an organism's genes using biotechnology. It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms. New DNA is obtained by either isolating and copying the genetic material of interest using molecular cloning methods or by artificially synthesizing the DNA. A construct is usually created and used to insert this DNA into the host organism. As well as inserting genes, the process can be used to remove, or "*knock out*", genes. The new DNA can be inserted randomly, or targeted to a specific part of the genome.

An organism that is generated through genetic engineering is considered to be genetically modified (GM) and the resulting entity is a genetically modified organism (GMO). The first GMOs were bacteria generated in 1973 and the first GM animals were mice in 1974. Insulin-producing bacteria were commercialized in 1982 and genetically modified food has been sold since 1994. **GloFish**, the first GMO designed as a pet, was sold in the United States in December 2003.

Genetic engineering techniques have been applied in numerous fields including research, agriculture, industrial biotechnology, and medicine. Enzymes used in laundry detergent and medicines such as insulin and human growth hormone are now manufactured in GM cells, experimental GM cell lines and GM animals such as mice or zebrafish are being used for research purposes, and genetically modified crops have been commercialized.

Terms Used In Genetic Engineering

Before discussing various applications of genetic engineering, it is essential to define various terms which are often used in this connection.

Recombinant DNA: The DNA which contains genes from different sources and can combine with DNA of any organism is called recombinant DNA. Since genetic engineering utilizes recombinant DNA, it is also known as recombinant DNA technology. Recombinant DNA is obtained by special techniques.

Gene Cloning: It is a technique of genetic engineering by which a gene sequence with many identical copies is replicated. Identical gene sequence is isolated by using restriction endonuclease enzyme. This can also be obtained by making a complementary DNA from mRNA template using reverse transcriptase. It is then inserted into a doing vector, i.e., a plasmid or bacteriophage. The hybrid is used to infect a cell (plant or bacteria) and replicated within the cell. Gene cloning is used for identification of molecular structure of genes.

Gene Sequencing: Gene sequencing refers to the determination of the order of bases of a DNA molecule making up a gene. The DNA is purified and then broken at a specific point using restriction endonuclease enzyme. Thus all strands have one identical end. These strands are then broken at a random distance from this end so that there are strands ending on every base present.

These strands are then separated; their end base identified and put in order of fragment size to determine the entire sequence.

Gene Splicing: In genetic engineering, the enzyme catalyzed joining of DNA fragments is referred to as gene splicing. In genetics, the joining of exons after the introns sequences have been removed to produce functional messenger RNA is called gene splicing. This occurs in the cell nucleus and is catalyzed by splicing enzymes such as ligase.

DNA Probes: The small segments of DNA with known base sequences, origin and function are called DNA probes. DNA probes can be obtained either through DNA template or can be produced by gene cloning technique. Now microprocessor based sophisticated equipment's are available which can produce DNA probes. These microprocessor based equipment's are known as gene synthesizing machines. DNA probes are very much useful in determining the nucleotide sequence in living as well as non-living systems.

10.3 TOOLS AND TECHNIQUES IN GENETIC ENGINEERING

Genetic engineering involves the manipulation of genetic material (DNA) to achieve the desired goal in a pre-determined way. The terms used to describe genetic engineering are- Gene manipulation, Recombinant DNA (rDNA) technology, Gene cloning, Genetic modifications or even new genetics. A genetic engineer is an individual who is involved in genetic manipulations. There are tools that are commonly used in genetic engineering:

1. Polymerase Chain Reaction (PCR)

PCR is known as polymerase chain reaction. It is efficient because it multiplies the DNA exponentially for each of the 25 to 75 cycles. A cycle takes only a minute or so and each new segment of DNA that is made can serve as a template for new ones.

Genetic and genome engineering is a useful tool for researchers, from producing proteins to understanding disease, and the polymerase chain reaction (PCR) has a vital role supporting this process by cloning the DNA fragments used to modify the genomes of the bacteria, yeasts, animals and plants used in biological, agricultural and medical research.

Fig 10.2 PCR machine

The role of PCR in genetic engineering

PCR is used to created millions or billions of copies of DNA through repeated cycles of denaturing, which separates the DNA into its two strands; annealing, which attaches specific primers that mark the beginning and end of the DNA to be copied; and extension/elongation,

where the DNA strands are used as templates to build two new strands of DNA. These cloned DNA fragments can then be inserted into the target organism, including microorganisms, plants or animals, using vectors such as bacteria and viruses. Some of these traits can be passed on to the next generation.

2. Restriction Enzymes (Molecular Scissor)

The discovery of enzymes known as restriction endonucleases has been essential to protein engineering. These enzymes cut DNA at specific locations based on the nucleotide sequence. Hundreds of different restriction enzymes, capable of cutting DNA at a distinct site, have been isolated from many different strains of bacteria. DNA cut with a restriction enzyme produces many smaller fragments, of varying sizes. These can be separated using gel electrophoresis or chromatography.

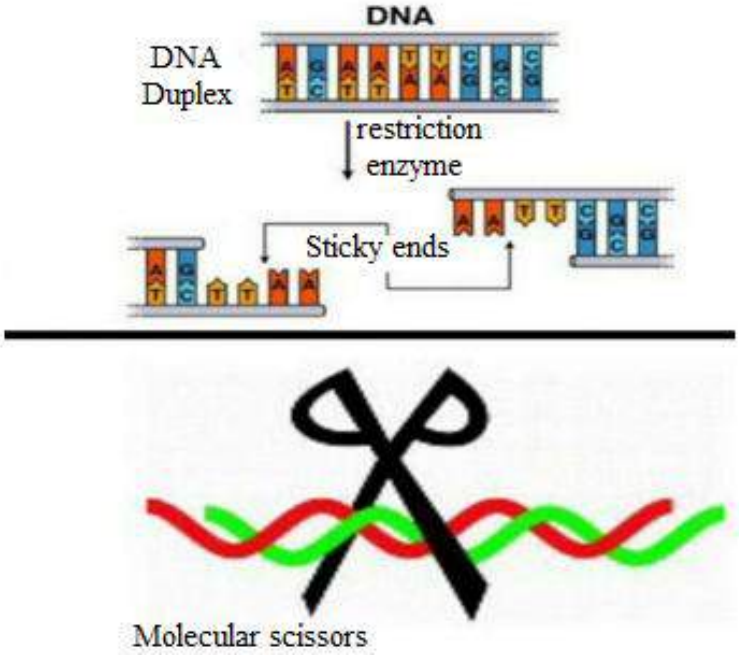

Fig.10.3 Function of Restriction endonuclease

3. Electrophoresis

Purifying DNA from a cell culture, or cutting it using restriction enzymes wouldn't be of much use if we couldn't visualize the DNA – that is, find a way to view whether or not your extract contains anything, or what size fragments you've cut it into. One way to do this is by gel electrophoresis. Gels are used for a variety of purposes, from viewing cut DNA to detecting DNA inserts and knockouts.

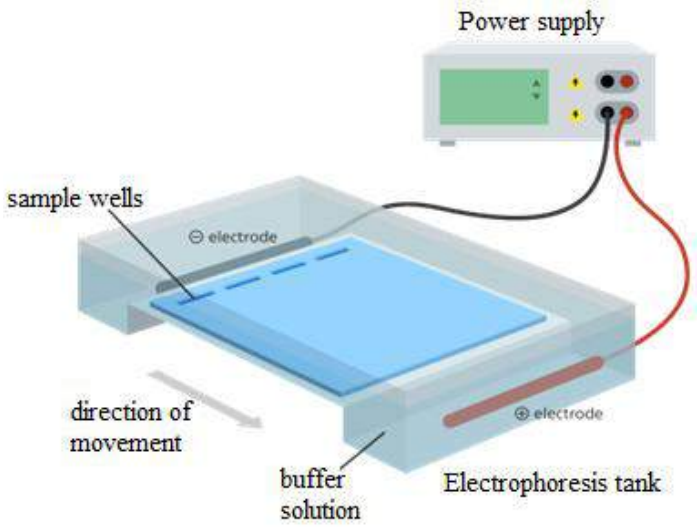

Fig.10.4 Gel Electrophoresis method

4. DNA Ligase

In genetic research it is often necessary to link two or more individual strands of DNA, to create a recombinant strand, or close a circular strand that has been cut with restriction enzymes. Enzymes called DNA ligases can create covalent bonds between nucleotide chains. The enzymes DNA polymerase I and polynucleotide kinase are also important in this process, for filling in gaps, or phosphorylating the 5′ ends, respectively.

DNA ligase is an enzyme which can connect two strands of DNA together by forming a bond between the phosphate group of one strand and the deoxyribose group on another. It is used in cells to join together the Okazaki fragments which are formed on the lagging strand during DNA replication.

In molecular biology, DNA ligase can be used to insert genes of interest into plasmid vectors, or to create fusion genes by joining one gene onto another. This process is called ligation (literally "tying a knot"). Ligation can be performed on lengths of DNA which have "blunt" or "sticky" ends following restriction digests. In "blunt end" ligation, the DNA fragments are joined directly together by the DNA ligase. There is less control over the orientation of the resultant insertion, however this can be improved by creating single base overhangs e.g."A-Tailing" with Taq Polymerase and dATP.

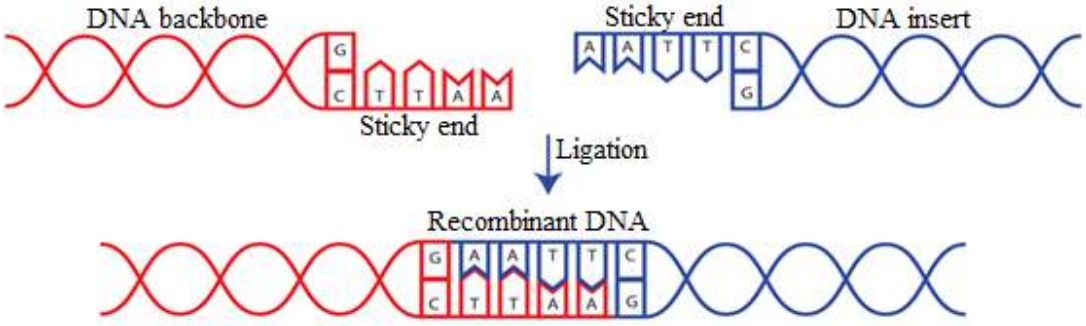

Fig.10.5 DNA Ligation

In "sticky end" ligation, overlapping regions of complementary single stranded DNA hydrogen bond to each other, and the DNA ligase enzyme connects the sugar phosphate backbones together. Through careful selection of restriction enzymes to create the sticky ends, a great deal of control can be exercised over the site of ligation.

5. Polymerases

The groups of enzymes that catalyze the synthesis of nucleic acid molecules are collectively referred to as polymerases. It is customary to use the name of the nucleic acid template on which the polymerase acts. The three important polymerases are given below.

a) DNA-dependent DNA polymerase that copies DNA from DNA.
b) RNA-dependent DNA polymerase (reverse transcriptase) that synthesizes DNA from RNA.
c) DNA-dependent RNA polymerase that produces RNA from DNA.

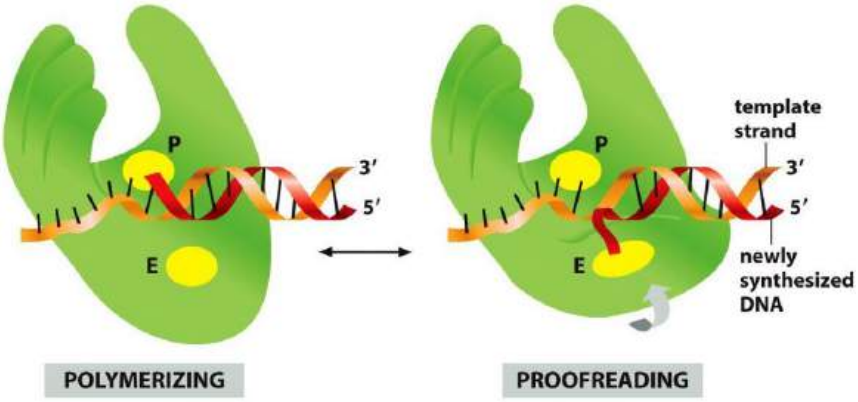

Fig.10.6 Polymerase enzyme action

6. Escherichia Coli- (A Prokaryotic Host)

The bacterium, *Escherichia coli* was the first organism used in the DNA technology experiments and continues to be the host of choice by many workers. Undoubtedly, E. coli, the simplest Gram negative bacterium (a common bacterium of human and animal intestine), has played a key role in the development of present day biotechnology.

Under suitable environment, *E. coli* can double in number every 20 minutes. Thus, as the bacteria multiply, their plasmids (along with foreign DNA) also multiply to produce millions of copies, referred to as colony or in short clone. The term clone is broadly used to a mass of cells, organisms or genes that are produced by multiplication of a single cell, organism or gene.

7. Eukaryotic Host

Eukaryotic organisms are preferred to produce human proteins since these hosts with complex structure (with distinct organelles) are more suitable to synthesize complex proteins. The most commonly used eukaryotic organism is the yeast, *Saccharomyces cerevisiae*. It is a non-pathogenic organism routinely used in brewing and baking industry. Certain fungi have also been used in gene cloning experiments.

8. Selection of Small Self-Replicating DNA

Small circular pieces of DNA that are not part of a bacterial genome, but are capable of self-replication, are known as plasmids. Plasmids are often used as vectors to transport genes between microorganisms. In biotechnology, once the gene of interest has been amplified and both the gene and plasmid are cut by restriction enzymes, they are ligated together generating what is known as a recombinant DNA. Viral (bacteriophage) DNA can also be used as a vector, as can Cosmids, recombinant plasmids containing bacteriophage genes.

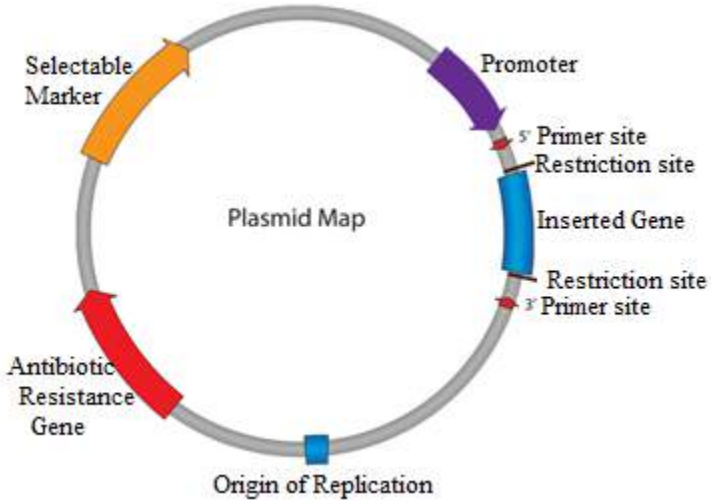

Fig.10.7 Plasmid vector

9. Method to Move a Vector into a Host Cell

The process of transferring genetic material on a vector such as a plasmid, into new host cells, is called transformation. This technique requires that the host cells are exposed to an environmental change which makes them "competent" or temporarily permeable to the vector. Electroporation is one such technique. The larger the plasmid, the lower the efficiency with which it is taken up by cells. Larger DNA segments are more easily cloned using bacteriophage, retrovirus or other viral vectors or cosmids in a method called transduction. Phage or viral vectors are often used in regenerative medicine but may cause insertion of DNA in parts of our chromosomes where we don't want it, causing complications and even cancer.

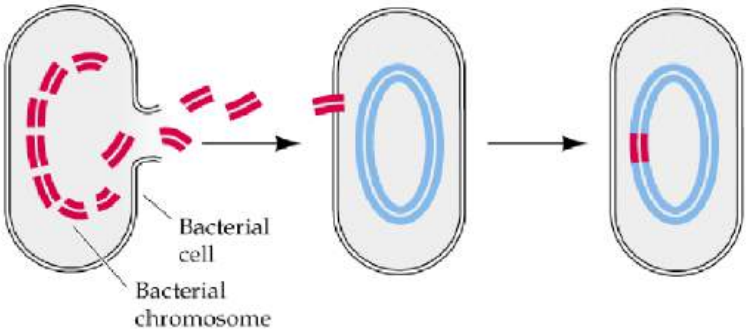

Fig.10.8 Transformation

10. Methods to Select Transgenic Organisms

Not all cells will take up DNA during transformation. It is essential that there be a method of detecting the ones that do. Generally, plasmids carry genes for antibiotic resistance and transgenic cells can be selected based on expression of those genes and their ability to grow on media containing that antibiotic. Alternative methods of selection depend on the presence of other reporter proteins such as the x-gal/lacZ system, or green fluorescence protein, which allow selection based on color and fluorescence, respectively.

Genetic Engineering Technique

The process for genetic engineering begins similar for any organism being modified:

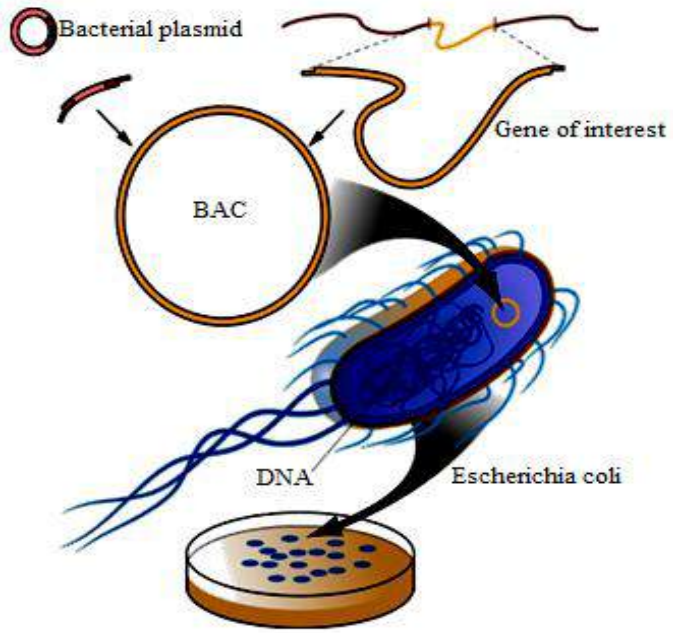

Fig.10.9 Recombination of DNA

1. Identify an organism that contains a desirable gene.
2. Extract the entire DNA from the organism.
3. Remove this gene from the rest of the DNA. One way to do this is by using a restriction enzyme. These enzymes search for specific nucleotide sequences where they will "cut" the DNA by breaking the bonds at this location.
4. Insert the new gene to an existing organism's DNA. This may be achieved through a number of different processes.

When modifying bacteria, the most common method for this final step is to add the isolated gene to a plasmid, a circular piece of DNA used by bacteria. This is done by "cutting" the plasmid with the same restriction enzyme that was used to remove the gene from the original DNA.

The new gene can now be inserted into this opening in the plasmid and the DNA can be bonded back together using another enzyme called ligase. This process creates a recombinant plasmid. In this case, the recombinant plasmid is also referred to as a bacterial artificial chromosome (BAC).

Once the recombinant DNA has been built, it can be passed to the organism to be modified. If modifying bacteria, this process is quite simple. The plasmid can be easily inserted into the bacteria where the bacteria treat it as their own DNA. For plant modification, certain bacteria such as *Agrobacterium tumefaciens* may be used because these bacteria permit their plasmids to be passed to the plant's DNA.

Techniques Used In Genetic Engineering

1. Agarose Gel Electrophoresis: Electrophoresis refers to the movement of charged molecules in an electric field. The negatively charged molecules move towards the positive electrode while the positively charged molecules migrate towards the negative electrode.

Gel electrophoresis is a routinely used analytical technique for the separation/purification of specific DNA fragments. The gel is composed of either polyacrylamide or agarose. Polyacrylamide gel electrophoresis (PAGE) is used for the separation of smaller DNA fragments while agarose electrophoresis is convenient for the separation of DNA fragments ranging in size from 100 base pairs to 20 kb pairs. Gel electrophoresis can also be used for the separation of RNA molecules.

2. Isolation and Purification of Nucleic Acids: Almost all the experiments dealing with gene manipulations require pure forms of either DNA or RNA, or sometimes even both. Hence there is a need for the reliable isolation of nucleic acids from the cells. The purification of nucleic acids broadly involves three stages.

(i) Breaking or opening of the cells to expose nucleic acids.

(ii) Separation of nucleic acids from other cellular components.

(iii) Recovery of nucleic acids in a pure form.

Analytical procedures involving a few steps to several steps are in use for the purification of nucleic acids. In fact, commercial kits are readily available these days to enable purification of either DNA or RNA from different sources.

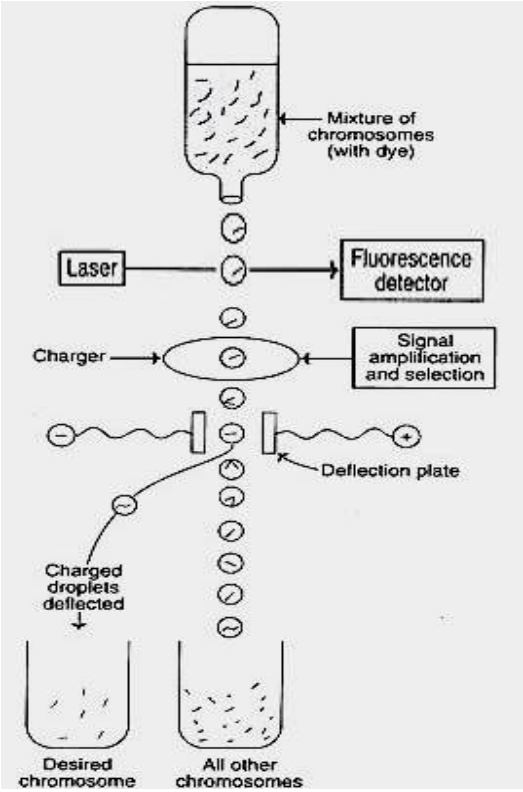

Fig.10.10 Separation of chromosomes by FACS

3. Isolation of Chromosomes: Separation of large chromosomes of eukaryotes is not possible by conventional electrophoresis. The individual chromosomes of eukaryotes can be separated by fluorescence-activated cell sorting (FACS), also known as flow Cytometry or flow Karyotyping.

Fluorescence-Activated Cell Sorting:

To carry out FACS, the dividing cells (with condensed chromosomes) are carefully broken open, and a mixture of intact chromosomes is prepared. These chromosomes are then stained with a fluorescent dye. The quantity of the dye that binds to a chromosome depends on its size. Thus, larger chromosomes (with more DNA) bind more dye and fluoresce more brightly than the smaller ones. The dye-mixed chromosomes are diluted and passed through a fine aperture that results in the formation of a stream of droplets. Each droplet contains a single chromosome. The fluorescence of the chromosomes is detected by a laser.

When the fluorescence indicates that the chromosome illuminated by the laser is the one desired, electrical charge is specifically applied to these droplets (and no others) which get charged. This results in the deflection of the droplets with the desired chromosome which can be separated from the rest and collected. Uncharged droplets that do not contain the desired chromosome pass through a waste collection vessel.

4. Nucleic Acid Blotting Techniques:

Blotting techniques are very widely used analytical tools for the specific identification of desired DNA or RNA fragments from thousands of molecules. Blotting refers to the process of immobilization of sample nucleic acids or solid support (nitrocellulose or nylon membranes). The blotted nucleic acids are then used as targets in the hybridization experiments for their specific detection. An outline of the nucleic acid blotting technique is depicted in Figure.

The most commonly used blotting techniques are listed below:

A. Southern blotting (for DNA)
B. Northern blotting (for RNA)
C. Dot blotting (DNA/RNA)
D. Colony and plaque hybridization (cells from colony).

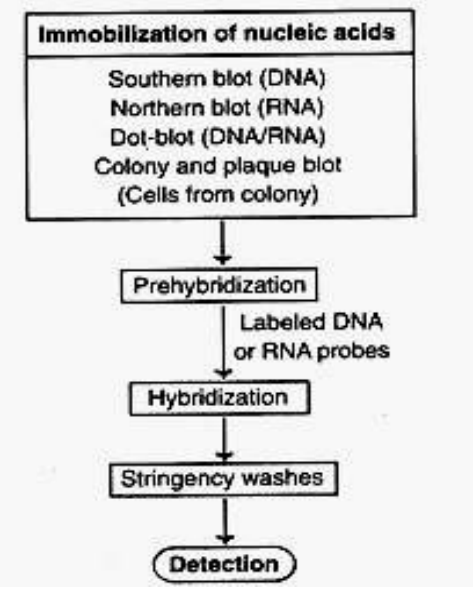

Fig.10.11 An outline of the nucleic acid blotting techniques

5. DNA Sequencing: Determination of nucleotide sequence in a DNA molecule is the basic and fundamental requirement in biotechnology. DNA sequencing is important to understand the functions of genes, and basis of inherited disorders. Further, DNA cloning and gene manipulation invariably require knowledge of accurate nucleotide sequence.

6. Chemical Synthesis of DNA: Advances in the laboratory techniques have made it possible to chemically synthesize DNA in a short period. Thus, oligonucleotides of about 100 bases can be produced in about 10 hours. Laboratory synthesis of DNA (recently by use of DNA synthesizers or gene machines) with specific sequence of nucleotides, rapidly and inexpensively, has significantly contributed to cloning. Chemical synthesis of DNA is based on the ability to protect the reactive -5′ and -3′ ends by blocking (protecting) them.

10.3.1 Genetic Engineering

Genetic engineering is the process by which scientists modify the genome of an organism. Creation of genetically modified organisms requires recombinant DNA. Recombinant DNA is a combination of DNA from different organisms or different locations in a given genome that would not normally be found in nature. In most cases, use of recombinant DNA means that you have added an extra gene to an organism to alter a trait or add a new trait. Some uses of genetic engineering include improving the nutritional quality of food, creating pest-resistant crops, and creating infection-resistant livestock.

Basic Steps of Genetic Engineering

There are three basic steps of genetic engineering:
(i) Isolation of a DNA fragment from a donor organism,
(ii) Insertion of an isolated donor DNA fragment into a vector genome, and
(iii) Growth of recombinant vector in an appropriate host
Having known the basic steps of genetic engineering we ought to know how these steps are carried on. There are many biological tools which are used to carry out manipulation of genetic material.

Definition

"Genetic engineering or genetic modification is defined as the humans direct manipulation in an organisms genetic material". It is basically an alteration of genetic makeup of an organism by using techniques in order to produce heritable material and then directly introduce into a cell or host that is then amalgamated or hybridized with the host.

In other words, genetic engineering is the technology used to prepare Recombinant DNA (genetically engineered DNA made by recombining fragments of DNA from different organisms) in vitro by pruning up DNA molecules and splicing (is a junction where two things (as paper, film or magnetic tape) have been joined together) together break ups from more than one organism. Genetic engineering, process that alters the genetic make-up of an organism by either removing or introducing DNA. DNA can be introduced directly into the host organism or into a cell that is then fused or hybridized with the host. This relies on recombinant nucleic acid techniques to form new combinations of heritable genetic material followed by the incorporation of that material either indirectly through a vector system or directly through micro-injection, macro-injection or micro-encapsulation.

Genetic engineering does not normally include traditional animal and plant breeding, in vitro fertilization, induction of polyploidy, mutagenesis and cell fusion techniques that do not use recombinant nucleic acids or a genetically modified organism in the process. However, some broad definitions of genetic engineering include selective breeding. Cloning and stem cell research, although not considered genetic engineering, are closely related and genetic engineering can be used within them. Synthetic biology is an emerging discipline that takes genetic engineering a step further by introducing artificially synthesized material into an organism.

Plants, animals or micro organisms that have been changed through genetic engineering are termed Genetically Modified Organisms or GMOs. If genetic material from another species is added to the host, the resulting organism is called **Transgenic**. If genetic material from the same species or a species that can naturally breed with the host is used the resulting organism is called **Cisgenic**. If genetic engineering is used to remove genetic material from the target organism the resulting organism is termed a knockout organism.

10.3.2 Genetically Modified Plants and Ethical Issues

Any organism whose genetic material has been altered or manipulated using genetic engineering techniques, such manipulated organisms are called genetically modified organisms (GMOs) or Transgenics. Over the past decade, GMOs have been in the news for their potential benefits and harms to society. Before human cloning became an issue, the emergence of transgenic plants - plants with foreign genes introduced to provide enhanced traits- was the hot topic in biotechnology. These engineered crops seek to provide better flavor, disease resistance, and increased nutritional value just like traditional breeding methods (Kishore 1993; Ye 2000). Biochemical and molecular biological procedures provide the technology to genetically engineer plants, as well as the methods used to detect them.

As far as plants are concerned, one common method of engineering plants uses recombinant DNA technology mediated by the action of a soil bacterium, *Agrobacterium tumefaciens*, to transfer DNA from bacteria to plants. Once the transformation has occurred, polymerase chain reaction (PCR) provides the ability to detect the genetically altered plants, allowing scientists to assess the success of the transformations, and in a different context, study the spread of transgenic crop species in the wild.

Agrobacterium tumefaciens contains a plasmid, a small circular piece of DNA that has its own origin of replication and is replicated independently of other nuclear material, that is key to its use in genetically modifying plants. This plasmid, called a tumor-inducing (Ti) plasmid, interacts with compounds released by fractured plant cells. When a wounded plant is exposed to *A. tumefaciens*, it integrates a stretch of its DNA, called transferred DNA (T-DNA), to the plant's genome.

Normally, the bacterium transfers its own T-DNA, but if the T-DNA is removed and replaced with another gene, *A. tumefaciens* can be used to introduce that gene into the plant genome, thereby providing a vector for scientists to engineer beneficial genes into plants. Studies have shown that native T-DNA genes are not necessary for this process. Inserted genes get transferred to plants as long as two repeated border sequences of 25 base pairs flanking the genes are present in the vector. The Ti plasmid itself can only hold a 25 kb gene fragment, so it can only be used for small genes. It can also be somewhat difficult to work with.

However, other vectors can be used to perform the transformation. Binary vectors, like the pBIN20 vector, are plasmids that contain the 25 base pair border sequences, allowing the new genes within them to be integrated into plant genomes, as well as marker genes that are used later in the process to select for successful gene transfer.

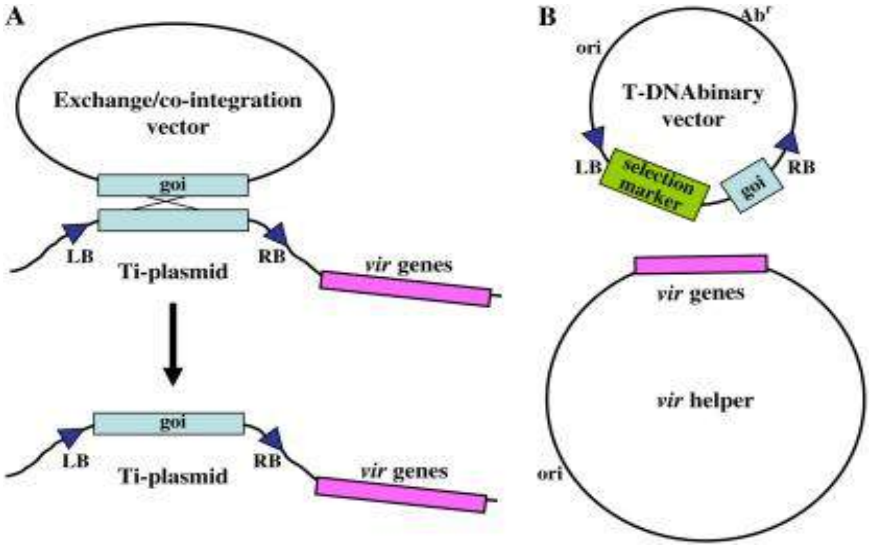

Fig. 10.12 Action of Binary vectors

The vectors also contain origins of replication for *A. tumefaciens* and Escherichia coli (Walden 1990), a bacterium commonly used in research. Therefore, the plasmids can replicate themselves in either E. coli or *A. tumefaciens*, allowing scientists to work with E. coli and then transfer the vectors to *A. tumefaciens* through bacterial conjugation, the process by which two bacteria exchange genetic information in the form of plasmids, when the genes are ready to be inserted into the plant genome.

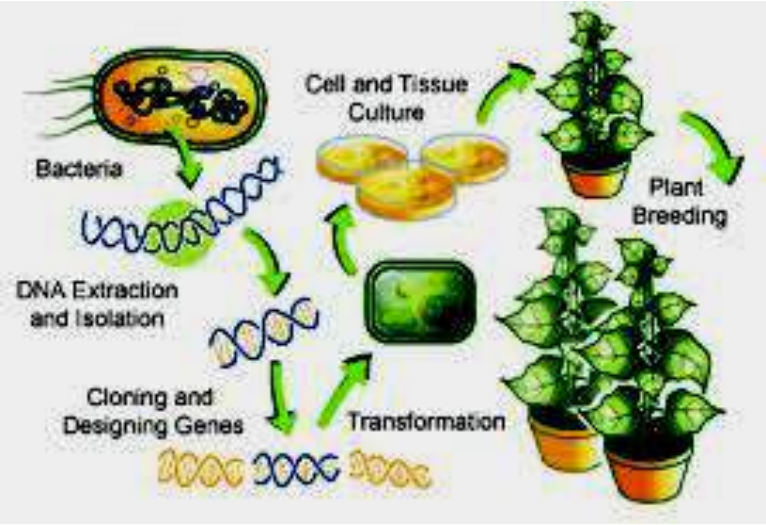

Fig. 10.13 Process of growing Transgenic plant

The Process of Genetic Modification

Production of GMOs is a multi-stage process which can be summarized as follows:

1. Identification of the gene interest,

2. Isolation of the gene of interest,
3. Amplifying the gene to produce many copies,
4. Associating the gene with an appropriate promoter and poly A sequence and insertion into plasmids,
5. Multiplying the plasmid in bacteria and recovering the cloned construct for injection,
6. Transference of the construct into the recipient tissue, usually fertilized eggs,
7. Integration of gene into recipient genome,
8. Expression of gene in recipient genome and
9. Inheritance of gene through further generations.

Genetically Modified Plants

Plants with favorable characteristics have been produced for thousands of years by conventional breeding methods. Desirable traits are selected, combined and propagated by repeated sexual crossings over numerous generations. This is a long process, taking up to 15 years to produce new varieties. Genetic engineering not only allows this process to be dramatically accelerated in a highly targeted manner by introducing a small number of genes, it can also overcome the barrier of sexual incompatibility between plant species and vastly increase the size of the available gene pool.

Transgenic (GM) plants are those that have been genetically modified using recombinant DNA technology. This may be to express a gene that is not native to the plant or to modify endogenous genes. The protein encoded by the gene will confer a particular trait or characteristic to that plant. The technology can be utilized in a number of ways, for example to engineer resistance to abiotic stresses, such as drought, extreme temperature or salinity, and biotic stresses, such as insects and pathogens, that would normally prove detrimental to plant growth or survival. The technology can also be used to improve the nutritional content of the plant, an application that could be of particular use in the developing world. New-generation GM crops are now also being developed for the production of recombinant medicines and industrial products, such as monoclonal antibodies, vaccines, plastics and biofuels.

Genetically modified material sounds a little bit like science fiction territory, but in reality, much of what we eat on a daily basis is a genetically modified organism (GMO). Whether or not these modified foods are actually healthy is still up for debate -and many times, you don’t even know that you are buying something genetically modified. It is not required to label GMOs in the U.S. and Canada, but there are substantial restrictions, and even outright bans, on GMOs in many other countries. Almost 85 % of corn grown in the U.S. is genetically modified. Even Whole Foods' brand of corn flakes was found to contain genetically modified corn. Many producers modify corn and soy as they are resistant to the herbicide **Glyphosate**, which is used to kill weeds.

The **Flavr Savr tomato** was the first commercial genetically engineered food product approved for human consumption by the Food and Drug Administration (FDA) in 1992. This

tomato was rendered more resistant to rotting and softening by transgenic insertion of an antisense gene that suppresses expression from a polygalacturonase gene, which blocks breakdown of pectin in the cell wall, hence reducing softening. However, in practice Flavr Savr tomatoes did not stay firm and had to be reaped just like normal wild-type varieties, hence they did not provide any significant advantage, and did not survive very long in the market.

The **Roundup Ready Soybean**, also from Monsanto, has been genetically engineered for increased resistance to the trademark herbicide of Monsanto, Glyphosate, which interferes with the synthesis of essential amino acids, and hence is detrimental to not only herbs but also crops. This chemical inhibits an enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which catalyzes a critical step in the synthesis of these essential amino acids.

The Roundup Ready variety is genetically engineered to synthesize a variant of this EPSPS enzyme that is not responsive to Glyphosate which protects the commercial crop from the herbicide. The major problem with the Roundup Ready use has been that although early on the system worked well and herbs were effectively removed with relatively less amount of Glyphosate, herbs (similar to bacteria and antibiotics) have gained resistance to this herbicide over the years, and the use of herbicide has gradually increased, effectively outweighing the advantages of the genetic modification (i.e., less use of herbicides). Some of the genetically modified plants are:

1. Soy: It is the most heavily genetically modified food in the countries like U.S. and China. It is modified to have a high level of oleic acid, which is naturally found in olive oil. Oleic acid is a monounsaturated omega-9 fatty acid that may lower LDL cholesterol (traditionally thought of as "bad" cholesterol) when used to replace other fats. Low-density lipoprotein (LDL) are one of the five major groups of lipoprotein which transport all fat molecules around the body in the extracellular water.

2. Golden Rice: It has been genetically engineered to synthesize beta-carotene in rice by a research team of the Swiss Federal Institute of Technology and University of Freiburg researchers, with the hope of improving the nutritional value of this staple crop for a large percentage of the world population that relies on rice for their diet, and who suffer from vitamin A deficiencies.

3. Yellow Crookneck Squash and Zucchini: Numbers of this GMO veggie are relatively small, but genetically modified yellow squash and zucchini can be found in two different species in the U.S. The species contain protein genes that protect against viruses. Just like their other GMO counterparts, you won't be able to tell the difference between non-GMO and GMO zucchini or squash.

4. Alfalfa: Cultivation of genetically engineered alfalfa was approved in 2011, and consists of a gene that makes it resistant to the herbicide Roundup, allowing farmers to spray the chemical without damaging the alfalfa.

5. Sugar Beets: A very controversial vegetable, sugar beets were approved in 2005, banned in 2010, then officially deregulated in 2012. Genetically modified sugar beets make up half of the U.S. sugar production, and 95 percent of the country's sugar beet market.

Methods for Transgenics

There are two principal methods for transgene insertion:

1. Gene Gun: In this method, microscopic pellets of gold or tungsten are coated with the transgene fragment and shot at high velocity into plant cells or tissues. In a small proportion of cases, the pellet will pass through the cells and the DNA fragment will remain behind and become incorporated into a plant chromosome in the cell nucleus.

2. Agrobacterium Tumefaciens (Natural Genetic Engineer): This method utilizes a biological vector, the soil dwelling bacterium *Agrobacterium tumefaciens*, which in nature transfers part of its DNA into plants and causes crown gall disease. Genetic engineers have taken advantage of this DNA transfer mechanism while disarming the disease-causing properties. Plant and bacterial cells are co-cultivated in a petridish under conditions that facilitate gene transfer. This allows incorporation of genes in a more controlled manner than with the gene gun; however, it does not work equally well in all plant species.

Genetically Modified Foods (Advantage & Disadvantage)

Genetically modified foods, often classified as GMOs, have changed the way that people view their food. Although genetic modifications have occurred throughout history with selective breeding and growing methods, scientific advances have allowed this practice to advance to the genetic level. In the modern GMO, plants can be resistant to specific pesticides and herbicides while becoming adaptive to changing environmental conditions.

The primary advantage of genetically modified foods is that crop yields become more consistent and productive, allowing more people to be fed. According to Oxfam, the world currently produced about 20% more food calories than what is required for every human being to be healthy.

GMOs are not without disadvantages. Although there are no conclusive links, Brown University concluded that changes to foods on a genetic level combine proteins that humans are not used to consuming. This may increase the chances of an allergic reaction occurring. Since 1999, the rates of food allergies in children have increased from 3.4% to 5.1%. Here are some of the additional advantages and disadvantages of genetically modified foods to think about.

Advantages of Genetically Modified Crop or Foods:

1. Food Supplies become Predictable: When crop yields become predictable, then the food supply becomes predictable at the same time. This gives us the ability to reduce the presence of food deserts around the world, providing a greater population with a well-rounded nutritional opportunity that may not have existed in the past.

2. Increased Flavor and Nutrition: Along with resistances to insects and disease, the genes of the crops can also be altered to have a better flavor and increased nutritional value. Genetic modifications do more than add pest resistance or weather resistance to GMO crops.

The nutritional content of the crops can be altered as well, providing a denser nutritional profile than what previous generations were able to enjoy. This means people in the future could gain the same nutrition from lower levels of food consumption. The UN Food and Agricultural Organization notes that rice, genetically modified to produce high levels of Vitamin A, have helped to reduce global vitamin deficiencies.

3. Longer Shelf Life: Genetically modified foods have a longer shelf life. This improves how long they last and stay fresh during transportation and storage. Instead of relying on preservatives to maintain food freshness while it sits on a shelf, genetically modified foods make it possible to extend food life by enhancing the natural qualities of the food itself. According to Environmental Nutrition, certain preservatives are associated with a higher carcinogen, heart disease, and allergy risk.

4. Medical Benefits from GMO crops: Through a process called "Pharming", it is possible to produce certain proteins and vaccines, along with other pharmaceutical goods, by the use of genetic modifications. This practice offers cheaper methods of improving personal health and could change how certain medications are provided to patients in the future. Imagine being able to eat your dinner to get a tetanus booster instead of receiving a shot in the arm – that's the future of this technology.

5. It creates foods that are more appealing to eat: Colors can be changed or improved with genetically modified foods so they become more pleasing to eat. Spoon University reports that deeper colors in foods changes how the brain perceives what is being eaten. Deeper red colors make food seem to be sweeter, even if it is not. Brighter foods are associated with better nutrition and improved flavors.

6. Genetically modified foods are easier to transport: Because GMO crops have a prolonged shelf life, it is easier to transport them greater distances. This improvement makes it possible to take excess food products from one community and deliver it to another that may be experiencing a food shortage. GMO foods give us the opportunity to limit food waste, especially in the developing world, so that hunger can be reduced and potentially eliminated.

7. Less use of Herbicides and Pesticides (Better For the Environment): Since GMOs require much less chemicals to thrive, the impact on the environment is lessened. The pesticides and other chemicals commonly used on non GMO crops emit green house gases and pollute the ground soil.

Herbicides and pesticides create certain hazards on croplands that can eventually make the soil unusable. Farmers growing genetically modified foods do not need to use these products as often as farmers using traditional growing methods, allowing the soil to recover its nutrient base over time. Because of the genetic resistance being in the plant itself, the farmer still achieves a predictable yield at the same time.

8. Keeps It Affordable: One of the biggest effects that the use of GMOs has had on our everyday life is the prices of produce and other foods. Since more crops can be yielded, the prices can be much lower.

9. Resistance to Disease: One of the modifications made to the crops is an added resistance to disease that would normally kill off the crops. This keeps the yields high and the prices for the consumers low.

10. Sustainability: GMOs provide a stable and efficient way to sustain enough crops to feed the ever growing population of people in the world. This was the main goal of GMO crops in the first place.

Disadvantages of Genetically Modified Foods

1. GMO crops may cause antibiotic resistance: The crops that have been genetically modified have antibiotic properties put into them in order to make them immune to certain diseases. When you eat these foods these properties are left in your body and can make many antibiotics less effective.

Iowa State University research shows that when crops are modified to include antibiotics and other items that kill germs and pests, it reduces the effectiveness of an antibiotic or other medication when it is needed in the traditional sense. Because the foods contain trace amounts of the antibiotic when consumed, any organisms that would be affected by a prescription antibiotic have built an immunity to it, which can cause an illness to be more difficult to cure.

2. Farmers growing genetically modified foods have a greater legal liability: Crops that are genetically modified will create seeds that are genetically modified. Cross-pollination is possible between GMO crops and Non-GMO crops as well, even when specified farming practices are followed. Because many of the crops and seeds that produce GMO crops are patented, farmers that aren't even involved in growing these foods are subjected to a higher level of legal liability. Farmers that do grow GMO crops could also face liabilities for letting seeds go to other fields or allowing cross-pollination to occur.

3. Genes go into different plant species or Cross Contamination: The pollen from the genetically modified plants is also contaminated. When this pollen is around other plants, even

things like grass or weeds, they cross pollinate. This could develop "**Super weeds**" that have the same resistance properties as the crops.

Crops share fields with other plants, including weeds. Genetic migrations are known to occur. What happens when the genes from an herbicide-resistant crop get into the weeds it is designed to kill? Interactions at the cellular level could create unforeseen complications to future crop growth where even the benefits of genetically modified foods may not outweigh the problems that they cause. One example- dozens of weed species are already resistant to **Atrazine**.

4. Allergies on the Rise: Ever since the introduction of GMO foods, the amount of childhood food allergies has risen significantly. The exact link to GMO has not yet been found, but many believe this is due to insufficient research in the area.

5. Not Enough Testing: There has been very little testing and research done on genetically modified foods and the long term effects have not been discovered yet. This makes many people feel uneasy at the high use of these foods. The advantages and disadvantages of genetically modified foods can spark a bitter debate. There is an advantage in providing the world with better food access, but more food should not come at the expense of personal health. We deserve to know what we're eating and how that food is grown. Knowing more about genetically modified foods allows us to do just that.

Applications of Genetic Engineering in Crop Improvement

Genetic engineering has several potential applications in crop improvement.
(i) Interspecific and inter-generic gene transfer (distant hybridization),
(ii) Development of transgenic plants,
(iii) Development of nodules in cereals,
(iv) Development of C4 plants, etc.

(i) Distant Hybridization: With the advancement of genetic engineering, it is now possible to transfer genes between distantly related species. The barriers of gene transfer between species or even genera have been overcome. The desirable genes can be transferred even from lower organisms through recombinant DNA technology.

(ii) Development of Transgenic Plants: Plants which contain foreign genetic material are known as transgenic plants. Resistance to diseases and insects can be achieved through genetic transformation and development of transgenic plants. In case of cotton, a gene has been transferred from prokaryote, viz., *Bacillus thuringiensis (or Bt)* which confers resistance against bollworms. This work has been done by Monsanto Company in USA. The transgenic plants secrete a toxin when the larvae injure the buds or bolls. This toxin inactivates the larvae

and ultimately leads to death of the insect. This inbuilt system of insect resistance will help in saving huge amount of money towards the use of insecticides in cotton crop.

(iii) Development of Nodules in Cereals: Leguminous plants have root nodules which contain nitrogen fixing bacteria called Rhizobium. These bacteria convert the free nitrogen available in the atmosphere into nitrates in the root nodules. These nitrogen fixing genes may be transferred to cereal crops like wheat, rice, maize, barley etc. through the techniques of genetic engineering. Development of nodules in cereals seems to be impossible.

(iv) Development of C4 Plants: Improvement in yield can be achieved by improving the photosynthetic efficiency of crop plants. The photosynthetic rate can be increased by conversion of C3 plants into C4 plants, which may be achieved either through protoplast fusion or recombinant DNA technology. C4 plants have higher potential rate of biomass production than C3 plants.

Most C4 crop plants are grown in tropical and subtropical zones (sorghum, sugarcane, maize, some grasses). Maize is also grown in temperate zones but as forage crop, because below 15°C temperature grain yield is significantly reduced. The chances of conversion of C3 plants into C4 plants seem to be still very remote.

Applications of Genetic Engineering in Medicine

Biotechnology especially genetic engineering plays an important role in the production of antibiotics, hormones, vaccines and interferon in the field of medicine. These are briefly described below:

(i) Production of Antibiotics: Penicillium and Streptomyces fungi are used for mass production of antibiotics penicillin and streptomycin. Genetically efficient strains of these fungi have been developed to greatly increase the yield of above antibiotics.

(ii) Production of Hormone: Insulin, a hormone, is usually extracted from the pancreas of cows and pigs. This insulin is slightly different in structure from human insulin. As a result, it leads to allergic reactions in about 5% of the patients. Human gene for insulin production has been incorporated into bacterial DNA and such genetically engineered bacteria are used for large scale production of this hormone. Since this is produced using human gene, it does not have allergic reactions.

(iii) Production of Vaccines: Vaccines are produced by multiplication of disease producing organisms on large scale, which is a dangerous process. Recombinant DNA technique permits production of vaccines by incorporation of specific gene into bacteria. In other words, vaccines are produced by transfer of antigen coding genes to bacteria. Such antibodies provide protection against infection by the same virus.

(iv) Production of Interferon: Interferons are virus induced proteins produced by virus infected cells. Interferons are anti-viral in action and act as first line of defense against viruses. Their response is much quicker than influenza. It also appears to be effective against cancer of breast and lymphatic system. Natural interferon is produced in very small quantity from human blood cells. Now it is possible to produce interferon by recombinant DNA techniques at much cheaper rate.

(v) Production of Enzymes: Some useful enzymes can also be produced by recombinant DNA technique. The enzyme Urokinase, which is used to dissolve blood clots, has been produced by genetically engineered microorganisms.

Industrial Applications

In industries, recombinant DNA technique will help in the production of chemical compounds of commercial importance, improvement of existing fermentation processes and production of proteins from waste.

This can be achieved by developing more efficient strains of micro-organisms. Specially developed micro-organisms may be used even to clean up the pollutants. Thus, biotechnology has several useful applications in crop improvement, medicine and industry.

Dangers of Genetic Engineering

Several dangers are associated with recombinant DNA technology.
(i) Spread of new diseases,
(ii) Effect on evolution, and
(iii) Biological warfare.

(i) Spread of New Diseases: New dangerous forms of micro-organisms can be developed through recombinant DNA technique either accidentally or deliberately. Escape of such micro-organisms from the research laboratory through drainage, laboratory glassware, laboratory personnel etc., may lead to the spread and origin of new type of diseases, which may pose a serious problem.

(ii) Effect on Evolution: Nature has provided several barriers for exchange of DNA between prokaryotes and eukaryotes. Recombinant DNA technology permits exchange of DNA between these two classes of organisms and thus interferes with the natural process of evolution.

(iii) Biological Warfare: There is a fear that genetic engineering techniques will be used for biological warfare. In such warfare, disease carrying microorganisms can be used against the enemy. This will lead to disaster. Thus, genetic engineering has several demerits. Many of the results achieved through genetic engineering, can be achieved through other less dangerous techniques.

Safety Measures: Dangers of recombinant DNA technique can be minimized:
(i) With increasing experience and knowledge,
(ii) By adopting safety measures to check the escape of new micro-organisms from laboratory,
(iii) By putting international ban on the work involving tumor viruses, and
(iv) By putting international ban on the use of such techniques in the warfare's.

Ethical Issues

The main reason genetically modified organisms are not more widely used is due to ethical concerns. Nearly 50 countries around the world, including Australia, Japan and all of the countries in the European Union, have enacted significant restrictions or full bans on the production and sale of genetically modified organism food products, and 64 countries have GMO (Genetically Modify Organism) labeling requirements. There are a number of ethical concerns over genetically modified (GM) foods and these have all affected public support of the products. The issues have also triggered controversy and regulations around GM foods and any company that produces these crops or products. Concerns range from the environment to risks to our food web or issues concerning disease, allergies and contamination. Some issues to consider when deciding whether to create and/or use GMOs include-

1. Safety: This generally arises in the case of GMO foods. Are the foods safe for human consumption? Is GMO feed healthy for animals? Many opponents of GMO foods say not enough independent testing is done before the food is approved for sale to consumers. In general, research has shown that GMO foods are safe for humans. Another safety consideration is the health of farmers and their families, animals and communities who are put at risk with exposure to chemicals used in tandem with GMO seeds.

2. Damage to the Environment: Damage to the environment is another ethical fear with regards to GM crops. Unfortunately, the technology is still new enough that there is much we do not know about the effect of GM crop production on the environment. Long-term studies take decades to complete and most studies of GM crop production involve short-term effects of the technology.

There are fears that if these crops do negatively impact the environment, they will spread in an out-of-control fashion and we will not be able to stop their damaging effects. For instance, one type of sugar beet that had been engineered to be resistant to a specific herbicide ended up unintentionally having the genes to resist a different herbicide. When farmers went to eliminate the crop, they still found that a small percentage had survived.

Consider that genetic engineers have the ability to create trees that grow faster than their unmodified counterparts. This seems like a great deal for the lumber or timber industry, but might some unintended consequences result? Being outdoors and grown in large quantities, the modified trees may cross-pollinate with unmodified trees to form hybrids outside of designated growing areas. This in return could create trees that could disrupt the ecosystem. For example,

they could overpopulate the area or grow so large that they smother other plant life. This same scenario has unintended and undesirable consequences when the pollen from GMO crops drifts into non-GMO fields.

3. Allergies and Disease: A key ethical concern about GM foods is their potential to trigger allergies or disease in humans. Given that a gene could be extracted from an allergenic organism and placed into another one that typically does not cause allergies, a person may unknowingly be exposed to an allergen. In turn, this could lead to an allergic reaction. There is also the fear that new allergies could occur from the mixing of genes from two organisms.

Disease is a major health worry with regards to GM foods. Given that some of the crops modified are done so with DNA from a bacterium or virus, there is concern that a new disease may occur in humans who consume the GM food. With some GM crops having antibiotic-resistant marker genes, there is also the worry that these genes could be passed on to microbes that cause disease and health problems in humans. With widespread antibiotic resistance currently already occurring, any new resistance could prove disastrous.

4. Humans: Should humans be genetically engineered? Doing so could have medical applications that reduce or prevent genetic disorders such as Down's syndrome. However, the bigger question is where should engineering humans stop? Should parents be allowed to decide their children's eye colors, heights or even genders before birth?

5. Cross-Pollination: Cross-pollination is a challenge for any crop growth but it can typically be managed if care is taken to use good growing practices. There is the possibility of genes from GM foods spreading to other plants and crops, which could create overzealous weeds that can't be contained at all.

6. Food Web and Risks: Risks to the food web are a very real ethical concern around GM technology. Any pesticide or herbicide from the crop could harm animals and other organisms in the environment. For example, GM sugar beets that were produced to be resistant to herbicides did successfully reduce weeds. However, Skylark birds that consume the seeds from this particular weed would now be required to find a new food source, thereby endangering their existence.

An animal could also consume the GM crop itself, which means that if the crop has been engineered to produce a pesticide, the animal may become ill and die. In one North American study, caterpillars of the monarch butterfly were killed when they fed on pollen from GM corn crops.

Addressing Ethical Concerns for Gm Foods

Unfortunately, the controversy and fears around GM foods and any company that produces these products still continue to persevere, although this could be viewed as a positive movement because it will challenge GM technology and help to make it safer and more regulated. In one public opinion poll, it was found that the more people read about GM foods, the more concerned they became about the technology.

Studies are ongoing into the many ethical concerns around GM foods but these are not conclusive and have thus far shown very mixed results. It is also very difficult to assess the long-term impact, thereby leaving many of the public fearing for the long-term safety of humans and the environment. For now, it is hoped that people will become more educated on the ethical concerns about GM foods, which will ideally fuel further research and accountability in the field.

10.4 SUMMARY

Genetic engineering is the process by which scientists modify the genome of an organism. Creation of genetically modified organisms requires recombinant DNA. Recombinant DNA is a combination of DNA from different organisms or different locations in a given genome that would not normally be found in nature. In most cases, use of recombinant DNA means that you have added an extra gene to an organism to alter a trait or add a new trait. Some uses of genetic engineering include improving the nutritional quality of food, creating pest-resistant crops, and creating infection-resistant livestock.

DNA technology has largely helped scientists to understand the structure, function and regulation of genes. The development of modern biotechnology is primarily based on the success of DNA technology. Thus, the present biotechnology (more appropriately molecular biotechnology) has its main roots in molecular biology. Biotechnology is an interdisciplinary approach for applications to human health, agriculture, industry and environment. The major objective of biotechnology is to solve problems associated with human health, food production, energy production and environmental control. It is an accepted fact that the recombinant DNA technology has entered the main stream of human life and has become one of the most significant applications of scientific research. Biotechnology is regarded as more an art than a science. After the successful sequencing of human genome, many breakthroughs in biotechnology are expected in future.

The use of genetic engineering and the creation of genetically modified crops has resulted in many benefits for the agricultural world. The most noticeable benefit is that genetic engineering has made it possible to produce more crops in a shorter time period. Due to the modifications that make crops resistant to diseases, it has been possible to increase overall yields. Many genetically modified crops are also designed to grow at a faster rate, which also helps increase overall yield.

Genetic engineering has also increased yield by making it possible to grow crops in regions that would otherwise be unsuitable for agriculture, such as areas with salty soil, areas that are drought prone and areas with low amounts of sunlight. Through genetic engineering, crops have been modified to tolerate salty soils, be more drought resistant and increase their rate of photosynthesis to take advantage of limited sunlight. In addition to increasing productivity, genetic engineering has had several other benefits to agriculture. By modifying crops so that they are resistant to diseases and insects, less chemical pesticides have to be used to combat diseases and pests. Also, if crops are genetically modified to include components of fertilizers, less chemical fertilizers have to be placed on the fields.

Although there are many benefits of genetically engineered crops, there are also some major issues and concerns associated with these types of crops. One major concern is that as pests experience constant exposure to the pesticide or herbicide that is genetically inserted into the crops, they will develop genetic resistance to the chemical. If the pests develop genetic resistance, eventually the genetically modified crops would no longer be successful at preventing harm and would become obsolete. Another major concern about genetic engineering is the long-term effects on human health and the environment. There is little known about the long-term effects of genetically engineered crops, and this makes many people cautious about their use.

10.5 GLOSSARY

Amalgamated: Combine or unite to form one organization or structure.

Antibiotic: A medicine (such as penicillin or its derivatives) that inhibits the growth of or destroys microorganisms.

Atrazine: Atrazine is an herbicide of the triazine class. Atrazine is used to prevent pre- and post emergence broadleaf weeds in crops such as maize

Biotechnology: The use of living systems and organisms to develop or make products, or "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use"

C3 plant: C3 plants are called temperate or cool-season plants. They reduce (fix) CO2 directly by the enzyme Ribulose Bisphosphate Carboxylase (RUBPcase) in the chloroplast.

C4 plant: A C4 plant is a plant that cycles carbon dioxide into four-carbon sugar compounds to enter into the Calvin cycle. These plants are very efficient in hot, dry climates and make a lot of energy.

Carcinogen: A carcinogen is any substance, radionuclide, or radiation that promotes carcinogenesis, the formation of cancer.

Chromatography: Chromatography is a physical method of separation that distributes components to separate between two phases, one stationary (stationary phase), the other (the mobile phase) moving in a definite direction.

Chromosome: Any of several threadlike bodies, consisting of chromatin, that carry the genes in a linear order A chromosome is a DNA molecule with part or all of the genetic material of an organism.

Cisgenic: A product designation for a category of genetically engineered plants

Cloning: Cloning is the process of producing similar populations of genetically identical individuals that occurs in nature when organisms such as bacteria, insects or plants reproduce asexually.

Cross pollination: Pollination of a flower or plant with pollen from another flower or plant.

Cytometry: The measurement of the characteristics of cells. Variables that can be measured by cytometric methods include cell size, cell count, cell morphology (shape and structure), cell cycle phase, DNA content, and the existence or absence of specific proteins on the cell surface or in the cytoplasm.

Distant hybridization: Hybridization between individuals from different species belonging the same genus (Interspecific hybridization) or two different genera of same family (intergeneric hybridization)

DNA probe: Are stretches of single-stranded DNA used to detect the presence of complementary nucleic acid sequences (target sequences) by hybridization.

DNA: Acronym for deoxyribonucleic acid, which is a molecule that contains an organism's complete genetic information.

Dot blotting: A technique in molecular biology used to detect biomolecules, and for detecting, analyzing, and identifying proteins. It represents a simplification of the northern blot, Southern blot, or western blot methods.

Electrophoresis: A technique used in laboratories in order to separate macromolecules based on size. The technique applies a negative charge so proteins move towards a positive charge. This is used for both DNA and RNA analysis.

Encapsulated: Enclose (something) in or as if in a capsule.

Endanger: Put (someone or something) at risk or in danger.

Endogenous: Substances and processes are those that originate from within an organism, tissue, or cell.

Enzyme: Enzymes are macromolecular biological catalysts. Enzymes accelerate chemical reactions. The molecules upon which enzymes may act are called substrates and the enzyme converts the substrates into different molecules known as products.

Ethical issue: A problem or situation that requires a person or organization to choose between alternatives that must be evaluated as right (ethical) or wrong (unethical).

Fermentation: A metabolic process that consumes sugar in the absence of oxygen. The products are organic acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation.

Fluorescence: The emission of light by a substance that has absorbed light or other electromagnetic radiation. It is a form of luminescence.

Gene gun: A gene gun or a biolistic particle delivery system, originally designed for plant transformation, is a device for delivering exogenous DNA to cells.

Gene: The molecular unit of an organism that contains information for a specific trait (specific DNA sequence).

Genome: An entire set of genes for an organism.

Glyphosate: A broad-spectrum systemic herbicide and crop desiccant. It is an organophosphorus compound, specifically a phosphonate. It is used to kill weeds, especially annual broadleaf weeds and grasses that compete with crops

GMO: Acronym for Genetically Modified Organism.

Herbicide: A substance that is toxic to plants, used to destroy unwanted vegetation.

Hybridization: The process of crossing two genetically different individuals to result in a third individual with a different, often preferred, set of traits.

Insecticide: An insecticide is a substance used to kill insects. They include ovicides and larvicides used against insect eggs and larvae, respectively.

Insulin: A peptide hormone produced by beta cells of the pancreatic islets, and it is considered to be the main anabolic hormone of the body

Interferon: (IFNs) are a group of signaling proteins made and released by host cells in response to the presence of several pathogens, such as viruses, bacteria, parasites, and also tumor cells.

Karyotyping: A test to identify and evaluate the size, shape, and number of chromosomes in a sample of body cells.

LDL: Low-density lipoproteins are one of the five major groups of lipoprotein which transport all fat molecules around the body in the extracellular water.

Leguminous: Relating to or denoting plants of the pea family (Leguminosae). These have seeds in pods, distinctive flowers, and typically root nodules containing symbiotic bacteria able to fix nitrogen.

Lymphatic system: Part of the circulatory system and a vital part of the immune system, comprising a network of lymphatic vessels that carry a clear fluid called lymph directionally towards the heart.

Mutagenesis: A process by which the genetic information of an organism is changed, resulting in a mutation. It may occur spontaneously in nature, or as a result of exposure to mutagens.

Northern blotting: (RNA blot), is a technique used in molecular biology research to study gene expression by detection of RNA (or isolated mRNA) in a sample.

Nucleotide: The building block of DNA.

PAGE: Polyacrylamide gel electrophoresis (PAGE), describes a technique widely used in biochemistry, forensics, genetics, molecular biology and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility.

PCR: Polymerase chain reaction is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Pesticide: Are substances that are meant to control pests (including weeds).

Plaque blotting: A technique used in Molecular biology for the identification of recombinant phages. The procedure can also be used for the detection of differentially represented repetitive DNA.

Plasmid: The circular DNA structure used by bacteria.

Polyploidy: Cells and organisms are those containing more than two paired (homologous) sets of chromosomes.

Protein: Large biomolecules used by an organism for a number of purposes; in this context, to express a desired trait.

Recombinant DNA: DNA to which a section has been removed and replaced (recombined) with a new sequence.

Restriction enzyme: An enzyme that "cuts" DNA when specific base pair sequences are present.

RNA: Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation, and expression of genes.

Southern blotting: A method used in molecular biology for detection of a specific DNA sequence in DNA samples.

Super weed: A weed which is extremely resistant to herbicides, especially one created by the transfer of genes from genetically modified crops into wild plants.

Sustainable: The property of biological systems to remain diverse and productive indefinitely. Long-lived and healthy wetlands and forests are examples of sustainable biological systems.

Transgene: A gene or genetic material that has been transferred naturally, or by any of a number of genetic engineering techniques from one organism to another. The introduction of a transgene (called "transgenesis") has the potential to change the phenotype of an organism.

Ti plasmid: A Ti or tumor inducing plasmid is a plasmid that often, but not always, is a part of the genetic equipment that *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* use to transduce their genetic material to plants.

Trait: A distinguishing characteristic.

Transgenic: relating to or denoting an organism that contains genetic material into which DNA from an unrelated organism has been artificially introduced.

Urokinase: Also known as Urokinase-type Plasminogen Activator (uPA), is a serine protease present in humans and other animals. It was originally isolated from human urine, and it is also present in the blood and in the extracellular matrix of many tissues.

Vector: In molecular cloning, a vector is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed (e.g.- Plasmid, Cosmid, Lambda phages). A vector containing foreign DNA is termed recombinant DNA.

10.6 SELF ASSESSMENT QUESTIONS

10.6.1 Short Answers Questions:

1. Who discovered recombinant DNA (rDNA) technology?
2. Restriction enzymes are?
3. The enzyme used to join the DNA fragments?
4. Ti plasmid used in genetic engineering is obtained from?
5. Introduction of recombinant DNA into bacterial cell by using current is called?
6. Enzyme which is used to remove or knockout genes is known as?
7. Cry genes or BT genes are obtained from?
8. Transgenic plants are developed by?
9. Which enzyme is useful in genetic engineering?
10. A molecule containing DNA from two different organisms is called?

10.6.2 Objective Type Questions

1. Plant transformation can be obtained by
(a) Combining plant and animal cells in culture
(b) Shooting DNA into plant cells with a gun
(c) Using the *E. coli* bacterium to infect plant roots
(d) Infecting plants with a tobacco mosaic virus

2. An expression vector
(a) Always contains an origin of replication
(b) Usually contains a gene that confers antibiotic resistance to the bacterial host
(c) Always contains DNA segments for the regulation of mRNA production
(d) All of the above

3. The unpaired nucleotides produced by the action of restriction enzymes are referred to have
(a) Sticky ends (b) Single strands
(c) Restriction fragments (d) Ligases

4. What enzyme forms covalent bonds between restriction fragments?
(a) DNA primase (b) DNA helicase
(c) DNA polymerase (d) DNA ligase

5. The transfer of antibiotic-resistant genes from genetically engineered bacteria to disease-causing bacteria.
(a) Would be of no concern if it occurred. (b) Has occurred
(c) Can never occur (d) Seems unlikely

6. First step of genetic engineering is
(a) Isolation of gene interest (b) Insertion of gene into vector
(c) Growth of GMO (d) Expression of gene

7. In first step of genetic engineering, special enzymes that are used to separate identified gene are called

(a) Expressed nucleases (b) Inserted nucleases
(c) Restriction end nucleases (d) Isolated nucleases

8. Particular enzyme produced by genetic engineering which is used to dissolve clots of blood is called

(a) Interferon (b) Urokinase
(c) Thymosin (d) Beta-endorphin

9. Second step of genetic engineering is

(a) Growth of GMO (b) Expression of gene
(c) Isolation of gene interest (d) Insertion of gene into vector

10. Cutting certain genes out of molecules of DNA requires the use of special

(a) Degrading nucleases. (b) Restriction endonucleases.
(c) Eukaryotic enzymes. (d) Viral enzymes.

11. Which of the following cannot be used as a vector?

(a) Phage (b) Plasmid
(c) Bacterium (d) All can be used as vectors.

12. A probe is used in which stage of genetic engineering?

(a) Cleaving DNA (b) Recombining DNA
(c) Cloning (d) Screening

13. The enzyme used in the polymerase chain reaction is

(a) Restriction endonuclease. (b) Reverse transcriptase.
(c) DNA polymerase. (d) RNA polymerase.

14. A method used to distinguish DNA of one individual from another is

(a) Polymerase chain reaction. (b) cDNA.
(c) Reverse transcriptase. (d) Restriction fragment length polymorphism

15. Which of the following is not an application of genetic engineering in plants?

(a) Nitrogen fixation (b) DNA vaccines
(c) Resistance to glyphosate (d) Production of insecticidal proteins in plants

16. First genetically modified organism generated was

(a) Fish (b) Bacteria

(c) Mice (d) Virus

17. Process of manipulating genes usually outside normal reproductive process is known as
(a) Genetic modification (b) Gene targeting
(c) Genome recombination (d) Gene linking

18. Which of the following is a plasmid?
(a) Sal I (b) Bam H1
(c) Eco R1 (d) pBR322

19. Taq polymerase is used in PCR because of its
(a) Low thermal stability (b) High fidelity
(c) High speed (d) High thermal stability

20. Which of the following is not a restriction endonuclease?
(a) Eco RI (b) DNA ligase
(c) Hind III (d) Bam H1

10.6.1. Answers Key: 1. Stanley Cohen and Herbert Boyer, 2**.** DNA cleaving enzymes, 3**.** DNA ligase, **4.** *Agrobacterium* Spp., **5.** Electroporation, 6**.** Nuclease, 7**.** *Bacillus thuringiensis*, **8.** Introducing foreign genes, **9.** Restriction endonuclease, **10.** Recombinant DNA

10.6.2 Answers Key: 1-(b), 2-(d), 3-(a), 4-(d), 5-(d), 6-(a), 7-(c), 8-(b), 9-(d), 10-(b), 11-(c), 12-(d), 13-(c), 14-(d), 15-(b), 16-(b), 17-(a), 18-(d), 19-(d), 20-(b)

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10.9 TERMINAL QUESTIONS

1. Give a detailed account of Restriction endonuclease.
2. What are GMOs? What are ethical issues associated with them
3. What is DNA sequencing?
4. How can plant and animal viruses be used as vectors? Discuss using suitable examples
5. Describe major steps of gene manipulation.
6. What is genetic engineering?
7. At the present level of advancement of biotechnology, what are the main techniques of genetic engineering?
8. What are the different tools used in genetic engineering?
9. What are DNA ligases? How do these enzymes participate in recombinant DNA technology?
10. What are restriction enzymes? How do these enzymes participate in recombinant DNA technology?
11. What is PCR? How does PCR works?

12. What is cloning? How is genetic engineering used to create bacteria capable of producing human insulin?
13. What is the main moral problem regarding the cloning of human individuals?
14. What are transgenic plants? Discuss some genetically modified plants?
15. What are the various ethical issues probably arise regarding genetic engineering?

UNIT-11 BIOTECHNOLOGY

11.1 OBJECTIVES

After reading this unit student will be able to understand:

- Meaning of Biotechnology
- Definition of Biotechnology
- Applications of Biotechnology in Health, Agriculture and Industry

11.2 INTRODUCTION

Contrary to its name, biotechnology is not a single technology. Rather it is a group of technologies that share two (common) characteristics, working with living cells and their molecules and having a wide range of practice uses that can improve our lives.

Biotechnology deals with techniques of using live micro-organisms, plant or animal cells or their components or enzymes from organisms to produce products and processes (services) useful to human beings. The term biotechnology was coined in 1917 by a Hungarian Engineer, **Karl Ereky** to describe a process for large scale production of pigs. Gene manipulation is a fast emerging science. It started with the development of recombinant DNA molecules. It is named variously as DNA manipulation biotechnology, recom-binant DNA technology and genetic engineering. The technology mostly involves cutting and pasting of desired DNA fragments.

Fig.11.1 Karl Ereky

It is based on two important discoveries in bacteria:

(i) Presence of plasmids in bacteria which can undergo replication along with and independent of chromosomal DNA.

(ii) Restriction endonucleases (**Arber, Nathan and Smith** 1970; Nobel Prize in 1978) which can break DNA at specific sites.

They are appropriately called molecular scissors. **Berg** (1972) was able to introduce a gene of SV-40 into a bacterium with the help of lambda phage. Berg is often considered "father of genetic engineering". He was awarded Nobel Prize in 1980.

In 1973 **Herbert Boyer**, of the University of California at San Francisco, and **Stanley Cohen**, at Stanford University, reported the construction of functional organisms that combined and replicated genetic information from different species. Their experiments dramatically demonstrated the potential impact of DNA recombinant engineering on medicine and pharmacology, industry and agriculture. The science of recombinant technology took birth when Cohen and Boyer were able to introduce a piece of gene containing foreign DNA into plasmid of *Escherichia coli*.


Fig.11.2 Stanley N. Cohen and Herbert Boyer

Old Biotechnology (Traditional Biotechnology)

Microorganisms were first used to produce some organic compounds like citric acid. They were also used to produce antibodies. The levels of production of penicillin yield has been improved But the types of products are not changed. They remain the same as those obtained from the natural strains or cell lines. In all these processes, only the natural capabilities of the organisms and cells are exploited. These activities are called old biotechnology.

Modern Biotechnology

Human insulin is also produced from a transgenic *Escherichia coli* stain that contains and expresses the insulin gene. Proteins produced by transgenes are called recombinant proteins. The production technologies based on genetic engineering are termed as modern biotechnology. It developed during 1970.

Biotechnology-(A Multidisciplinary Growing Tree)

Biotechnology is an interdisciplinary pursuit with multidisciplinary applications, and it may be represented as a growing biotechnology tree. This figure gives an overview of biotechnology with special reference to the fundamental principles and scientific foundations, biotechnological tools and applications of biotechnology.

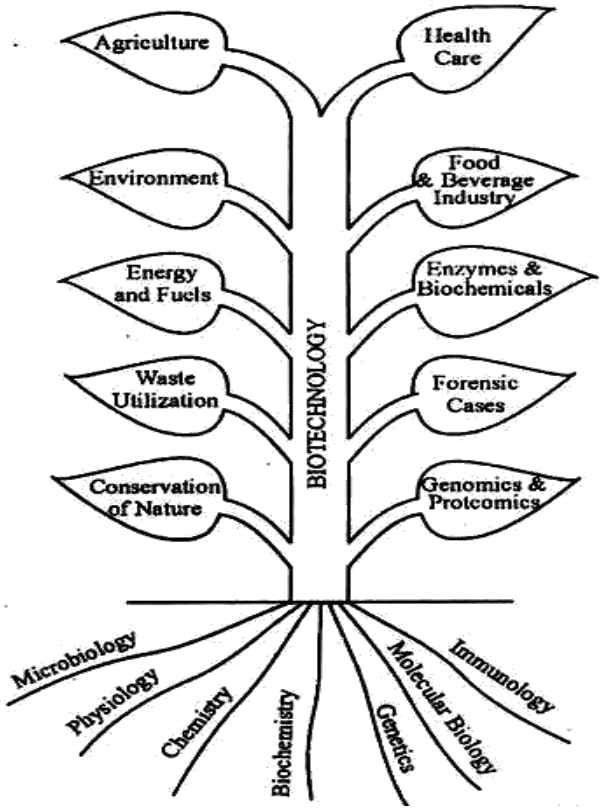

Fig.11.3 A Multidisciplinary Growing Tree

Biotechnological Tools

Several methods, techniques or procedures which may be collectively called as Biotechnological tools have been developed for transforming the scientific foundations into biotechnological applications. These tools include protein engineering, bioprocess/fermentation technology, cell and tissue culture technology, transgenesis and antisense technology.

11.3 DEFINITION OF BIOTECHNOLOGY

Biotechnological practices are being followed since time immemorial; however it emerged as a new discipline in late 1970s. Making yoghurt from the milk, and making wine by the fermentation of grapes, are some of the biotechnological processes used since long. Biotechnology, is the use of biological processes, organisms, or systems to manufacture products intended to improve the quality of human life. The earliest biotechnologists were farmers, who developed improved species of plants and animals by cross-pollination or cross-breeding. In recent years, biotechnology has expanded in sophistication, scope, and applicability.

The definition was almost ignored for many years. For most people, biotechnology represented two aspects of engineering- Industrial fermentation and Study of the efficiency at

work place. Biotechnology is a term which is a combination of two individual terms- Biology and Technology. As the name suggests "It is the assembly of technology in science of biology". Most simply it may be defined as- "The regulated and controlled use of the biological agents for the manufacture of useful products or for generating beneficial services". These biological agents may be microorganisms, animals or plants or their cellular components. However, it is not easy to define biotechnology in a single sentence because of its wide and multidisciplinary applications.

The fact that biotechnology is interdisciplinary in nature, with a wide range of applications has created some confusion with regard to its definition. This is mainly because scientists from each discipline have described the term from their own perspective. Various definitions have been given by different scientific organizations.

- **U.S. National Science Federation** says that "Biotechnology is the controlled use of biological agents such as microorganisms or cellular components for beneficial use."
- According to **IUPAC (International Union of Pure and Applied Chemistry)**, biotechnology means "The application of biochemistry, biology, microbiology and chemical engineering to industrial processes and products and on environment."
- The **European Federation of Biotechnology (EFB)** broadly considers biotechnology as "*The integration of natural sciences and organisms, cells, parts thereof, and molecular analogues for products and services*". In whichever way the term biotechnology has been defined, it essentially represents the use of microbial, animal or plant cells or enzymes to synthesize, breakdown or transform materials.

A definition of biotechnology which covers both traditional views and modern molecular biotechnology has been given by EFB. According to which- " Biotechnology is the integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application of the capabilities of microorganisms, cultured tissues or cells and parts thereof ". Thus definition of biotechnology involves two common factors- First the use of biological agents and second, the product or service is generated for the well being of humans.

Development of Biotechnology

The development of biotechnology, in the first half of twentieth century is associated with the fields of applied microbiology and industrial fermentations (production of penicillin, organic solvents etc.) The development of modern biotechnology is closely linked with the advances made in molecular biology. A selected list of historical foundations that contributed to the advancement of biotechnology is given below:

6000BC: Wine preparation (using yeast)

4000BC: Bread making (employing yeast)

1876: **Louis Pasteur** identified role microorganisms in fermentation.

1914: Production of industrial chemicals (acetone, butanol, glycerol) by using bacteria.

1917: The term biotechnology was coined by **Karl Ereky**.

1928: Discovery of penicillin by **Alexander Flaming**.
1973: Establishment of recombinant DNA technology by **Boyer and Cohen**.
1975: Production of monoclonal antibodies by **Kohler and Milstein**.
1976: **Sanger and Gilbert** developed techniques to sequence DNA.
1978: Production of human insulin in E. coli.
1983: Use of Ti plasmids to genetically transform plants.
1988: Development of polymerase chain reaction (PCR).
1990: Official launching of human genome project (HGP).
1992: First chromosome (of yeast) sequenced.
1996: First eukaryotic organism (*Saccharomyces cerevisiae*) sequenced.
1997: The first mammalian sheep, **Dolly** developed by nuclear cloning.
2000: First plant genome (of *Arabidopsis thaliana*) sequenced.
2001: Human genome, the first mammalian genome, sequenced.
2002: First crop plant (rice, *Oryza sativa*) genome sequenced.

The biotechnology revolution began in the 1970s and early 1980s when the scientists understood the genetic constitution of living organisms. A strong foundation of genetic engineering and modern biotechnology was laid down by Cohen and Boyer in 1973 when they could successfully introduce the desired genes of one organism into another, and clone the new genes. It is an acknowledged fact that of all the scientific development, related recombinant DNA technology (rDNA technology) trigged the most significant and profound advancements in biotechnology. Thus, rDNA technology laid firm foundations for genetic engineering.

The science of biotechnology can be broken down into sub disciplines called red, white, green, and blue.

Red biotechnology: involves medical processes such as getting organisms to produce new drugs, or using stem cells to regenerate damaged human tissues and perhaps re-grow entire organs.

White biotechnology: (also called gray), involves industrial processes such as the production of new chemicals or the development of new fuels for vehicles.

Green biotechnology: applies to agriculture and involves such processes as the development of pest-resistant grains or the accelerated evolution of disease-resistant animals**.**

Blue biotechnology: rarely mentioned, encompasses processes in marine and aquatic environments, such as controlling the proliferation of noxious water-borne organisms.

11.4 APPLICATIONS OF BIOTECHNOLOGY

The fruits of biotechnological research have wide range of applications. In fact, there is no other branch of science which has as many applications as biotechnology. Biotechnology has benefited medical and health sciences (diagnostics, vaccines, therapeutics, and foods), agricultural sciences

(improved crop yield, food quality, and improved animal health) and environmental sciences (pollution control, environmental monitoring, and bioremediation).

It is desirable to describe at least one example of biotechnological achievement that has helped the mankind. Prior to 1982, insulin required for the treatment of diabetics was obtained from pig and cow pancreases. The procedure was tedious, and the use of animal insulin was frequently associated with complications. Then the human gene for insulin was isolated, cloned and expressed in microorganisms for the large scale production of insulin. Insulin was the first pharmaceutical product of recombinant DNA technology approved for human use. Millions of diabetics over the world are benefited by the biotechnology of insulin production.

Biotechnology is such a branch of science which has advanced rapidly and has emerged as a potential science for providing benefits in all the fields of human welfare. It has a great impact in almost all the domains of human life, may it be health, environment, foods or agriculture. Recent advancements have led to a multidisciplinary applicability of biotechnology. Various areas in which this discipline is very frequently used on a large scale are as follows:

1. Agriculture
2. Food and Beverage Industry
3. Environment
4. Health care and Medicines
5. Energy and Fuels.
6. Enzymes and Biochemical.
7. Other Industrial applications.
8. Forensic cases
9. Conservation of Nature

11.4.1 Applications in Health

The world has witnessed extraordinary advances in science over the last few decades. Biotechnology, one such area of growth - is an umbrella term covering a broad spectrum of scientific applications used in many sectors, such as health. It involves the use of living organisms, or parts of living organisms, to provide new methods of production and make new products.

From new vaccines to prevent disease to genetically modified plants with resistance to pests; from replacement heart valves that are better accepted by the body to treatments for human infertility; and from bacteria (*Pseudomonas*) capable of cleaning up oil spills to environmentally friendly biofuels - biotechnology, like any new technology, offers us potential benefits and potential risks.

Biotechnology has been used in many areas related to food and nutrition. In fact, the first commercially produced genetically engineered product was human insulin. Next was an enzyme **Chymosin** also called **Rennin**, produced with biotechnology that replaced the **Rennet** (a complex of enzymes produced in the stomachs of ruminant mammals) used in cheese production.

A more recent focus of biotechnology has been to enhance the nutrient content of food. This area includes the development of oils with reduced saturated fat content and rice that has been modified to have high carotene (a vitamin A precursor) levels. There are foods and crops produced using biotechnologies that are available in the markets.

1. Tomatoes with delayed ripening traits that have better flavor, remain fresh longer and withstand transport better than traditional tomatoes.
2. Soybeans, canola, corn, cotton and potatoes resistant to either insects, herbicides or both.
3. Squash resistant to a virus that often kills the vegetable on the vine.
4. Soybeans and canola that produce reduced saturated fat cooking oil.

All of these foods have undergone rigorous testing.

In medical field, the contribution of biotechnology is most frequent. It not only helps in the cure of diseases but also aids in detection and prevention of disease. It also helps in curing genetic disorders by means of gene therapy.

1. DNA probes and Monoclonal antibodies are used as tools for diagnosis of diseases.
2. Many valuable drugs and antibiotics are also produced on large scale by using biotechnological processes.
3. Human Insulin was the first therapeutic product to be made commercially by genetically engineered bacterium.
4. Cloning of human leukocyte interferon gene, Hepatitis B virus gene, Human Growth Hormone (HGH) genes, etc. have also helped in the production of vaccines.
5. Gene therapy is the method of curing genetic diseases (or acquired diseases) by the replacement of an abnormal gene by a therapeutic gene. Diseases like Tay-sachs disease, Cystic fibrosis, etc. can be cured by gene therapy. Currently biotechnologists are also making trials for using gene therapy to cure tumors, cancers, etc.

The type of gene therapy which is done at the level of germ cells like sperms, or eggs is called as germ line gene therapy. In this type of gene therapy, the functional genes are introduced into the genome of germ cells.

The changes so occurred are passed on to the forthcoming generations also, i.e., the changes are heritable in case of germ line gene therapy. Other type of gene therapy is the somatic cell gene therapy. It involves the correction of genetic defects by introduction of therapeutic gene into the somatic cells of body. The changes so occurred are not heritable.

6. Genetic engineering aids in the high speed and high quantity production of antibiotics by certain microorganisms.

A few important antibiotics and their sources are given below:

S. No.	Antibiotics	Source
1.	Penicillin	*Penicillium chrysogenum* (or *Penicillium notatum*),
2.	Streptomycin	*Streptomyces griseus*
3.	Aureomycin or	*Streptomyces aureofaciens*

	(Chlortetracycline)	
4.	Chloromycetin or (Chloramphenicol)	*Streptomyces venezuelae*, *Streptomyces lavendulae*
5.	Erythromycin	*Saccharopolyspora erythraea* (or *Streptomyces erytheraeus*)
6.	Griseofulvin	*Penicillium griseofulvum*
7.	Oxytetracycline	*Streptomyces rimosus*

Healthcare Biotechnology - (Red Biotech)

The healthcare sector, which includes diagnostic systems and innovative therapies, constitutes the leading segment of the whole biotechnological industry on an international level. In the last decades, the use of biotech in medicine has led to a series of important developments in several fields.

Therapy

The recombinant human insulin produced by genetically modified bacteria was the first biotech drug (1982). Since then, the use of biotechnology has led to the marketing of nearly 200 biotechnology products, including drugs, vaccines and advanced therapies. About 50% of all new drugs and therapies in development for the foreseeable future will originate from biotechnology, and the proportion is growing in the most innovative treatments such as vaccines, monoclonal antibodies for the treatment of cancer and inflammatory diseases/infectious diseases, cell therapy, gene therapy and regenerative medicine. In particular, a decisive contribution of biotechnology is placed on prevention through the development of vaccines: infectious diseases such as polio, measles, hepatitis A, B and C, diphtheria, tetanus, cholera, rabies, meningitis, tuberculosis, and many others have been addressed (and in some cases even eradicated) with the aid of biotech vaccines.

Diagnostics

Today for many diseases you can make an early diagnosis in time to locate and treat them with highly specific methods. A major problem with which we had to face in recent decades was the question of the "window period", the period of time that elapses between the first infection of a virus and the time at which a diagnostic test detects the antibody response. During the "window period" which can vary from a few weeks to several months, the tests were negative: in this way, a person with HIV or Hepatitis B was considered healthy while, on the contrary, they had been infected.

Thanks to biotechnology it had been possible to develop techniques such as PCR, which allowed the immediate identification of the presence of viral DNA in the infected patient. Other biotechnology techniques, such as ASO (allele specific oligonucleotide), FRET (fluorescence resonance energy transfer) and OLA (Oligonucleotide Ligation Assay), are used by laboratories

around the world to identify mutations in the genome, confirming the suspicions of a certain disease or making a definite clinical diagnosis.

Nano-Biotechnology

In the development of increasingly targeted and effective products, the convergence of nanotechnology and biotechnology is a powerful tool available to researchers in the diagnosis and treatment of a large number of diseases, in the development of means for the controlled release of drugs and in the field of biomaterials with a variety of applications in the life sciences and in the engineering of connective tissues of the human body, until the realization of the vital organs (e.g. regenerative medicine). The Nano-biotechnology is a highly multidisciplinary field of investigation, involving fields of research ranging from molecular biology to chemistry, materials science to physics, both applied and basic, to engineering mechanics and electronics.

Cosmetics

Between the healthcare and wellness, there are many uses of molecular biology in cosmetics: **Hyaluronic acid** and other fillers commonly used are in fact biotech-sourced. In particular, the new frontier of anti-aging has been disclosed with the advent of natural active ingredients modified with biotechnology, often extracted according to the so-called biocorrelation systems, which allow you to make the most of the active ingredients with no waste, saving trees and plants.

Biotechnology for Animal Healthcare

In the field of veterinary medicine, biotechnology is of considerable importance. Global trade, migration and climate changes may increase the spread of highly infectious diseases and zoonoses such as avian influenza. **Zoonoses** are infectious diseases of animals (usually vertebrates) that can naturally be transmitted to humans. Major modern diseases such as **Ebola virus** disease and **salmonellosis** are zoonoses.

In these cases, vaccination is the only way to protect animals against infectious diseases and help protect consumers from zoonotic pathogens. In recent years, there have been many vaccines produced using biotechnological techniques (e.g., vaccines against rabies, *epizoonotic apthae* and **Rinderpest virus** also cattle plague or steppe murrain); intervening with these vaccines and specific therapies can ensure that the animals reach their maximum weight in good health, while allowing farmers to maintain the productivity of their livestock.

11.4.2 Applications in Agriculture

Modern biotechnology holds considerable promise to meet challenges in agricultural production. These techniques can transform ideas into practical applications, viz, certain crops can be genetically altered to increase their tolerance to certain herbicides. Biotechnology can be used to develop safer vaccines against viral and bacterial diseases. It also offers new ideas and techniques applicable to agriculture and also develops a better understanding of living systems of our environment and ourselves. It has a tremendous potential for improving crop production.

New approaches in biotechnology can develop high yielding and more nutritious crop varieties, improve resistance to disease and also reduce the need for fertilizer and other expensive agricultural chemicals. It could also improve forestry and its products, fiber crops and chemical feedstock. They can also help in propagating plant species which contain useful and biologically active substances, e.g., food additive, pigment, pharmaceuticals, biopesticides, etc. Organ tissue and cell culture could be more efficient than conventional extraction.

Biotechnology helps to isolate the gene, study its function and regulation, modify the gene and reintroduce it into its natural host of another organism. It helps unlocking the secrets of diseases resistance, regulates growth and development or manipulates communication among cells. It is a comparatively new technique and is used in the field of agriculture and horticulture. This mainly involves manipulation in the genetic code (which includes processes like gene transfer), tissue culture, monoclonal antibody preparation and protoplast fusion. These processes help in increasing yield, producing better quality products both in plants and animals, increasing resistance to pests and herbicides, micro propagation in several crops etc. are some of the advantages of using biotechnological methods.

As far as biotechnology is concerned, the size of the farm is immaterial. So whether you farm vegetables in your backyard or cultivate in a large farm extending to several thousand acres, the key point is to have a bio-rational way of cultivation. That is the best way to grow not only healthier plants for ensuring food security, but also to protect our planet for future generations. Of course biotechnology is a science that can help bring about this transformational change. Another useful application of agricultural biotechnology is to give plants the ability to grow in a wider range of environments. Some plants do well only in certain climates or soil conditions. By introducing genes from other organisms, scientists can alter these plants so that they'll grow in climates that normally would be too harsh for them. Land previously unsuited for crops can be reclaimed for food production.

Genetic manipulation can lead to plants that are toxic to pests but still safe for human consumption. Alternatively, scientists can develop genes that will make crops resistant to pesticides and herbicides so that farmers can treat their crops with chemicals.

Agricultural biotechnology is supposed to be the answer to a hungry world’s food supply. The applications of biotechnology in agriculture rank second (first being in medicines). Biotechnological approaches are used valuably in the fields of horticulture and floriculture also. Major applicability’s of biotechnology in the field of agriculture and horticulture are- Manufacture of Biofertilizers which prove to be more beneficial than other synthetic and chemical fertilizers. Biofertilizers are cost effective, harmless for plants and they also increase soil fertility.

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants. Use of biofertilizers is one of the important components of integrated nutrient

management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers

The Nitrogen-fixation and assimilation efficiency of the crops are also being increased by the practices like cloning of nif (nitrogen fixing) genes or by transferring such genes Production of Transgenic plants or Genetically Modified plants (GMPs) – The plants whose genome has been modified by introduction of foreign gene(s) of an unrelated organism, are called transgenic plants or GMPs e.g. Bt cotton, Flavr Savr Tomato, golden Rice, etc. The transgenic plants may provide one or more characteristics of the following:

(a) Resistance to insects, fungi, bacteria and virus
(b) Highly resistant to herbicides, pesticides and other chemicals.
(c) Drought, resistance, flood resistance, Salinity resistance, etc.
(d) High productivity.
(e) Crop plants with improved quality.

Plant Tissue Culture

It aims at the in-vitro culture of plants. It is very beneficial for agriculture. This is evident by the following points:

(a) Clonal propagation helps in rapid production of commercially important plants and trees like timber trees, ornamental plants, orchids, fruits, rubber plants, etc.
(b) Production of somatic hybrids by hybridization of protoplasts compatible plant species. Such somatic hybrids have characters of both unrelated species.
(c) Production of artificial seeds, etc.

Applications of Biotechnology in Agriculture

The following points highlight the four main applications of biotechnology in agriculture:-

1. Micropropagation
2. Induction and Selection of Mutant
3. Production of Somatic Hybrids
4. Production of Transgenic Plants.

1. Micropropagation: Mass propagation of crop and forest plants is an important applica-tion of micro-propagation technique. The deve-lopment of embryos from somatic cells in culture resulted in artificial seed production.

This technique involves three stages:

(a) Establishment of culture
(b) Regeneration of plants
(c) Transfer of plants from test tube to soil

Regeneration of plantlets in cultured plant cell and tissues has been achieved in many trees of high economic value. Many of the studies are aimed at large scale micropropagation of important trees yielding fuel, pulp, timber, oils and fruits. Therefore, Clonal forestry and horticulture are gaining an increasing recognition as an alternative for tree improvement. In recent years, the interest has aroused in commercializing the in-vitro propagation of forest trees.

This will bring about refinement in the existing procedures to make micropropagation more cost effective. For betterment and improvement of tree plants of high economic value, genetic transformation and in-vitro regeneration have been done in many angiospermic and gymnospermic plants.

2. Induction and Selection of Mutant: Different physical and chemical mutagens are used in the plant explants of different species to generate mutants. Now the mutants can be used to select out the variant cell lines which are resistant to antibiotics, amino acid analogues, chlorate, nucleic acid base analogue, fungal toxin, environmental stresses (salinity, chilling, high temperature, aluminum toxicity) and herbi-cides, etc.

Single cell or the protoplast culture systems have proved to be valuable for mutagenesis since the presence of discrete cells in these substances is more effective to cause mutation, and isolation of mutant line is more easier.

3. Production of Somatic Hybrids: The protoplasts can undergo fusion under certain favorable conditions and the fused product can give rise to somatic hybrid plant which offers:

(a) The possibility of hybrid formation of widely unrelated forms,

(b) An asexual means of gene transfer either of whole genome or of partial genome.

Through successful production of hybrid plants at the tetraploid and hexaploid levels, both for inter- and intra-specific fusions, characters from sexually incompatible wild species are transferred to the cultivar.

Other approaches to genetic manipulation include the irradiation of donor protoplasts with useful characters, to fragment their genomes, followed by fusion to tetraploid acceptor protoplasts. Protoplast fusion also provides a means of transferring cytoplasmic traits into another genomic background.

Intergeneric somatic hybrids have been produced in many genera like '**Raphanobrassica**', obtained through fusion between Raphanus sativus and Brassica campestris, '**Solanopersicon**', obtained through fusion between *Solanum tuberosum* and *Lycopersicon esculentum*, etc. The technique of hybrid production has been utilized for transfer of cytoplasmic male sterile character, as has been done in case of Nicotiana, Brassica and Petunia.

4. Production of Transgenic Plants: Genetic engineering can be used to introduce genes into a plant, which do not exist in any member of the same plant family. If genetically engineered plants are to be used commercially, then the following criteria are to be satisfied:

(a) Introduction of the genes of interest to all plant cells,

(b) Stable maintenance of the new genetic information,
(c) Transmission of the new gene to subsequent generations, and
(d) Expression of the cloned genes in the correct cells at the correct time.

A number of useful traits, mostly single gene, that have been transferred to get the transgenic for various purposes are:

(i) Insect-pest resistance plants: Using gene transfer technique the Bt. gene (Cry I protein from *Bacillus thuringiensis*) has been transferred to many crop plants like rice, cotton, tomato, potato, etc. and insect resistant plants (Bt. crops) have been developed.

(ii) Herbicide resistant plants: Using biotechnological approaches many herbicide resistant crop plants have been obtained as in Brassica, tomato, corn, cotton, soybean, etc. which are resistant against glyphosate (Roundup), Glufosinate or L-phosphinothricin (herbicide-BASTA), etc.

(iii) Virus resistant plants: Viral coat protein genes can be introduced to get the virus resistant plants as has been done in tomato, potato, squash, papaya, etc.

(iv) Resistance against bacterial and fungal pathogens: Several examples are available where the transgenic plants against bacterial and fungal pathogens have been developed. The Chitinase gene have been introduced in tobacco to get the resistance against brown spot; acetyl transferase gene has been introduced in tobacco to get the resistance against wild fire disease.

(v) Improvement in nutritional quality: Nutritional quality can be improved by introduc-ing the genes for production of cyclodextrins, vita-mins, amino acids, etc.

Transgenic potato has been obtained to produce cyclodextrin molecule; the transgenic rice named as '**Golden rice'** has been obtained to produce pro vitamin-A which has opened the way for improving the nutritional stan-dards; Ama-I gene has been introduced in potato. Starch content has been increased in transgenic potato.

(vi) Quality of seed-protein and seed-oil: Recombinant DNA technology has been used successfully for improvement of protein quality in seed as has been done in pea plant which is rich in sulphur containing amino acids; lysine rich cereals have also been produced.

Oilseed rape has been made transgenic which has the modified seed oil quality, i.e., low erucic acid. Reduced linolenic acid containing flax and high stearic acid containing soybean and safflower also have been produced.

(vii) Improvement of quality for food- processing: 'Flavr-Savr' variety of tomato has been raised which shows bruise resistance as well as delayed ripening.

(viii) Male sterility and fertility restoration in transgenic plants: Male sterile transgenic plants have been produced with 'Barnase' gene which has the cytotoxic product tagged with anther spe-cific TA-29 promoter, and another set of plants have been produced to restore the fertility factor with the help of 'Barstar' gene tagged with the same promoter. F1 hybrids from these two sets of transgenic should facilitate the hybrid seed production for crop improvement.

(ix) Production of stress tolerant: Several projects are going on for transgene application to develop the tolerance against different abiotic stresses, e.g., cold (tobacco), drought (mustard), salt (rice).

11.4.3 Applications in Industries

The very first expression of industrial applications of biotechnology was found in the production of beer, wine, cheese, bread and other fermented products. Over the years, such applications have expanded to include a very wide range of products in the food, chemical and pharmaceutical industries. Genetic engineering and molecular biology have proved invaluable not only for the development of a host of products, but also for introducing new and more effective bioprocesses.

Industrial biotechnology is one of the most promising new approaches to pollution prevention, resource conservation, and cost reduction. It is often referred to as the third wave in biotechnology. If developed to its full potential, industrial biotechnology may have a larger impact on the world than health care and agricultural biotechnology. It offers businesses a way to reduce costs and create new markets while protecting the environment. Also, since many of its products do not require the lengthy review times that drug products must undergo, it's a quicker, easier pathway to the market. Today, new industrial processes can be taken from lab study to commercial application in two to five years, compared to up to a decade for drugs.

From the beginning, industrial biotechnology has integrated product improvements with pollution prevention. Nothing illustrates this better than the way industrial biotechnology solved the phosphate water pollution problems in the 1970s caused by the use of phosphates in laundry detergent. Biotechnology companies developed enzymes that removed stains from clothing better than phosphates, thus enabling replacement of a polluting material with a non-polluting biobased additive while improving the performance of the end product. This innovation dramatically reduced phosphate-related algal blooms in surface waters around the globe, and simultaneously enabled consumers to get their clothes cleaner with lower wash water temperatures and concomitant energy savings.

Industrial biotechnology involves working with nature to maximize and optimize existing biochemical pathways that can be used in manufacturing. The industrial biotechnology revolution rides on a series of related developments in three fields of study of detailed information derived from the cell: genomics, proteomics, and bioinformatics. As a result, scientists can apply new techniques to a large number of microorganisms ranging from bacteria, yeasts, and fungi to marine diatoms and protozoa.

Industrial biotechnology companies use many specialized techniques to find and improve nature's enzymes. Information from genomic studies on microorganisms is helping researchers capitalize on the wealth of genetic diversity in microbial populations. Researchers first search for enzyme-producing microorganisms in the natural environment and then use DNA probes to search at the molecular level for genes that produce enzymes with specific biocatalytic capabilities. Once isolated, such enzymes can be identified and characterized for their ability to

function in specific industrial processes. If necessary, they can be improved with biotechnology techniques.

Many biocatalytic tools are rapidly becoming available for industrial applications because of the recent and dramatic advances in biotechnology techniques. In many cases, the biocatalysts or whole-cell processes are so new that many chemical engineers and product development specialists in the private sector are not yet aware that they are available for deployment. This is a good example of a "technology gap" where there is a lag between availability and widespread use of a new technology. This gap must be overcome to accelerate progress in developing more economic and sustainable manufacturing processes through the integration of biotechnology.

The application of biotechnology to industrial processes is not only transforming how we manufacture products but is also providing us with new products that could not even be imagined a few years ago. Because industrial biotechnology is so new, its benefits are still not well known or understood by industry, policymakers, or consumers.

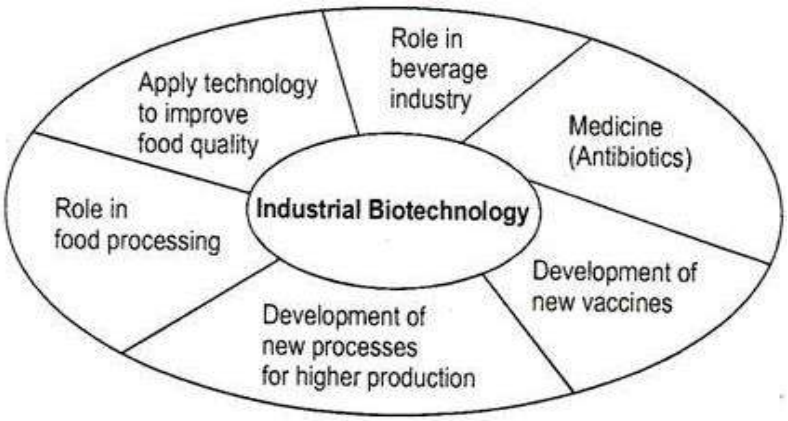

Fig.11.4 Role of Industrial Biotechnology in Human life

Industrial Applications of Biotechnology

The industrial application of molecular biotechnology is often subdivided, so that we speak of red, green, gray or white biotechnology. This distinction relates to the use of the technology in the medical field (in human and animal medicine), agriculture, the environment and industry. Some companies also apply knowledge deriving from molecular biotechnology in areas that cut across these distinctions (e.g., in red and green biotechnology, sequencing services). According to an investigation by Ernst and Young relating to the German biotech industry, 92% of companies in 2004, working in the field of red biotechnology, 13% in green, and 13% in gray or white biotechnology.

Biotechnology in Food and Beverage Industry

A number of microorganisms are used beneficially in the production of certain foods and beverages like cheese, wine curd beer, vinegar, etc. The underlying process behind such productions is fermentation Natural fermentation has played a vital role in human development and it is the oldest form of production of wine. Fermentation may be defined as the process which involves the biochemical activity of microorganisms to produce an economically important product like food, beverages or pharmaceuticals. In other words, it is the use of microorganisms for production of commercial products. Natural fermentation is the part of traditional biotechnology.

Several modifications are also done in the genome of microbes by gene transfer methods to achieve better results and this is involved in modern biotechnology. The fermentation may be performed by yeasts, bacteria, molds or by combination of these organisms. Yeasts are of primary importance in manufacture of bread, beer, wine and distilled liquors. Molds are important in the preparation of some cheeses and oriental foods A few fermented products are listed below along with the substrate name and the name of microorganism involved:

Table-1

Product	Substrate	Microorganism
1. Beer	Barley malt	*Saccharomyces uvarum, S. cerevisiae*
2. Cheese	Milk	*Streptococcus lactis*
3. Ginger Beer	Sugar solution flavoured with ginger	*Saccharomyces pyriformis and Lactobacillus vermiformis*
4. Grape wine	Juice of grapes.	*S. cerevisiae, S. ellipsoides* (Also called wine yeast)
5. Idli	Rice & black gram mungo soaked and ground	*Leuconostoc mesenteroides, Streptococcus faecalis, Pediococcus cerevisiae*
6. Miso	Steamed polished rice mash	*Aspergillus oryzae, Saccharomyces rouxii*
7. Soy Sauce	Mixture of wheat bran, soybean flour or rice	*A. oryzae, S. rouxii, Bacillus subtilis*
8. Tempeh	Boiled soybeans without seed coat	*Rhizopus* spp.
9. Tofu	Paste of soaked soybeans	*Mucor* spp.
10. Vinegar	Juices of fruits or starchy vegetables	*S. cerevisiae, S. ellipsoides and Acetobacter*
11. Yoghurt	Concentrated milk	*Streptococcus thermophilus and Lactobacillus bulgaricus.*

Distilled liquors

Liquors or spirits of interest produced by distillation of an alcoholically fermented product.

(a) **Rum**—Distillate from alcoholically fermented sugarcane juice or molasses

(b) **Whiskey**—Distillate from fermented grain mashes

(c) **Brandy**—Distillate from wine

(d) **Gin**—Distillate from fermented rye malt.

Single Cell Proteins (SCP)

It is the term which designates the high protein food from microorganisms like algae, filamentous fungi, bacteria and yeast. Genetic engineering is used to select and produce the high protein content or desirable composition of food by improving the microbial strains. SCP is, actually, the total microbial biomass which is free from any type of toxins and contaminants.

It is high in protein content so it can be used to replace the conventional vegetable and animal protein sources. Bio technological approaches have been in use for the mass-cultivation of SCP by improving the source microorganisms.

Some such microbial sources are:

Algae: *Chlorella, Spirulina, Scenedesmus*

Mushrooms: *Agaricus campestris*; *Morchella crassipes*

Yeasts: *Candida utilis, Saccharomyces fragilis, Rhodotorula*

Bacteria: *Pseudomonas, Cellulomonas*.

Biotechnology in Production of Enzymes and Biochemical Compounds

Many commercially important enzymes and other biochemical compounds can be obtained on large scale by modifying the source microbial strains using gene transfer methods. Such modified microbes are termed as GEMs (Genetically Engineered Microbes). Similarly, many other compounds like vitamins, steroids, secondary metabolites, organic acids, etc. may be extracted and derived from the activity of other GMOs (Genetically Modified Organisms). Bio-fertilizers, Bio-herbicides, Bio-insecticides etc. are certain biologically produced chemical compounds which involve the utilization of microbial activities. Some Examples of enzymes with their source microorganisms are:

Table-2

S. No.	Enzymes	Source
1.	Pectinase	*Aspergillus niger, Bacillus subtilis*
2.	Glucanase	*A. niger, B. subtilis*
3.	Lipase	*A. niger, Mucor spp.*
4.	Cellulase	*A. niger, Rhizopus, Trichoderma*
5.	α- amylase	*B. licheniformis, B. amyloliquefaciens*
6.	Invertase	*Saccharomyces cerevisiae, S. fragilis*
7.	Rennet	*Mucor spp*.
8.	Urate oxidase	*Aspergillus flavus*
9.	Protease	*Bacillus licheniformis*
10.	Glucose oxidase	*Penicillium notatum*

Other Industrial Applications: Microorganisms are of great importance for production of various substances having great scope in different industries. Microbial strains can be improved biotechnologically to get the desired product in sufficient quantity. For this purpose, the

microbes can be improved using genetic engineering (recombinant DNA technology). Different products of interest which are frequently produced in this manner are vitamins, enzymes, organic acids, amino acids, etc. Using genetic engineering techniques, it has become possible to obtain the mutants of microorganisms which can produce a much higher amount of product of interest than the natural ones. Genetic engineering not only results into enhanced metabolite production but may also help in product modification, or producing a completely new product of interest. Different uses of genetically engineered microbes (GEM) in various industries can be enlisted as follows:

(a) Vitamins like- A, B, C, etc.
(b) Alcohols like Ethanol, Butanol, Amyl Alcohol.
(c) Amino Acids e.g. L-Glutamate, Glycine, L-Lysine, L-Valine, eta
(d) Antibiotics e.g. Penicillin, Tetracyclic Streptomycin, etc.
(e) Enzymes from fungi, bacteria (e.g. L-amylase, lipase, penicillinase, protease, invertase)
(f) Bio-fertilizers, Bio-insecticides and Bio-herbicides from biotechnologically improved bacterial, fungal, protozoan strains. These are of great benefit in agriculture.
(g) Extraction of minerals like copper, uranium from ores through leaching by using improved bacterial strains.

Scope of Biotechnology

Biotechnological approaches are applied to accomplish goals for the benefit of mankind. Scientists have achieved many such goals and a few fields are also there in which they are trying for success. Following are a few programmes being undertaken by the biotechnologists:

(1) Development of effective antiviral vaccines.
(2) Bio-control of plant diseases
(3) Genetically improving the pharmaceutical microorganisms.
(4) Large scale production of bio-pesticides and bio-fertilizers.
(5) Production of Human Interferon's.
(6) Upgrading the photosynthetic efficiency of plants.
(7) Production of secondary metabolites from plants on large scale.
(8) Improved production of vitamins.
(9) Developing efficient biofuels.
(10) Developing methods for curing cancer.
(11) Better gene therapy practices for human.
(12) Production of transgenic animals and plants with better qualities.
(13) Protection of threatened species.

Biotechnology has become a very happening branch of science today. Developed countries and even some developing countries also, are pushing the researches in this field biotechnology has a great commercial potential It has revolutionized the industries specially the

pharmaceuticals. This revolution is clearly reflected by the emergence of a number of biotechnological companies all over the world.

Biotechnology in India

Like other developing countries, biotechnology has become a major thrust in India also for promotion and planning of various biotechnological programmes in India, there is present a separate department called Department of Biotechnology (DBT). It was set up in 1986 under the Ministry of Science & Technology.

DBT funds some important centres for exploiting biotechnological approaches and also for promotion of post-graduate education and research in the field of biotechnology. Apart from DBT there are some other agencies also which work under the Indian Government for promotion of biotechnological approaches in various fields like industry, agriculture and environment. A few important of them are:

DST—Department of Science and Technology, New Delhi
CSIR—Council for Scientific and Industrial Research, New Delhi
ICMR—Indian Council of Medical Research, New Delhi
IARI—Indian Agricultural Research Institute, New Delhi

There are many other centres in India which function, in one way or the other for promoting biotechnology in India. Some of these centres are: NDRI—National Dairy Research Institute, Karnal, Haryana
CDRI—Central Drug Research Institute, Lucknow, U.P.
IVRI—Indian Veterinary Research Institute, Izatnagar. U.P.
CFTRI—Central Food and Technological Research Institute, Mysore
CIMAP—Central Institute of Medicinal and Aromatic Plants, Lucknow, U.P.
IITs—Kanpur, Madras, Bombay, New Delhi.
NBPGR—National Bureau of Plant Genetic Resources, New Delhi

In addition to all the above mentioned centres, there are also a number of companies in private sector of India which have been showing keen interest in the production of modern biotechnological products.

11.5 SUMARRY

The term biotechnology represents a fusion or an alliance between biology and technology. Biotechnology, broadly defined, includes any technique that uses living organisms, or parts of such organisms, to make or modify products, to improve plants or animals, or to develop microorganisms for specific use. It ranges from traditional biotechnology to the most advanced modern biotechnology. Biotechnology is a frontline technologies today being developed and used to understand and manipulate biological molecules for applications in medical, agricultural,

industrial and environmental sectors of the national economy. Recent advances in biotechnology provide good opportunities for immediate benefits to developing countries. Biotechnology is not a separate science but rather a mix of disciplines (genetics, molecular biology, biochemistry, embryology, and cell biology) converted into productive processes by linking them with such practical disciplines as chemical engineering, information technology, and robotics.

The progress of biotechnology, to a great extent, is driven by economics. This is so since the ultimate objective of biotechnology is the development of commercial products. Due to high stakes in biotechnology, the business and research are closely associated. It is a fact that many biotechnology companies (besides the government-run institutions) have significantly contributed to the development of present day biotechnology. Most of the commercial developments of biotechnology have been centered in the United States and Europe. Humans are the ultimate beneficiaries of biotechnology. This may be through healthcare, transgenic plants and animals, pesticides, fertilizers, in vitro cultures etc. The public perceptions of biotechnology will significantly influence the rate and direction of future growth of biotechnology.

The use of recombinant DNA technology has raised safety concerns. The public attitudes to biotechnology are mostly related to matters of imaginary dangers of genetic manipulations. Some people argue against genetic engineering, and many times, the public and politicians are misled. There is a need for the biotechnology community to frequently interact with the media and public to clear the unwarranted fears about the genetic engineering and biotechnology.

Biotechnology, with much fanfare, has become a comprehensive scientific venture from the point of academic and commercial angles, within a short time with the sequencing of human genome and genomes of some other important organisms. The future developments in biotechnology will be exciting. It may be rather difficult to make any specific predictions, since new technical innovations are rapidly replacing the existing technologies. It is expected that the development in biotechnology will lead to a new scientific revolution that could change the lives and future of the people. It has happed through industrial revolution and computer revolution. And now, it is the turn of biotechnology revolution that promises major changes in many aspects of modern life.

11.6 GLOSSARY

Antibiotics: Any of a large group of chemical substances, as penicillin or streptomycin, produced by various microorganisms and fungi, having the capacity in dilute solutions to inhibit the growth of or to destroy bacteria and other microorganisms, used chiefly in the treatment of infectious diseases.

Antisense Technology: A tool that is used for the Inhibition of gene expression. The principle behind it is that an antisense nucleic acid sequence base pairs with its complementary sense RNA strand and prevents it from being translated into a protein.

ASO: Allele Specific Oligonucleotide

Barnase: ("**BA**cterial" "**R**ibo**N**ucle**ASE**") is a bacterial protein that consists of 110 amino acids and has ribonuclease activity. It is synthesized and secreted by the bacterium Bacillus amyloliquefaciens, but is lethal to the cell when expressed without its inhibitor Barstar.

Barstar: A small protein synthesized by the bacterium *Bacillus amyloliquefaciens*. Its function is to inhibit the ribonuclease activity of its binding partner Barnase.

Biofertilizers: A substance which contains living microorganism which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.

Biofuels: A fuel that is produced through contemporary biological processes, such as agriculture and anaerobic digestion, rather than a fuel produced by geological processes such as those involved in the formation of fossil fuels, such as coal and petroleum, from prehistoric biological matter.

Bioinformatics: An interdisciplinary field that develops methods and software tools for understanding biological data.

Biomass: Material produced by or remaining after the death of organisms (e.g., bacteria, plants, and animals).

Biopesticides: A contraction of 'biological pesticides', include several types of pest management intervention: through predatory, parasitic, or chemical relationships.

Bioremediation: The use of organisms, usually microorganisms, to break down pollutants in soil, air or groundwater.

Biotechnology: A collection of technologies that use living cells and/or biological molecules to solve problems and make useful products.

Blue Biotechnology: The application of molecular biological methods to marine and freshwater organisms.

Cancer: A malignant and invasive growth or tumor, especially one originating in epithelium, tending to recur after excision and to metastasize to other sites.

Cloning: Cloning is the process of producing similar populations of genetically identical individuals that occurs in nature when organisms such as bacteria, insects or plants reproduce asexually.

Cross-pollination: Pollination of a flower or plant with pollen from another flower or plant.

Cultivar: A plant variety that has been produced in cultivation by selective breeding.

Cystic fibrosis: A genetic disorder that affects mostly the lungs, but also the pancreas, liver, kidneys, and intestine.

DNA: (Deoxyribonucleic acid), the chemical molecule that is the basic genetic material found in all cells.

Enzymes: A protein that accelerates the rate of chemical reactions. Enzymes are catalysts that promote reactions repeatedly, without being damaged by the reactions.

Fermentation: A metabolic process that consumes sugar in the absence of oxygen. The products are organic acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation.

Floriculture: A discipline of horticulture concerned with the cultivation of flowering and ornamental plants for gardens and for floristry, comprising the floral industry.

FRET: Fluorescence Resonance Energy Transfer

GEMs: Genetically Engineered Microbes

Gene therapy: Altering DNA within cells in a living organism to treat or cure a disease. It is one of the most promising areas of biotechnology research. New genetic therapies are being developed to treat diseases such as cystic fibrosis, AIDS and cancer.

Gene: A unit of hereditary information. A gene is a section of a DNA molecule that specifies the production of a particular protein.

Genetic disorder: A genetic problem caused by one or more abnormalities in the genome, especially a condition that is present from birth (congenital).

Genetic engineering: The technique of removing, modifying or adding genes to a DNA molecule in order to change the information it contains. By changing this information, genetic engineering changes the type or amount of proteins an organism is capable of producing.

GMO: Genetically modified organism**, a**n organism that has been modified, or transformed, using modern techniques of genetic exchange is commonly referred to as a genetically-modified organism.

Genome: The complete set of an organisms genetic information. In humans this corresponds to twenty-three pairs of chromosomes.

Green Biotechnology: The application of biological techniques to plants with the aim of improving the nutritional quality, quantity and production economics.

Hepatitis: Inflammation of the liver, caused by a virus or a toxin and characterized by jaundice, liver enlargement, and fever.

Herbicides: A substance or preparation for killing plants, especially weeds.

Hexaploid: Having a chromosome number that is six times the haploid number.

HGH: Human Growth Hormone also known as somatotropin, a peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals.

Horticulture: The cultivation of a garden, orchard, or nursery; the cultivation of flowers, fruits, vegetables, or ornamental plants.

Hyaluronic acid: Also called hyaluronan, is an anionic, non-sulfated Glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues.

Hybrid: The offspring of two animals or plants of different breeds, varieties, species, or genera, especially as produced through human manipulation for specific genetic characteristics.

Infertility: Infertility is the inability of a person, animal or plant to reproduce by natural means.

Inflammatory: Relating to or causing inflammation of a part of the body.

Insulin: A polypeptide hormone, produced by the beta cells of the islets of Langerhans of the pancreas that regulates the metabolism of glucose and other nutrients.

Interferons: Any of various proteins, produced by virus-infected cells, that inhibit reproduction of the invading virus and induce resistance to further infection.

In-vitro: Made to occur in a laboratory vessel or other controlled experimental environment rather than within a living organism or natural setting.

Leukocyte: Also called White blood cells (WBCs), are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders.

Microbiology: The branch of biology dealing with the structure, function, uses, and modes of existence of microscopic organisms.

Molecular biology: A branch of biology concerned with studying the chemical structures and processes of biological phenomena at the molecular level molecule.

Monoclonal: Pertaining to cells or cell products derived from a single clone.

Mutant: A new type of organism produced as the result of mutation.

OLA: Oligonucleotide Ligation Assay

PCR: Polymerase chain reaction is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Pesticides: Are substances that are meant to control pests (including weeds).

Pharmaceuticals: Pertaining to pharmacy or pharmacists.

Pharmacology: The science dealing with the preparation, uses, and especially the effects of drugs.

Plant breeding: It is the technique involving crossing plants to produce varieties with particular characteristics (traits) which are carried in the genes of the plants and passed on to future generations.

Plasmid: The circular DNA structure used by bacteria.

Proliferation: The growth or production of cells by multiplication of parts.

Protoplast: The contents of a cell within the cell membrane, considered as a fundamental entity.

Recombinant DNA (rDNA) technology: The laboratory manipulation of DNA in which DNA, or fragments of DNA from different sources, are cut and recombined using enzymes. This recombinant DNA is then inserted into a living organism. rDNA technology is usually used synonymously with genetic engineering.

Recombinant DNA: DNA that is formed by combining DNA from two different sources. Humans direct formation of recombinant DNA through selective breeding and genetic engineering.

Red Biotechnology: The use of organisms for the improvement of medical processes. It includes the designing of organisms to manufacture pharmaceutical products like antibiotics and vaccines, the engineering of genetic cures through genomic manipulation, and its use in forensics through DNA profiling.

Regenerative medicine: A branch of translational research in tissue engineering and molecular biology which deals with the "process of replacing, engineering or regenerating human cells, tissues or organs to restore or establish normal function".

Rennet: A complex of enzymes produced in the stomachs of ruminant mammals. Chymosin (Rennin), its key component, is a protease enzyme that curdles the casein in milk. This helps

young mammals digest their mothers' milk. Rennet can also be used to separate milk into solid curds for cheese making and liquid whey.

Rennin: A coagulating enzyme occurring in the gastric juice of the calf, forming the active principle of rennet and able to curdle milk.

Rhizosphere: Area of soil that surrounds the roots of a plant and is altered by the plant's root growth, nutrients, respiration, etc.

SCP: Single-cell protein (SCP) refers to protein derived from cells of microorganisms such as yeast, fungi, algae, and bacteria, which are grown on various carbon sources for synthesis.

Stearic acid: One of the useful types of saturated fatty acids that comes from many animal and vegetable fats and oils.

Stem cells: An undifferentiated cell of a multicellular organism which is capable of giving rise to indefinitely more cells of the same type, and from which certain other kinds of cell arise by differentiation.

Tay-sachs: A disease is a genetic disorder that results in the destruction of nerve cells in the brain and spinal cord.

Tetraploid: Having a chromosome number that is four times the basic or haploid number.

Therapeutic: Of or relating to the treating or curing of disease; curative.

Tissue culture: The technique of cultivating living tissue in a prepared medium outside the body.

Transgenic plant: Genetically engineered plant or offspring of genetically engineered plants. Transgenic plants result from the insertion of genetic material from another organism so that the plant will exhibit a desired trait. Recombinant DNA techniques are usually used.

Tumor: An uncontrolled, abnormal, circumscribed growth of cells in any animal or plant tissue; neoplasm.

Vaccines: Any preparation used as a preventive inoculation to confer immunity against a specific disease, usually employing an innocuous form of the disease agent, as killed or weakened bacteria or viruses, to stimulate antibody production.

Veterinary: Of or relating to the medical and surgical treatment of animals, especially domesticated animals.

White Biotechnology: Focuses mainly on the industrial application of biotechnological research.

Zoonoses: Infectious diseases of animals (usually vertebrates) that can naturally be transmitted to humans. Major modern diseases such as Ebola virus disease and salmonellosis are zoonoses.

11.7 SELF ASSESSMENT QUESTIONS

11.7.1 Objective type question:

1. Process of using natural bacteria in mining industry is known as

(a) Biodegradation (b) Biogenomics

(c) Bioremediation (d) Bioleaching

2. Mold *Penicillin* was discovered by
(a) Alexander Fleming (b) Louis Pasteur
(c) Charles Darwin (d) Chaim Weizmann

3. Most common example of fermented cereal product is
(a) Pickles (b) Bread
(c) Yogurt (d) Cheese

4. Production of different products by using mass culture of microorganisms is classified as
(a) *Bacillus* production (b) Reproduction
(c) Fermentation (d) Pollination

5. The mechanism which has the ability to engineer new organisms is known as
(a) Totipotency (b) Molecular cloning
(c) Genetic Engineering (d) Splicing

6. Most commonly used method for transformation of plants is
(a) Protoplast method (b) *Agrobacterium* mediated
(c) Microinjection (d) None of these

7. Golden rice is a transgenic crop of the future with the following improved trait.
(a) Insect resistance (b) High protein content
(c) High vitamin A content (d) High lysine content

8. The technique of obtaining large number of plantlet by tissue culture method is called
(a) Plantlet culture (b) Micropropagation
(c) Organ culture (d) Macro propagation

9. Maximum application of animal cell culture technology today is in the production of
(a) Insulin (b) Interferons
(c) Edible proteins (d) Vaccines

10. Two bacteria found to be very useful in genetic engineering experiments are
(a) *Nitrobacter* and *Azotobacter* (b) *Rhizobium* and *Diplococcus*
(c) *Nitrosomonas* and *Klebsiella* (d) *Escherichia* and *Agrobacterium*

11. A genetically engineered microorganism used successfully in bioremediation of oil spills is a species of
(a) *Trichoderma* (b) *Bacillus*
(c) *Xanthomonas* (d) *Pseudomonas*

12. First cloned animal
(a) Dolly sheep (b) Dog
(c) Mule (d) Cat

13. Transgenic animals are those which are
(a) Foreign RNA in all its cells (b) Foreign DNA in some of its cells
(c) Foreign DNA in all its cells (d) Both a and b

14. The first transgenic plant was
(a) Tobacco (b) Pea
(c) Flax (d) Cotton

15. Interferons are
(a) Anti-bacterial proteins (b) Anti-viral proteins
(c) Bacteriostatic proteins (d) All of these

11.7.1Answers: 1. (d), 2. (a), 3. (b), 4. (c), 5. (c), 6. (b), 7. (c), 8. (b), 9. (d), 10. (d), 11. (d), 12. (a), 13. (c), 14. (a), 15. (b)

11.8 SUGGESTED READING

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11.10 TERMINAL QUESTIONS

1. What is Biotechnology? Describe its brief areas of Interest.
2. What is the definition of Biotechnology? Discuss the various definition given by different scientific organization?
3. Differentiate the traditional biotechnology from modern biotechnology.
4. Write an essay note on development of Biotechnology. Also write about the contribution of scientist Boyer and Cohen in Biotechnology?
5. What are the various sub-disciplines of Biotechnology? Write in details.
6. Discuss the applications of biotech in health. Also write about the biotech in animal healthcare.
7. What are Biofertilizers? Write about the Role of biotech in Agriculture.
8. What are the various applications of biotech in agriculture?
9. Write a short on:
   a. SCP
   b. Scope of biotechnology
   c. Role of Biotechnology in medicine.
   d. Plant tissue culture
10. What do you understand by Industrial biotechnology? What are different industrial applications of biotechnology?

UNIT-12 PLANT TISSUE CULTURE

12.1 OBJECTIVES

After reading this unit students will be able to understand the-

- Basics of plant tissue culture (Tools, techniques, media and culture type)
- Methods of gene transfer
- Transgenic plants
- Concept of Gene Bank
- Nif Gene, node gene and mycoprotein

12.2 INTRODUCTION

One of the stellar achievements of twentieth century plant biology was the genetic transformation of somatic cells enabling the regeneration of whole plants that were stably transformed and capable of transmitting the inserted genetic material to subsequent generations. This achievement grew out of three independent lines of research initiated early in the twentieth century: plant tissue culture, regeneration of plants from single somatic cells, and the study of crown gall disease. Plant tissue culture is a bio-technique based on the promise that an organ, tissue or cell of a plant can be *in vitro* manipulated to grow back in to a complete plant. Therefore, plant tissue culture is the foundation and in most cases the bottle-neck step for plant genetic engineering.

Culturing of plants cells, tissue and organs under sterile conditions on the nutrient medium is known as plant tissue culture. The technique relies on the ability of plants cells to regenerate a whole plant (Totipotency). When introducing a foreign gene into a target genome in plant tissue, you need to grow the transgenic cell to a complete plant. This is done by plant tissue culture, a bio-technique based on the concept that an organ, tissue or cell of a plant can be manipulated to grow back into a complete plant. After delivering a foreign gene into a target genome, you need to bring the transgenic cell to a complete plant. This step has to be done by plant tissue culture. In the recent years, large numbers of crops have been modified to suite human needs through genetic engineering. Since the genetic transformation is a costly affair plant tissue culture is a boon for the propagation of these transgenic/genetically altered plants. The plant tissue culture can be used to form large number of clones of these plants. Gene banks are remarkable in providing the raw material for the tissue culture as they can be used as the store house for the various explants used in tissue culture.

The history of plant tissue culture can be traced back to near the turn of the 20th century when **Gottlieb Haberlandt** (father of plant tissue culture) reported his culture of leaf mesophyll tissue and hair cells, though the cultured cells did not divide. Haberlandt's student Kotte (1922) reported *in vitro* growth of isolated root tips. P. R. White (1934) repeatedly reported subculture of root tip-derived tissues of tomato. Techniques for plant tissue culture progressed rapidly during the 1930s due to the discovery of the necessity of B vitamins and auxin for the growth of

isolated meristem tissues. The works of **F. K. Skoog** (plant physiologist) and his associates on the nutritional requirements of tobacco tissue culture led to not only the discovery of plant growth hormones, kinetin and cytokinins, but also to the formation of an important plant tissue culture medium. Since the 1960s, tissue and cell culture has increasingly been used as a tool by plant scientists and biotechnologists.

12.3 PLANT TISSUE CULTURE

The term "Plant tissue culture" broadly refers to the *in vitro* cultivation of plant parts under aseptic conditions. Such parts as meristems, apices, axillary buds. Young inflorescence, leaves, stems, and roots have been cultured. A controlled aseptic environment and suitable nutrient medium are the two chief requirements for successful tissue culture. These essential nutrients include inorganic salts, a carbon and energy source, vitamins and growth regulators. The basic technology can be divided into five classes, depending on the material being used: Callus, organ, meristem, and protoplast and cell culture. The technique of embryo, ovule, ovary, anther and microspore culture are used and can yield genotypes that cannot easily be produced by conventional methodology.

Plant tissue culture is a collection of techniques used to sustain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is used to produce clones of plant in a method called micropropagation. Plant tissue culture relies on the fact that many plant cells have the ability to regenerate into a whole plant in a process called **Totipotency**. Single cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant on culture media given the required nutrients and hormones. The plant part obtained from a plant to be cultured is called explant while the main plant it is obtained from is called mother plant. **Explant** can be taken from different plant parts such as shoots, leaves, stems, flowers, roots, single undifferentiated cells etc.

A small tissue excised from any part of the plant is called explant which is the starting point. It can be initiated from any part of plant- root, stem, petiole, leaf or flower, choice of explant varies with species. Meristems are more responsive and give better success as they are actively dividing. The physiological state of the plant also has an influence on its response to initiate tissue culture. Therefore, the parent plant must be healthy and free from obvious signs of disease or decay. Aseptic environment during culture is required to avoid contamination from microorganisms. Since plant cell division is slower compared to the growth of bacteria, fungi and even minor contaminants can easily overgrow the plant tissue culture. Therefore, all the materials like glassware, instruments, medium, explant etc. to be used in culture work must be freed of microbes. Laminar flow is a mandatory prerequisite for any tissue culture laboratory for contamination free work.

Totipotency

Capacity of higher organism cell to differentiate into entire organism, totipotent cell contains all genetic information necessary for complete development. When an explant from differentiated tissue is used for culture on a nutrient medium, the non-dividing quiescent cells first undergo certain changes to achieve a meristematic state. The phenomenon of the reversion of mature cells to the meristematic state leading to the formation of callus is called "dedifferentiation". The component cells of the callus have the ability to form a whole plant a phenomenon described as "dedifferentiation". These two phenomenon's of dedifferentiation and "redifferentaition" are inherent in the capacity described as "cellular Totipotency", a property found only in plant cell and not in animal cells. In other words, while a differentiated plant cell retain its capacity to give rise to whole plant, an animal cell loses its capacity of regeneration after differentiation. Although, generally a callus phase is involved before the cell can undergo redifferentaition leading to regeneration of whole plant, but rarely, the dedifferentiated cells give rise to whole plant directly without an intermediate callus phase.

Tools and Technique Used For Plant Tissue Culture

1. **pH and pH Meter:** An approximate idea of the pH of a solution can be obtained using indicators. These are organic compounds of natural or synthetic origin whose Colour is dependent upon the pH of the solution. Indicators are usually weak acids, which dissociate in solution. A standard pH meter has two electrodes, one glass electrode for measuring pH and the other calomel reference electrode
2. **Autoclave:** Autoclave is used to sterilize medium, glassware and tools for the purpose of plant tissue culture. Sterilization of material is carried out by increasing moist heat (121 °C) due to increased pressure inside the vessel (15-22 psi or 1.02 to 1.5 kg/cm2) for 15 minutes for routine sterilization. Moist heat kills the microorganism and makes the material free from microbes.
3. **Plant Growth Chamber:** Plant growth chambers can be constructed in a suitable sized room or can be purchased as commercially available equipment. Thermal insulation of walls increases the efficiency of the cooling system. Essentially plant growth chamber has three environmental control systems:
   (i) Light-intensity and duration cycle control.
   (ii) Temperature control and regulation.
   (iii) Humidity control and regulation.
4. **Colorimeter:** The most commonly used method for determining the concentration of biochemical compounds is colorimetry. It uses the property of light such that when white light passes through a coloured solution, some wavelengths are absorbed more than others.

5. Laminar Air Flow Bench: Laminar air flow (LAF) bench is the main working table for aseptic manipulations related to plant tissue culture. This is equipment fitted with High Efficiency Particulate Air (HEPA) Filters, which allow air to pass but retain all the particles and micro-organisms.

Fig.12.3 Laminar flow
(Tissue culture work in sterilized conditions)

6. Methods of Sterilization: Plant tissue culture requires contamination free environment, tools and cultures or strict maintenance of germ free system in all the operations, known as **asepsis**.

7. Microscopy:

(a) Electron Microscopy: Electron microscopy permits a detailed study of sub-cellular organelles as its resolving power is much greater than that of the light microscope.

(b) SEM: Scanning electron microscope (SEM) provides surface views of whole structure of specimen. Normally, specimens are coated with a thin film of metal under vacuum.

(c) Light Microscopy: Bright field microscopy is absolutely indispensable tool for cell biologists. This is required for routine observations of cells, cellular differentiation and pigmentation.

8. Centrifugation: A centrifuge is an instrument which produces centrifugal force by rotating the samples around a central axis with the help of an electric motor.

9. Chromatography: (meaning 'colored writing') is a technique to separate molecules on the basis of differences in size, shape, mass, charge and adsorption properties.

10. Thermometer: It is a device that measures temperature or temperature gradient using a variety of different principles.

11. Hygrometer: The instruments used for measuring humidity. A simple form of a hygrometer is specifically known as a "Psychrometer" and consists of two thermometers, one of which includes a dry bulb and the other of which includes a bulb that is kept wet to measure wet-bulb temperature.

12. LUX Meter: Lux is a measurement of the overall intensity of light within an environment for any given area or distance from the source or lux is the amount of light in an environments perceived by the human eye.

13. Medium and Its Preparation: The media used by earlier workers were based on Knop's solution. Subsequently media developed by White (1943) and Heller (1953) were used.

Murashige and Skoog's medium (Murashige and Skoog, 1962) is a land mark in plant tissue culture research and is the most frequently used medium for all types of tissue culture work.

Plant Growth Regulators

Plant growth regulators (also called plant hormones) are numerous chemical substances that profoundly influence the growth and differentiation of plant cells, tissues and organs. Plant growth regulators function as chemical messengers for intercellular communication. There are currently five recognized groups of plant hormones: auxins, gibberellins, cytokinins, abscisic acid (ABA) and ethylene. They work together coordinating the growth and development of cells. Ethylene is mainly involved in abscission and flower senescence in plants and is rarely used in plant tissue culture. In addition to the five principal growth regulators, two other groups sometimes appear to be active in regulating plant growth, the brassino steroids and polyamines.
Hormones used in Plant Tissue Culture:

1. Auxins
2. Cytokinins
3. Gibberellins
4. Abscisic Acid (ABA)
5. Polyamines

1. Auxins: Auxins stimulate cell elongation and influence a host of other developmental responses, such as root initiation, vascular differentiation, tropic responses, apical dominance and the development of auxiliary buds, flowers and fruits. Auxins are synthesized in the stem and root apices and transported through the plant axis. The principal auxin in plants is Indole-3-acetic acid (IAA). Several other indole derivatives, all as precursors to IAA, are known to express auxin activity, probably by converting to IAA in the tissue. Auxins in plant tissue culture are used to induce callus from explants, and cause root and shoot morphogenesis . Auxins are often most effective in eliciting their effects when combined with cytokinins.

2. Cytokinins: Cytokinins are able to stimulate cell division and induce shoot bud formation in tissue culture. They usually act as antagonists to auxins. Cytokinins most used in tissue culture include Zeatin, Adenine, 6-(γ,γ-Dimethylallylamino)purine (2iP) and Kinetin. Cytokinins often inhibit embryogenesis and root induction.

3. Gibberellins: The main effect of gibberellins in plants is to cause stem elongation and flowering. They are also prominently involved in mobilization of endosperm reserves during early embryo growth and seed germination. There exist over 80 different gibberellins compounds in plants but only Giberrellic acid (GA3) and GA4+7 are often used in plant tissue culture. In tissue culture, gibberellins are used to induce organogenesis, particularly adventitious root formation.

4. Abscisic Acid: Abscisic acid (ABA) in plants is a terpenoid involved primarily in regulating seed germination, inducing storage protein synthesis and modulating water stress. In plant tissue culture, it is used to help somatic embryogenesis, particularly during maturation and germination.

5. Polyamines: PAs (Polyamines) are known as a group of natural compounds with aliphatic nitrogen structure, present in almost all living organisms, play important roles in many physiological processes, such as cell growth and development and respond to environmental stresses. Many polyamines were found to be physiologically active and implicated in a number of physiological effects in plants. Their role in controlling plant growth and development is being increasingly recognized now.

However, polyamines have not yet been given status of plant hormones by plant physiologists unequivocally. Polyamines differ from plant hormones in two respects:

1. While plant hormones are present in very small concentration, the polyamines are present in abundance.
2. The polyamines are poorly translocated.

Culture Media

Culture medium is the most important part of plant tissue culture. A successful plant tissue culture system largely relies on a right culture medium formula. Plant tissue culture media usually contains inorganic elements, organic compounds and a support matrix. Culture media provides the cultures the necessary inorganic nutrients that are usually available from soil. In addition, they also provide the cultures the necessary organic compounds such as vitamins, and carbon source, which are usually produced in plants. Sometimes, plant growth regulators are added to the medium to stimulate cell division and/or differentiation.

Another important function of a culture medium is creating a necessary environment for plants to grow. For example, solid media, functioning like the soil, provides a physical support for the cultures to keep contact with air for respiration, and for regenerated plantlets to root into. A liquid medium enables explants to keep constant, maximum contact with nutrient supplies. A selective reagent may also be included in a culture medium to restrict the growth of certain cultures. Therefore, medium formulas vary depending upon the purpose of plant tissue culture. Different media may also be used during a plant tissue culture process.

Culture Technique in Plant Tissue Culture

Preparation of plant tissues for tissue culture is performed under aseptic conditions under HEPA (High efficiency particulate air) filtered air provided by laminar flow cabinet. The tissue is grown in sterile containers inside Petridish, test tube or flasks in a growth room with controlled temperature and light intensity. Living plant materials are usually contaminated on their surfaces (or sometimes interior) with microorganisms, so their surfaces are stressed in chemical solutions (e.g. alcohol and sodium hypochlorite). The sterile explant are placed on the solid and liquid

media are generally composed of inorganic salts, organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of gelling agent (agar). The composition of the medium, particularly the plant hormones and the nitrogen source have profound effects on the morphology of the tissues that grow from the initial explant. For instance, an excess of auxin will result in a proliferation of roots while an excess of cytokinin may yield to shoots proliferation. A balance of both auxin and cytokinin will often produce an unorganized growth of cells called callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition.

Classification of Plant Tissue Culture Technique

1. Embryo Culture: For embryo culture, embryos are excised from immature seeds, usually under a 'hood', which provides a clean aseptic and sterile area. Sometimes, the immature seeds are surface sterilized and soaked in water for few hours, before the embryos are excised. The excised embryos are directly transferred to a culture dish or culture tube containing synthetic nutrient medium. Entire operation is carried out in the 'laminar flow cabinet' and the culture plates or culture tubes with excised embryos are transferred to a culture room maintained at a suitable temperature, photoperiod and humidity. The frequency of excised embryos that gives rise to seedlings generally varies greatly and medium may even have to be modified made for making Interspecific and Intergeneric crosses within the tribe Triticeae of the grass family. The hybrids raised through culture have been utilized for

a) Phylogenetic studies and genome analysis.
b) Transfer of useful agronomic traits from wild genera to the cultivated crops and
c) To raise synthetic crops like triticale by producing amphiploids from the hybrids.

Embryo culture has also been used for haploid production through distant hybridization followed by elimination of chromosomes of one of the parent in the hybrid embryos cultured as above.

Application of Embryo Culture

i) Recovery of distant hybrids.
ii) Recovery of haploid plants from Interspecific crosses.
iii) Propagation of orchids.
iv) Shortening the breeding cycle
v) Overcoming dormancy.
In addition ovule and ovary can also be cultured.

2. Meristem Culture: In attempts to recovery pathogen free plants through tissue culture techniques, horticulturists and pathologists have designated the explants used for initiating cultures as 'shoot –tip', tip, meristem and meristem tip. The portion of the shoot lying distal to the youngest leaf primordium is called the **apical meristem**. The apical meristem together with one to three young leaf primordia constitute the shoot apex. In most published works explants of

larger size have been cultured to raise virus-free plant. The explants of such a size should be infect referred to as shoot-tips.

However, for purpose of virus or disease elimination the chances are better if cultures are initiated with shoot tip of smaller size comprising mostly meristematic cells. Therefore, the term 'meristem' or meristem-tip' culture is preferred for *in vitro* culture of small shoot tips. The *in vitro* techniques used for culturing meristem tips are essentially the same as those used for aseptic culture of plant tissues. Meristem tips can be isolated from apices of the stems, tuber sprouts , leaf axils , sprouted buds or cuttings or germinated seeds.

Application of Meristem Culture

i) Vegetative propagation
ii) Recovery of virus free stock.
iii) Germplasm exchange
iv) Germplasm conservation

3. Anther or Pollen Culture: Angiosperms are diploid and the only haploid stage in their life cycle being represented by pollen grains. From immature pollen grains we can sometimes raise cultures that are haploid. These haploid plants have single completes set of chromosomes. Their phenotype remains unmasked by gene dominance effects. When pollen grains of angiosperm are cultured, they undergo repeated divisions.

In *Datura innoxia* the pollen grains from cultured anther can form callus when grown on a media supplemented with yeast extract or casein hydrolysate. Similarly, when isolated anthers are grown on media containing coconut milk or kinetin, they form torpedo- shaped embryoids which in due course grow into small haploid plantlets. The usual approach in anther culture is that anthers of appropriate development stage are excised and cultured so that embryogenesis occurs. Alternatively pollen grains may be removed from the anther, and the isolated pollen is then cultured in liquid medium. Cultured anthers may take up to two months to develop into plantlets.

Application of Anther or Pollen Culture

Pollen culture or anther culture is useful for production of haploid plants. Similarly, haploid plants are useful in plant breeding in variety of ways as follows:
i) Releasing new varieties through F1 double haploid system.
ii) Selection of mutants resistant to diseases.
iii) Developing asexual lines of trees or perennial species.
iv) Transfer of desired alien gene.
v) Establishment of haploid and diploid cell lines of pollen plant.

4. Tissue and Cell Culture: Single cells can be isolated either from cultured tissues or from intact plant organs, the former being more convenient than the latter. When isolated from culture

tissues, the latter is obtained by culturing an organized tissue into callus. The callus may be separated from explant and transferred to fresh medium to get more tissue. Pieces of undifferentiated calli are transferred to liquid medium, which is continuously agitated to obtain a suspension culture. Agitation of pieces break them into smaller clumps and single cells, and also maintains uniform distribution of cells clumps in the medium. It also allows gases exchange. **Suspension cultures** with single cells can also be obtained from impact plant organs either mechanically or enzymatically. Suspension cultures can be maintained in either of the following two forms:

A. Batch culture: are initiated as single cells in 100-250 ml flasks and are propagated by transferring regularly small aliquots of suspension to a fresh medium.

B. Continuous culture: are maintained in steady state for long periods by draining out the used medium and adding fresh medium, in this process either the cells separated from the drained medium are added back to suspension culture or addition of medium is accompanied by the harvest of an equal volume of suspension culture.

Application of Cell Culture

i) Mutant selection
ii) Production of secondary metabolites or biochemical production.
iii) Biotransformation
iv) Clonal propagation
v) Somaclonal variations

Practical Applications of Plant Tissue Culture

The use of plant cells to generate useful products and/or services constitutes plant biotechnology. In plant biotechnology, the useful product is a plantlet. The plantlets are used for the following purposes.

1. Rapid Clonal Propagation: A clone is a group of individuals or cells derived from a single parent individual or cell through asexual reproduction. All the cells in callus or suspension culture are derived from a single explant by mitotic division. Therefore, all plantlets regenerated from a callus/suspension culture generally have the same genotype and constitute a clone. These plantlets are used for rapid Clonal propagation. This is done in oil palm.

2. Somaclonal Variation: Genetic variation present among plant cells of a culture is called Somaclonal variation. The term Somaclonal variation is also used for the genetic variation present in plants regenerated from a single culture. This variation has been used to develop several useful varieties.

3. Transgenic Plants: A gene that is transferred into an organism by genetic engineering is known as transgene. An organism that contains and expresses a transgene is called transgenic

organism. The transgenes can be introduced into individual plant cells. The plantlets can be regenerated from these cells. These plantlets give rise to the highly valuable transgenic plants.

4. Induction and Selection of Mutations: Mutagens are added to single cell liquid cultures for induction of mutations. The cells are washed and transferred to solid culture for raising mutant plants. Useful mutants are selected for further breeding. Tolerance to stress like pollutants, toxins, salts, drought, flooding, etc. can also be obtained by providing them in culture medium in increasing dosage. The surviving healthy cells are taken to solid medium for raising resistant plants.

5. Resistance to Weedicides: It is similar to induction of mutations. Weedicides are added to culture initially in very small concentrations. Dosage is increased in subsequent cultures till the desired level of resistance is obtained. The resistant cells are then regenerated to form plantlets and plants.

12.4 METHODS OF GENE TRANSFER

The uptake of foreign DNA or the recombinant DNA by cells is called gene transfer or transformation. Conventionally, the gene transfer necessary for crop improvement is obtained through sexual and vegetative propagation. However, biotechnological approaches like soma-Clonal variation, protoplast fusion etc. has successfully speeded up the process of generating genetic variation and introgression of foreign genes. The most potential biotechnological approach for transferring recombinant DNA is based on genetic engineering which involves various techniques for gene transfer discussed ahead. The transferred gene is called transgene and the plants that carry these stably integrated transgenes are called transgenic plants**.**

Various gene transfer techniques used are grouped into two broad categories:

I. Vector-mediated gene transfer

II. Direct or vector less DNA transfer

Table.1 Gene transfer (DNA delivery) methods in plants

Method	*Salient features*
I. Vector–mediated gene transfer	
Agrobacterium (Ti plasmid)-mediated gene transfer	Very efficient, but limited to a selected group of plants
Plant viral vectors	Ineffective method, hence not widely used
II. Direct or vectorless DNA transfer	
(A) Physical methods	
Electroporation	Mostly confined to protoplasts that can be regenerated to viable plants. Many cereal crops developed.
Microprojectile (particle bombardment)	Very successful method used for a wide range of plants/ tissues. Risk of gene rearrangement high.
Microinjection	Limited use since only one cell can be microinjected at a time. Technical personnel should be highly skilled.
Liposome fusion	Confined to protoplasts that can be regenerated into viable whole plants.
Silicon carbide fibres	Requires regenerable cell suspensions. The fibres, however, require careful handling.
(B) Chemical methods	
Polyethylene glycol (PEG)-mediated	Confined to protoplasts. Regeneration of fertile plants is frequently problematical.
Diethylaminoethyl (DEAE) dextran-mediated	Does not result in stable transformants.

I. Vector-Mediated Gene Transfer

Vector-mediated gene transfer is carried out either by *Agrobacterium*-mediated transformation or by use of plant viruses as vectors.

Agrobacterium-Mediated Gene Transfer:

Agrobacterium tumefaciens is a soil-borne, Gram-negative bacterium. It is rod shaped and motile, and belongs to the bacterial family of Rhizobiaceae. *A. tumefaciens* is a phytopathogen, and is treated as the nature's most effective plant genetic engineer. Some workers consider this bacterium as the natural expert of inter-kingdom gene transfer. In fact, the major credit for the development of plant transformation techniques goes to the natural unique capability of *A. tumefaciens*. Thus, this bacterium is the most beloved by plant biotechnologists. There are mainly two species of Agrobacterium:

1. *A. tumefaciens* that induces crown gall disease.
2. *A. rhizogenes* that induces hairy root disease.

Plant Transformation Technique Using Agrobacterium:

Agrobacterium-mediated technique is the most widely used for the transformation of plants and generation of transgenic plants. The important requirements for gene transfer in higher plants through *Agrobacterium* mediation are listed.

1. The explants of the plant must produce phenolic compounds (e.g. Acetosyringone) for activation of virulence genes.
2. Transformed cells/tissues should be capable to regenerate into whole plants.

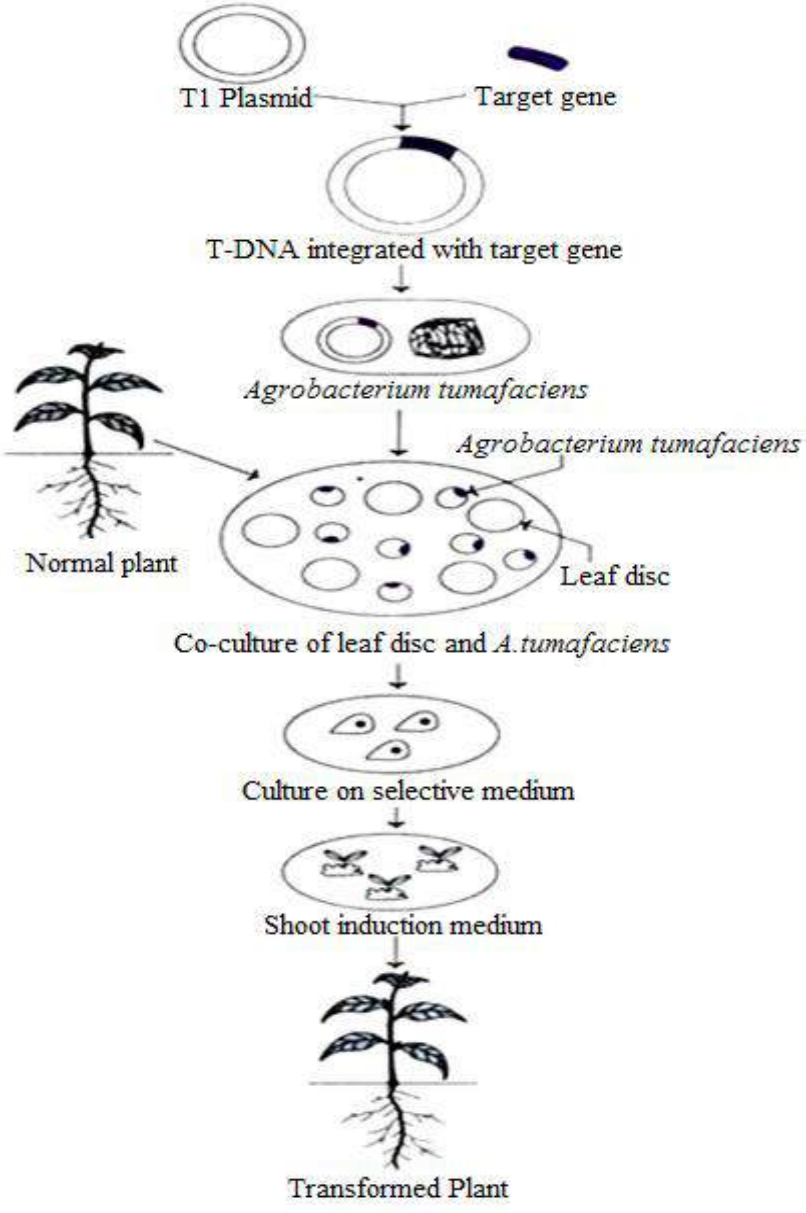

Fig. 12.4 Transformation technique using Agrobacterium-mediated gene transfer

In general, most of the *Agrobacterium*-mediated plant transformations have the following basic protocol

1. Development of *Agrobacterium* carrying the co-integrate or binary vector with the desired gene.
2. Identification of a suitable explant e.g. cells, protoplasts, tissues, calluses, organs.
3. Co-culture of explants with *Agrobacterium*.

4. Killing of *Agrobacterium* with a suitable antibiotic without harming the plant tissue.
5. Selection of transformed plant cells.
6. Regeneration of whole plants.

Advantages of Agrobacterium- mediated transformation:

1. This is a natural method of gene transfer.
2. Agrobacterium can conveniently infect any explant (cells/tissues/organs).
3. Even large fragments of DNA can be efficiently transferred.
4. Stability of transferred DNA is reasonably good.
5. Transformed plants can be regenerated effectively.

Limitations of *Agrobacterium*- mediated transformation:

1. There is a limitation of host plants for *Agrobacterium*, since many crop plants (monocotyledons e.g. cereals) are not infected by it. In recent years, virulent strains of *Agrobacterium* that can infect a wide range of plants have been developed.
2. The cells that regenerate more efficiently are often difficult to transform, e.g. embryonic cells lie in deep layers which are not easy targets for *Agrobacterium*.

II. Direct or Vector-less DNA Transfer:

The term direct or vector less transfer of DNA is used when the foreign DNA is directly introduced into the plant genome. It is a process where no vector is involved and can be applied to any species or genotype. Direct DNA transfer methods rely on the delivery of naked DNA into the plant cells. This is in contrast to the *Agrobacterium* or vector-mediated DNA transfer which may be regarded as indirect methods. Majority of the direct DNA transfer methods are simple and effective. And in fact, several transgenic plants have been developed by this approach.

Limitations of direct DNA transfer:

The major disadvantage of direct gene transfer is that the frequency of transgene rearrangements is high. This results in higher transgene copy number, and high frequencies of gene silencing.

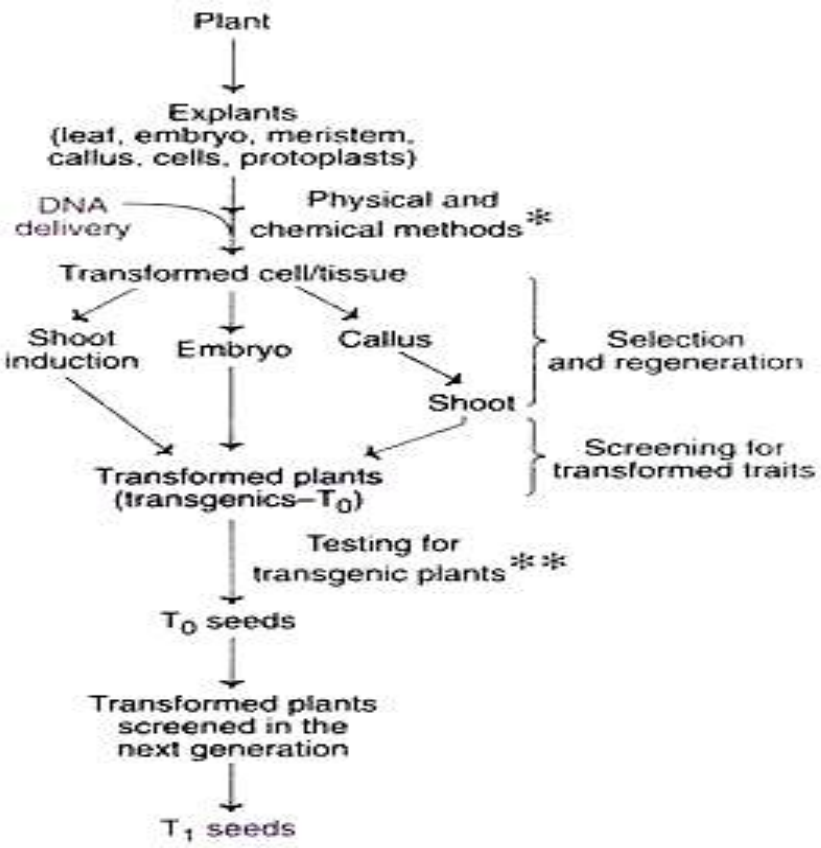

Fig.12.5 An overview of the protocol for the production of transgenic plants using direct DNA delivery methods

Types of direct DNA transfer:

The direct DNA transfer can be broadly divided into three categories.

1. Physical gene transfer methods—electro- portion, particle bombardment, microinjection, liposome fusion, silicon carbide fibres.
2. Chemical gene transfer methods—Polyethylene glycol (PEG)-mediated, diethyl amino ethyl (DEAE) dextran-mediated, calcium phosphate precipitation.
3. DNA imbibition by cells/tissues/organs.

(A) Physical Gene Transfer Methods

An overview of the general scheme for the production of transgenic plants by employing physical transfer methods is depicted in Fig.12.5. Some details of the different techniques are described.

1. Electroporation: is a process where the cells are exposed to electrical impulses of high voltage to reversibly make cell membranes permeable for uptake of DNA. This technique can be used for the delivery of DNA into intact plant cells and protoplasts. Recently, transformation of intact plant cells of sugarbeet and rice has been successfully reported. This method is convenient,

simple and quick. The plant material is incubated in a buffer solution containing the desired foreign/target DNA, and subjected to high voltage electrical impulses. This results in the formation of pores in the plasma membrane through which DNA enters and gets integrated into the host cell genome.

In the early years, only protoplasts were used for gene transfer by Electroporation. Now a days, intact cells, callus cultures and immature embryos can be used with suitable Pre- and Post-Electroporation treatments. Electroporation has been successfully used for the production of transgenic plants of many cereals e.g. rice, wheat, maize. However, Electroporation cannot be applied to all the tissues, cell viability drops due to electric shock. Also, regeneration of plants from protoplasts is still difficult.

Advantages of Electroporation

1. This technique is simple, convenient and rapid, besides being cost-effective.
2. The transformed cells are at the same physiological state after Electroporation.
3. Efficiency of transformation can be improved by optimizing the electrical field strength, and addition of spermidine.

Limitations of Electroporation

1. Under normal conditions, the amount of DNA delivered into plant cells is very low.
2. Efficiency of Electroporation is highly variable depending on the plant material and the treatment conditions.
3. Regeneration of plants is not very easy, particularly when protoplasts are used.

2. Particle Bombardment (Biolistics): Particle (or micro projectile) bombardment is the most effective method for gene transfer, and creation of transgenic plants. This is a relatively recent development but is widely used and is effective in introduction of DNA into plant cells. This method is versatile due to the fact that it can be successfully used for the DNA transfer in mammalian cells and microorganisms. The micro projectile bombardment method was initially named as Biolistics by its inventor Sanford (1988). Biolistics is a combination of biological and ballistics. There are other names for this technique- particle gun, gene gun, bio-blaster.

The technique involves coating 1μm diameter particles of tungsten or gold known as micro-projectiles (Micro carriers) with DNA, carried by macro carriers (macro projectiles). These macro-carriers are inserted into the apparatus and pushed downward by rupturing the disc. The stopping plate does not permit the movement of macro carrier while the micro carriers (with DNA) are accelerated to high speed using a pulse of high pressure helium into an evacuated chamber containing the target tissues. Here, the DNA coated particles penetrate through the cell wall releasing DNA from particles which can express transiently or get integrated into nuclear genome of that cell. With appropriate tissue culture and selection, transgenic plants can be regenerated. Particle bombardment has been used for transformation of monocotyledonous crop plants such as maize, rice, wheat etc.

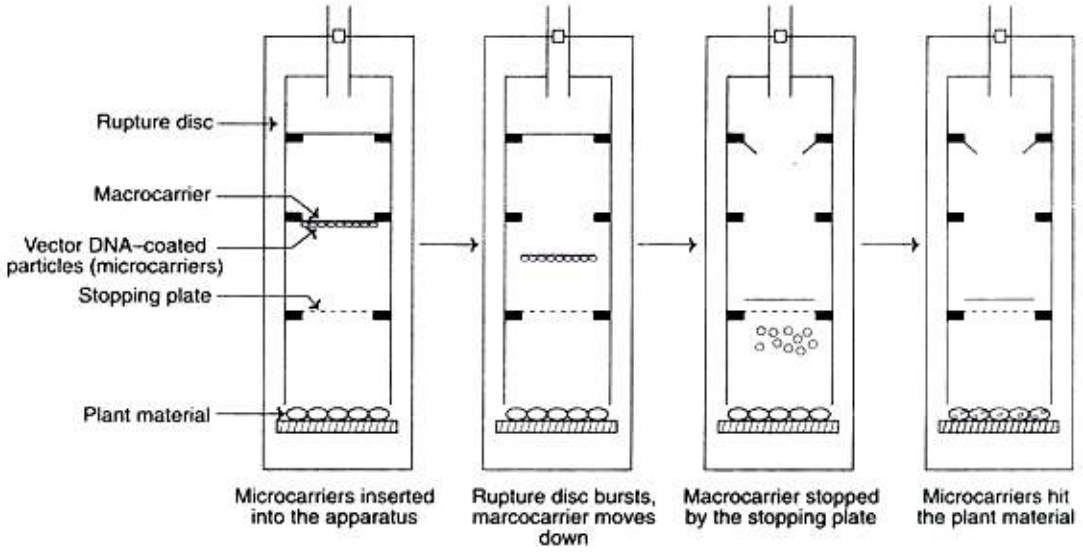

Fig.12.6 Diagrammatic representation of Particle Bombardment (Biolistics) system for gene transfer in plants

Plant material used in bombardment: Two types of plant tissue are commonly used for particle bombardment.

1. Primary explants which can be subjected to bombardments that are subsequently induced to become embryogenic and regenerate.
2. Proliferating embryonic tissues that can be bombarded in cultures and then allowed to proliferate and regenerate.

In order to protect plant tissues from being damaged by bombardment, cultures are maintained on high osmoticum media or subjected to limited plasmolysis.

Transgene integration in bombardment

It is believed (based on the gene transfer in rice by Biolistics) that the gene transfer in particle bombardment is a two stage process.

1. In the pre-integration phase, the vector DNA molecules are spliced together. This results in fragments carrying multiple gene copies.
2. Integrative phase is characterized by the insertion of gene copies into the host plant genome.

The integrative phase facilitates further transgene integration which may occur at the same point or a point close to it. The net result is that particle bombardment is frequently associated with high copy number at a single locus. This type of single locus may be beneficial for regeneration of plants.

Advantages of particle bombardment:

1. Gene transfer can be efficiently done in organized tissues.
2. Different species of plants can be used to develop transgenic plants.

Limitations of particle bombardment:

1. The major complication is the production of high transgene copy number. This may result in instability of transgene expression due to gene silencing.
2. The target tissue may often get damaged due to lack of control of bombardment velocity.
3. Sometimes, undesirable chimeric plants may be regenerated.

3. Microinjection: Microinjection is a direct physical method involving the mechanical insertion of the desirable DNA into a target cell. The target cell may be the one identified from intact cells, protoplasts, callus, embryos, meristems etc. Microinjection is used for the transfer of cellular organelles and for the manipulation of chromosomes.

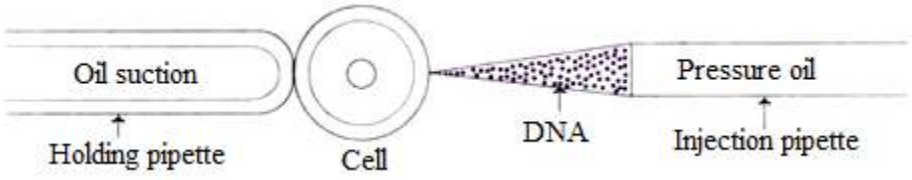

Fig.12.7 Microinjection of DNA by holding pipette method

4. Liposome-Mediated Transformation: Liposomes are artificially created lipid vesicles containing a phospholipid membrane. They are successfully used in mammalian cells for the delivery of proteins, drugs etc. Liposomes carrying genes can be employed to fuse with protoplasts and transfer the genes. The efficiency of transformation increases when the process is carried out in conjunction with polyethylene glycol (PEG). Liposome-mediated transformation involves adhesion of liposomes to the protoplast surface, its fusion at the site of attachment and release of plasmids inside the cell.

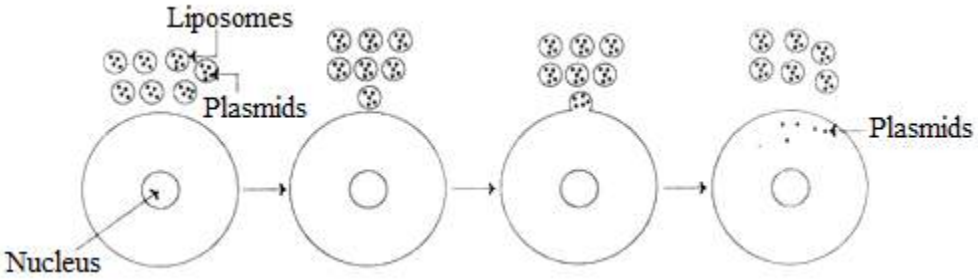

Fig.12.8 Diagrammatic representation of fusion of plasmid-filled Liposomes with protoplast

5. Silicon Carbide Fibre-Mediated Transformation: The silicon carbide fibres (SCF) are about 0.3-0.6 pm in diameter and 10-100 pm in length. These fibres are capable of penetrating the cell wall and plasma membrane, and thus can deliver DNA into the cells. The DNA coated silicon carbide fibres are vortexed with ‘plant material (suspension culture, calluses). During the mixing, DNA adhering to the fibres enters the cells and gets stably integrated with the host genome. The silicon carbide fibres with the trade name Whiskers are available in the market.

B) Chemical Gene Transfer Methods:

1. Polyethylene glycol (PEG)-mediated transfer: Polyethylene glycol (PEG), in the presence of divalent cations (using Ca2+), destabilizes the plasma membrane of protoplasts and renders it permeable to naked DNA. In this way, the DNA enters nucleus of the protoplasts and gets integrated with the genome.

The procedure involves the isolation of protoplasts and their suspension, addition of plasmid DNA, followed by a slow addition of 40% PEG-4000 (w/v) dissolved in mannitol and calcium nitrate solution. As this mixture is incubated, protoplasts get transformed.

Advantages of PEG-mediated transformation

1. A large number of protoplasts can be simultaneously transformed.
2. This technique can be successfully used for a wide range of plant species.

Limitations of PEG-mediated transformation

1. The DNA is susceptible for degradation and rearrangement.
2. Random integration of foreign DNA into genome may result in undesirable traits.
3. Regeneration of plants from transformed protoplasts is a difficult task.

2. DEAE-Dextran-Mediated transfer: Diethylaminoethyl (DEAE)-dextran is a poly-cationic derivative of the carbohydrate polymer dextran, and it is one of the first chemical reagents used to transfer nucleic acids into cultured mammalian cells (Vaheri and Pagano, 1965). The desirable DNA can be complexed with a high molecular weight polymer diethyl amino ethyl (DEAE) dextran and transferred. The major limitation of this approach is that it does not yield stable trans-formants.

12.5 TRANSGENIC PLANTS

Transgenic plants are the ones, whose DNA is modified using genetic engineering techniques. The aim is to introduce a new trait to the plant which does not occur naturally in the species. A transgenic plant contains a gene or genes that have been artificially inserted. The inserted gene sequence is known as the **transgene**, it may come from an unrelated plant or from a completely different species. The purpose of inserting a combination of genes in a plant is to make it as useful and productive as possible. This process provides advantages like improving shelf life, higher yield, improved quality, pest resistance, tolerant to heat, cold and drought resistance, against a variety of biotic and abiotic stresses. Transgenic plants can also be produced in such a way that they express foreign proteins with industrial and pharmaceutical value.

Generation of transgenic plants and their identification

The advantage of recombinant DNA technique and transformation methods for plants has given agricultural scientists a powerful new way of incorporating defined genetic changes into plants

and thus generating transgenic plants. The continued development of *Agrobacterium* based transfer systems for improving its efficiency and applicability to more crops is rapidly replacing other methods for generation of transgenic plants.

Agrobacterium based generation of transgenic plants

There are few prerequisites for *Agrobacterium* mediated gene transfer which includes:

1. In order to induce *vir* genes, plants must produce acetosyringone or *Agrobacterium* can be pre induced with synthetic acetosyringone. **Acetosyringone** is a phenolic compound secreted by wounded plant tissue and is known to be a potent inducer of *Agrobacterium vir* genes.
2. Following induction, the agrobacteria should have access to cells that are competent for transformation. Thus, wounded and dedifferentiated cells, fresh explants are used which have replicating DNA or are undergoing mitosis.
3. Transformation competent cells should be able to regenerate in whole plants.

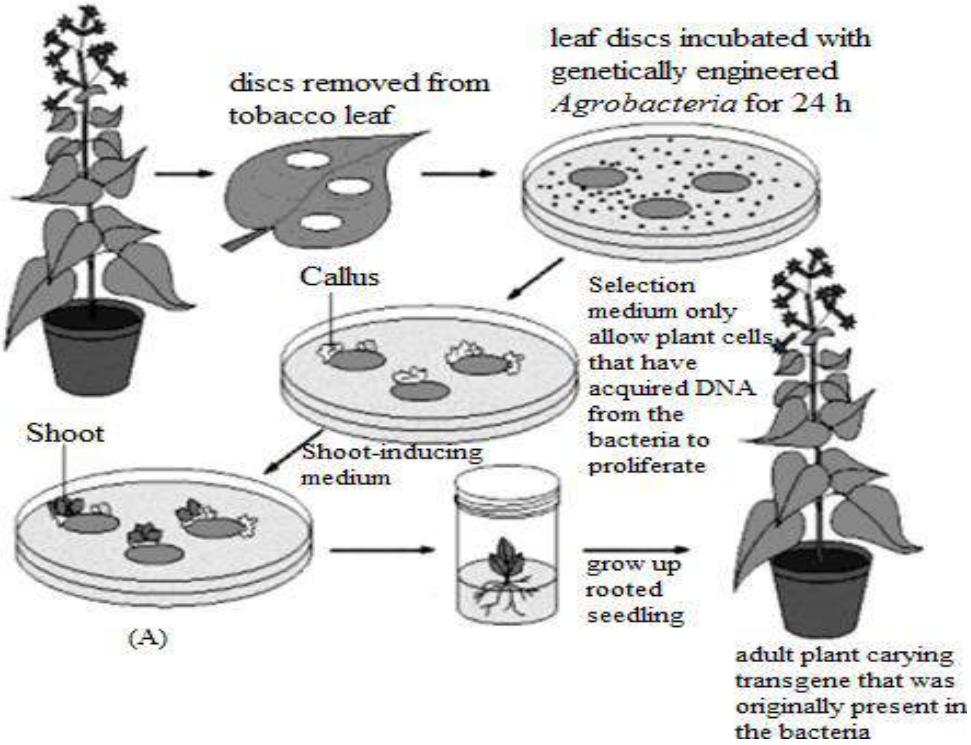

Fig.12.9 *Agrobacterium* mediated development of transgenic plants in tobacco

Procedure for *Agrobacterium* mediated gene transfer

The explants used for inoculation or co-cultivation with *Agrobacterium* carrying the vector include protoplasts, callus, tissue slices, sections of organs like leaf discs etc. In practice, the procedure can be performed with any tissue explant that provides a good source for initiation of whole plant differentiation.

1. A tissue segment like leaf disc is excised and is incubated in *Agrobacterium* suspension for few hours to 3-4 days followed by culturing on a media for bacterial growth to take place.

2. Tissue explants are then transferred to media containing **carbenicillin** or **cefotaxime** which eliminate bacteria.
3. After explants are inoculated with *Agrobacterium* carrying the vector with gene of interest, they are moved to media designed for selection of transformed plant cells.
4. Selection is facilitated by selectable marker genes present in vector which is usually resistance to herbicide or antibiotics like **kanamycin**.
5. Following selection, the transformed calli is put in regeneration medium for development of shoots and roots.
6. The second level of selection for transformed tissue is done by expression of reporter gene or scorable marker gene like β-glucuronidase (GUS). These transgenic plants are then tested for stable integration and expression of genes by PCR or by Southern hybridization.

Types of Transgenic plants

Transgenic plants have genes inserted into them, deriving from other species. The inserted genes can come from species within the same kingdom (plant to plant) or between kingdoms (bacteria to plant). In many cases, the inserted DNA has to be modified slightly in order to correctly and efficiently express in the host organism. Transgenic plants are used to express proteins, like the cry toxins from *Bacillus thuringiensis*, herbicide resistant genes and antigens for vaccinations. Cisgenic plants are made up of using genes, found within the same species or a closely related one, where conventional plant breeding can occur. Some breeders and scientists argue that cisgenic modification is useful for plants that are difficult to crossbreed by conventional means (such as potatoes). Those plants in the cisgenic category should not require the same level of legal regulation as other genetically modified organisms.

Applications of transgenic plants

1. Resistance to biotic stresses i.e. resistance to diseases caused by insects, viruses, fungi and bacteria.
2. Resistance to abiotic stresses-herbicides, temperature (heat, chilling, freezing), drought, salinity, ozone, intense light.
3. Improvement of crop yield, and quality e.g. storage, longer shelf life of fruits and flowers.
4. Transgenic plants with improved nutrition.
5. Transgenic plants as bioreactors for the manufacture of commercial products e.g. proteins, vaccines, and biodegradable plastics.

12.6 GENE BANK

Conserving the genetic diversity of our crops, landraces and related wild species is essential to ensure future plant breeders can access this variation, especially in view of increased food demand by a growing world population and climate change. Gene banks are a type of

biorepository which preserve genetic material, where biological material is collected, stored, catalogued and made available for redistribution. For plants, this could be by freezing cuttings from the plant, or stocking the seeds (e.g. in a seed bank). For animals, this is the freezing of sperm and eggs in zoological freezers until further need. With corals, fragments are taken which are stored in water tanks under controlled conditions.The main role of plant gene banks is to preserve genetic diversity, in the form of seeds or cuttings in the case of plants reproduced vegetatively, and subsequently make this material, together with associated information, available for future use in research and plant breeding. Plant genetic material in a 'Gene Bank' is preserved at -196° Celsius in Liquid Nitrogen as mature seed (dry). In plants, it is possible to unfreeze the material and propagate it, however, in animals, a living female is required for artificial insemination. While it is often difficult to use frozen animal sperm and eggs, there are many examples of it being done successfully.

The place where seeds and cuttings from a large variety of plants are kept is called gene banks or seed banks. Gene banks and seed banks can be setup at a local level, or they can be setup on a national or even international level. One famous seed bank is located in the Arctic Circle called the **Svalbard Global Seed Vault**. This international seed bank, located between Norway and the North Pole, can hold a maximum of 2.5 billion seeds representing 4.5 million crop varieties (500 seeds from each crop). The seeds at Svalbard are kept at -18 degrees Celsius to keep them viable for a long time. This global seed bank was built as a backup to the world's more than 1,700 gene banks located in different countries. If something were to happen to the other seed banks such as an economic or natural disaster, then the world would still have the seeds stored at Svalbard to use if needed.

Types of Gene Banks

Gene bank refers to a place or organization where Germplasm can be conserved in living state. Gene banks are also known as Germplasm banks. The Germplasm is stored in the form of seeds, pollen or in vitro cultures, or in the case of a field gene bank, as plants growing in the field. Gene banks are mainly of two types, namely:

1) Seed gene banks, and
2) Field gene banks.

1. Seed Gene Bank: A place where Germplasm is conserved in the form of seeds is called seed gene bank. Seeds are very convenient for storage because they occupy smaller space than whole plants. However, seeds of all crops cannot be stored at low temperature in the seed banks. Spores and Pteridophytes are conserved in seed banks, but other seedless plants, such as tuber crops cannot be preserved this way. The Germplasm of only orthodox species (whose seed can be dried to low moisture content without losing variability) can be conserved in seed banks. The largest seed bank in world is the Millennium Seed Bank (MSB), in West Sussex, near London. In the seed banks, there are three types of conservation, viz.

a) Short term,

b) Medium term,
c) Long term.
Base collections are conserved for long term (50 years or more) at 18 or 20^0C. Active collections are stored for short term (3-5 years) at 5-10 0 C. The main advantages of gene banks are as follows:
1. Large number of Germplasm samples or entire variability can be conserved in a very small space.
2. In seed banks, handling of Germplasm is easy.
3. Germplasm is conserved under pathogen and insect free environment.

There are some disadvantages of Germplasm conservation in the seed banks. These are listed below:
1. Seeds of recalcitrant species cannot be stored in seed banks.
2. Failure of power supply may lead to loss of viability and thereby loss of Germplasm.
3. It requires periodical evaluation of seed viability. After some time multiplication is essential to get new or fresh seeds of storage.

2. Field Gene Banks: Also called plant gene banks, are areas of land in which Germplasm collections of growing plants are assembled. This is also ex-situ conservation of Germplasm. Those plant species that have recalcitrant seeds or do not produce seeds readily are conserved in field gene bank. **Recalcitrant seeds** (subsequently known as unorthodox seeds) are seeds that do not survive drying and freezing during ex-situ conservation and vice versa. This is a method of planting plants for the conservation of genes. For this purpose, an ecosystem is created artificially. Through this method one can compare the differences among plants of different species and can study them in detail. It needs more land, adequate soil, weather, etc.

In field gene banks, Germplasm is maintained in the form of plants as a permanent living collection. Field gene banks are often established to maintain working collections of living plants for experimental purposes. Germplasm of important crops are conserved through this method. They are used as source of Germplasm for species such as coconut, rubber, mango, cassava, yam, and cocoa. 42,000 varieties of rice are conserved in the Central Rice Research Institute in Orissa. Field gene banks have been established in many countries for different crops.
Field gene banks have some advantages and disadvantages which are discussed below:
There are two main advantages:
1. It provides opportunities for continuous evaluation for various economic characters.
2. It can be directly utilized in the breeding programmes.

There are three main demerits of field gene banks as given below:
1. Field gene banks can not cover the entire genetic diversity of a species. It can cover only a fraction of the full range of diversity of a species.

2. The Germplasm in field gene banks is exposed to pathogen and insects and sometimes is damaged by natural disasters such as bushfires, cyclones, floods, etc.
3. Maintenance of Germplasm in the field gene banks is costly affair.

Table-1: Field Gene Banks in Some Countries

Name of Country	Crop Species for which Field Gene Bank is Established
Malaysia	Oil palm has been conserved on 500 hectares.
Indonesia	Earmarked 1000 hectares area for coconut and other perennial crops.
Philippines	South East Asian Germplasm of banana has been conserved.
India	Global collection of coconut has been conserved in Andaman ad Nicobar.

Meristem Gene Banks: Germplasm of asexually propagated species can be conserved in the form of meristem. This method is widely used for conservation and propagation of horticultural species. In vitro method can be used in two ways, first, for storage of tissue under slow growth conditions. Second, for long term conservation of Germplasm by Cryopreservation.

In **Cryopreservation**, the tissues are stored at very low temperatures i.e. at **-196 °C** in liquid nitrogen. At these temperatures, all biological processes virtually come to a stop. Based on status of Research Institutes, gene banks are again of two types, viz.
1) National gene banks, and
2) International or global gene banks.
National gene banks are maintained by each country and **Global gene banks** are located in International Crop Research Institutes/Centres. In India, gene banks are maintained by concerned crop research institute of **ICAR**. National Bureau of Plant Genetic Resources (**NBPGR**), New Delhi is also maintaining Germplasm of various field crops.

Cryobank: In this technique, a seed or embryo is preserved at very low temperatures. It is usually preserved in liquid nitrogen at -196 °C. This is helpful for the conservation of species facing extinction. Cryobanks are utilized for the Cryo-preservation Cryo-conservation of animal genetic resources

Storage of pollen: This is a method in which pollen grains are stored. We can make plants which are facing extinction in the present world. Using this technique, we can make plants with one set of chromosomes. The pollen is stored in liquid nitrogen. This method is useful for crossbreeding.

12.7 NIF GENE

The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms. The nif genes code for proteins essential in nitrogen fixation, such as nitrogenase and certain regulatory proteins. The primary enzyme encoded by the nif genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen (N_2) to other nitrogen forms such as ammonia (NH_3) which the organism can use for various purposes. Besides the nitrogenase enzyme, the nif genes also encode a number of regulatory proteins involved in nitrogen fixation. They are found in nitrogen-fixing bacteria. They occur as an operon in free-living anaerobic nitrogen-fixing bacteria such as *Klebsiella pneumoniae, Rhodospirillum rubrum*, and *Rhodobacter capsulatus*. These genes may also be found on plasmids (together with the other genes, e.g. nod genes) in symbiotic bacteria, such as in Rhizobium inhabiting the roots of leguminous plants.

The expression of the nif genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations (the low oxygen concentrations are actively maintained in the root environment of host plants). NifA protein regulated the nif genes transcription. NifA protein is in turn regulated by Nitrogen regulator NtrC. The expression of NifA protein is triggered when fixed nitrogen and oxygen levels are low. In contrast, a sufficient concentration of nitrogen or oxygen would stimulate the protein NifL. The latter inhibits the activity of NifA and this inhibits the formation of nitrogenase. The first Rhizobium genes for nitrogen fixation (nif) and for nodulation (nod) were cloned in the early 1980s by Gary Ruvkun and Sharon R. Long in Frederick M. Ausubel's laboratory. Nitrogen fixation is important because many living organisms are unable to metabolize directly the atmospheric nitrogen and would require the nitrogen fixation capability of certain bacteria in order to produce a form of nitrogen (e.g. ammonia) that can be readily utilized.

Organization of Nif Genes

Nitrogen fixation is carried out by three groups of genes. These are-

a. Nod gene (responsible for nodule formation)

b. Nif gene (responsible for nitrogen fixation)

c. Hup gene (responsible for nitrogen uptake).

All these three types of genes are present in a group on a single chromosome. This makes their copying and transfer mechanism simple for genetic engineering purposes. Though the mechanism of nodule formation is complex, nod gene is responsible for nodule formation as well as host reorganization and specificity. However, a few genes located on plasmids can produce nodules. Plasmid of *R. leguminosarum* is less than 10 kb even then it has property to recognize host and nodule formation.

Nif genes: This gene is responsible for nitrogen fixation and present in the genome of symbiotic and non-symbiotic nitrogen fixing bacteria. In symbiotic bacteria Rhizobium, it is present near nod genes on the mega-plasmid, while in non-symbiotic Cyanobacteria it is present on the main DNA. Initially Nif gene has been transferred in E. coli. In higher plants, chloroplast is a cell organelle which might have been originated from prokaryotes, therefore attempt are made to transfer Nif gene into chloroplast. Easy availability of ATP and $NADPH_2$ in chloroplast also makes them ideal recipient for this gene transfer. Most of the cereal plants are monocots and any such effort to transfer such Nif gene will revolutionize the yield, economics and environmental pollution. However, there are many difficulties in transferring, integration and expression of a prokaryotic gene into a monocot.

Klebsiella pneumoniae strain M5 a1 (Enterobacteriaceae) is a free living bacteria which has been studied extensively for genetics of nitrogen fixation. This bacterial genome is quite similar to that of *E. coli* and *Salmonella typhimurium*. Therefore most of the techniques of genetic engineering can be applied to *Klebsiella*.

Examples in nature

The expression and regulation of nif genes, while sharing common features in all or most of the nitrogen-fixing organisms in nature, have distinct characters and qualities that differ from one diazotroph to another. **Diazotrophs** are bacteria and archaea that fix atmospheric nitrogen gas into a more usable form such as ammonia. A diazotroph is an organism that is able to grow without external sources of fixed nitrogen. Examples of organisms that do this are rhizobia and Frankia (in symbiosis) and Azospirillum. Examples of nif gene structure and regulation in different diazotrophs include:

Klebsiella pneumonia- a free-living anaerobic nitrogen-fixing bacterium. It contains a total of 20 nif genes located on the chromosome in a 24-Kb region. Nif H, nif D, and nif K encode the nitrogenase subunits, while *nif E, nif N, nif U, nif S, nif V, nif W, nif X, nif B, and nif Q* encode proteins involved the assembly and incorporation of iron (Fe) and molybdenum (Mo) atoms into the nitrogenase subunits. Nif F and Nif J encode proteins related to electron transfer taking place in the reduction process and nif A and nif L are regulatory proteins in charge of regulating the expression of the other nif genes.

Rhodospirillum rubrum- Free-living anaerobic photosynthetic bacterium which, in addition to the transcriptional controls described above, regulates expression of the nif genes also in a metabolic way through a reversible ADP-ribosylation of a specific arginine residue in the nitrogenase complex. The ribosylation takes place when reduced nitrogen is present and it causes a barrier in the electron transfer flow and thereby inactivates nitrogenase activity.

Rhodobacter capsulatus- A free-living anaerobic phototroph containing a transcriptional nif gene regulatory system. It regulates nif gene expression through nif A in the same manner described before, but it uses a different nif A activator which initiates the NtrC. NtrC activates a different expression of nif A and the other nif genes.

***Rhizobium* spp.-** Gram-negative, symbiotic nitrogen fixing bacteria that usually form a symbiotic relationship with legume species. In some rhizobia, the nif genes are located on plasmids called '*sym* plasmids' (*sym* =symbiosis) which contain genes related to nitrogen fixation and metabolism, while the chromosomes contain most of the housekeeping genes of the bacteria. Regulation of the nif genes is at the transcriptional level and is dependent on colonization of the plant host.

Regulation of Nitrogen Fixation

In most bacteria, regulation of nif genes transcription is done by the nitrogen sensitive **Nif A** protein. When there isn't enough fixed nitrogen available for the organism's use, the nitrogen regulators **NtrC** triggers NifA expression, and NifA activates the rest of the nif genes. In the absence of ammonia or organic nitrogen the NtrC protein is phosphorylated by the **NtrB** protein. If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein is activated i.e. **Nif L** which inhibits Nif A activity resulting in the inhibition of nitrogenase formation. The nif genes can be found on bacterial chromosomes, but in symbiotic bacteria they are often found on plasmids or symbiosis islands with other genes related to nitrogen fixation (such as the nod genes). The greater study of the infectious process and the genes involved and the physiological aspects will lead to better nitrogen use by plants grown on nitrogen-poor soils in agriculture and can reduce the requirement for chemical fertilizers.

Transgenes with Nif Genes

It is important to develop transgenic plants containing Nif genes to solve the problem of nitrogen fertilizer supplement to crop plants. This will have beneficial effects of economics and environment also. For this purpose, Ti based plasmid and Cauliflower mosaic virus based promoter (CAM promoter) was used to transfer Nif genes in to non-legume plants. To test the efficacy of this system, phaseolin gene from legume (pulses) has been transferred to sunflower where it was expressed and produced phaseolin. Protoplasts isolation from root nodules and preparations of rhizobia were used to develop hybrids by protoplasts fusion and organelles uptake. Major contributions were made by groups headed by Prof. E.C. Cocking, Prof. M.R. Davey, Dr. **Ingo Potrykus** (inventor of *Golden rice*) and Prof. I. K. Vasil. However, true nitrogen fixing hybrids are yet to be obtained by this method.

12.8 NOD GENE

Most of the biological nitrogen fixing bacteria contains a large plasmid called mega-plasmid. In several functions it is similar to Ti plasmid and contains genes responsible for auxin and cytokinin production. Excess production of these plant growth regulators helps in nodule formation. According to **Rosenberg** (1981) several special genes are present along with nod genes. Such plasmids are absent in non-symbiotic bacteria. A nod gene is a group of genes containing Nod A, B, C, D genes having 8.5 kb length. These genes form polypeptides of different lengths (196, 197, 402, 211 amino acid). Nod genes of different Rhizobium species have almost 70% homologies which are called **Common Nod Genes**.

Nodulation (Nod) factors are signaling molecules produced by bacteria known as Rhizobia during the initiation of nodules on the root of legumes. A symbiosis is formed when legumes take up the bacteria. The rhizobia produce nitrogen for the plant, and the legumes produce Leghemoglobin (also leghaemoglobin or legoglobin) to carry away any oxygen that would inhibit nitrogenase activity.

Nod gene expression is induced by the presence of certain flavonoids in the soil, which are secreted by the plant to attract the bacteria. These chemicals induce the formation of NodD, which in turn activates other genes involved in the expression of nod factors and their secretion into the soil. Nod factors induce root-hair curling such that it envelops the bacterium. This is followed by the localized breakdown of the cell wall and the invagination of the plant cell membrane, allowing the bacterium to form an infection thread and enter the root hair. The end result is the nodule, the structure in which nitrogen is fixed. Nod factors act by inducing changes in gene expression in the legume, most notable the nodulin genes, which are needed for nodule organogenesis.

The establishment of the nitrogen-fixing symbiosis between rhizobia and legumes requires an exchange of signals between the two partners. In response to flavonoids excreted by the host plant, rhizobia synthesize Nod factors (NFs) which elicit, at very low concentrations and in a specific manner, various symbiotic responses on the roots of the legume hosts. NFs from several rhizobial species have been characterized. They all are lipo-chitooligosaccharides, consisting of a backbone of generally four or five glucosamine residues N-acylated at the non-reducing end, and carrying various O-substituents. The N-acyl chain and the other substituents are important determinants of the rhizobial host specificity. A number of nodulation genes which specify the synthesis of NFs have been identified. All rhizobia, in spite of their diversity, possess conserved nod A B C genes responsible for the synthesis of the N-acylated oligosaccharide core of NFs, which suggests that these genes are of a monophyletic origin. Other genes, the host specific nod genes, specify the substitutions of NFs. The central role of NFs and nod genes in the Rhizobium-legume symbiosis suggests that these factors could be used as molecular markers to study the evolution of this symbiosis.

Nodulation Process

Nodule Development

1. Plant releases flavonoids in the rhizosphere.
2. NodD recognizes flavonoids and return to Rhizobia to activate Nod factor production.
3. Nod factors are released from Rhizobia and bind to receptors on plant root hair.
4. Plant root hair curling occurs and traps Rhizobia.
5. Infection thread forms and induces cortical cell division in the plant primordium.
6. Rhizobia fix nitrogen within a fully formed nodule.

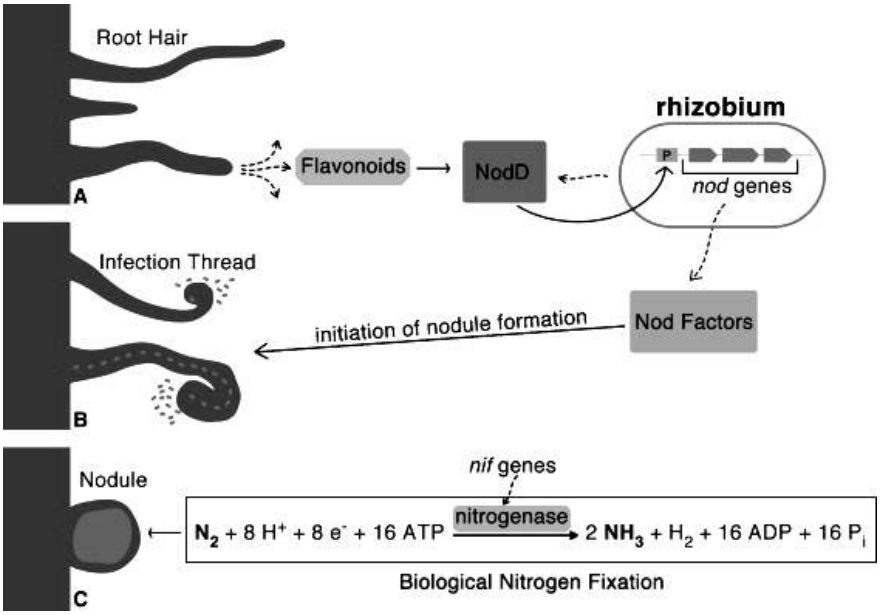

Fig.12.9 The process of nodule formation in root

Function of Nod Factors

Nod factors are the important signaling molecules in the symbiotic interaction between rhizobia and leguminous plants. When they bind to the root hairs of the leguminous plants, they cause root hair deformations, activation of plant genes, initiation of cortical cell division and nodule formation. Each of these processes are necessary for the proper formation of root nodules, which enable the symbiotic interaction between rhizobia and leguminous plants. Nod factors control the specificity of the interaction between the two organisms and they specifically cause morphological and physiological changes in leguminous plant. Additionally, Nod factors can affect other plants in the soil surrounding them. When Nod factors are present in mixed crop fields, they have the ability to stimulate seed germination, promote plant growth, increase photosynthetic rates, and increase grain yields of legume and non-legume crops. Thus, Nod factors do not only benefit leguminous plants, but can contribute to the growth rate of many different types of crops.

Nod factors are lipo-chitooligosaccharides (LCOs) and have three to five N-acetyl-glucosamines. The substitutions on the lipo-chitooligosaccharides side chains determine specific recognition by the Nod factor receptors found in leguminous root hairs. The specific structure of Nod factors is determined by modifications made by Nod genes, which are found in the Rhizobium genome. The Nod genes encode for proteins that modify Nod factors by adding or removing different chemical structures such as sulfates, fatty acids, acetyl groups, and methyl groups to the original lipo-chitooligosaccharide structure.

The most extensively studied Nod genes are the common Nod genes (NodA, NodB and NodC). The enzymes encoded by the common Nod genes have specific functions. NodC is an enzyme that synthesizes the N-acetyl glucosamine backbone of the Nod factor. NodB is a deacetylase that removes an acetyl group from the Nod factor and NodA is an acyl-transferase that adds a fatty acid chain to the site deacetylated by NodB.

12.9 MYCOPROTEINS

Mycoprotein is a form of single-cell protein, also known as fungal protein, is defined as- Protein derived from fungi, especially as produced for human consumption. "*Myco*" is from the Greek word for "*fungus*". Mycoprotein is a healthy, meat-free form of high quality protein, and as the main ingredient, it plays an important role in determining the nutritional value of **Quorn products** (the only mycoprotein on sale in Europe and North America). Mycoprotein is a type of fungi (*Fusarium venenatum*), discovered more that 40 years ago. The nutritional characteristics of mycoprotein have been found to deliver a unique combination of health benefits, showing that while 'vegetable' in origin, mycoprotein contains all nine essential amino acids and so falls into the category of being a source of first class protein, comparable with other protein sources such as meat and fish. However, unlike meat proteins, mycoprotein is free from cholesterol, is low in fat and saturates and contains no trans fats. It also possesses a unique fibre, comprising about 25 percent of its dry weight, composed mainly of polymeric n-acetyl glucosamine (chitin) and beta 1-3 and 1-6 glucans. Studies suggest that mycoprotein helps maintain normal blood cholesterol levels and can possibly even lower LDL (low-density lipoprotein) cholesterol levels.

Synthesis

The fungus is grown in vats using glucose syrup as food. A fermentation vat is filled with the growth medium and then inoculated with the fungal spores. The *F. venenatum* culture respires aerobically, so for it to grow at an optimum rate, it is supplied with oxygen, and carbon dioxide is drawn from the vat. To make protein, nitrogen (in the form of ammonia) is added and vitamins and minerals are needed to support growth. The vat is kept at a constant temperature, also optimized for growth; the fungus can double its mass every five hours. When the desired amount of mycoprotein has been created, the growth medium is drawn off from a tap at the bottom of the fermenter. The mycoprotein is separated and purified. It is a pale yellow solid with a faint taste of mushrooms. Different flavors and tastes can be added to the mycoprotein to add variety.

Characteristics of Mycoprotein

In today's 'obesogenic' environment, in which there is an abundance of foods high in fat and/or sugar available to consumers, there is growing interest in foods that are both nutritious and satiating, but that are of low-energy density, and are low in saturates, salt and sugar. Mycoprotein has a favorable fatty acid profile (being relatively low in saturates), a fibre content that is comparable with other vegetarian protein sources, and a naturally low sodium content. Mycoprotein is a good source of zinc and selenium but the levels of iron and vitamin B12 in mycoprotein are low in comparison to red meat. Mycoprotein is typically 12% water, 3% fat, 3% available carbohydrates, 6% fiber, and 2% ash. B complex vitamins and mineral nutrients are also present in small amounts.

A number of other features make mycoprotein of interest beyond being simply a protein source.

1. It can be modified so as to function as either a fat replacer or a cereal replacer.
2. Clinical studies have found that it can help control blood lipids and blood glucose as well as appetite, effects that may relate to its dietary fiber content.
3. Mycoprotein is a food ingredient that can be used in whole foods or as a whole food itself. It is composed of the hyphae of a fungal organism that is grown through a continuous fermentation process.
4. Mycoprotein is very favorable nutrient profile and its excellent taste characteristics make it suitable and beneficial for use in a variety of products. For example, mycoprotein can be used in dairy and cereal products, and its structure and texture make it a particularly suitable alternative to traditional sources of protein in the human diet.
5. Role of mycoprotein in lowering blood cholesterol concentrations, reducing energy intakes and controlling blood sugar levels has generated a small number of human studies investigating the effects of mycoprotein on cholesterol reduction, satiety and insulinaemia/glycaemia.
6. Furthermore, mycoprotein has a significant history of use in Europe, where it has been sold under the trade name Quorn for fifteen years.

Health Concerns: Some strains of *F. venenatum* produce a variety of mycotoxins such as type *A Trichothecenes*. Mycotoxins-producing genes like isotrichodermin, sambucinol, culmorin, and enniatin B can be found in cultures of *F. venenatum*. Specific strands that do not produce mycotoxins under optimal conditions can be selected to reduce the danger to human consumers. Testing at six-hour intervals can be done to monitor Mycotoxins presence. There is continual testing for concerns of allergic reactions, which can range from abdominal pain, nausea and vomiting to severe asthmatic reactions, especially when crossed with inhaled mold spores.

12.10 SUMMARY

The present chapter deals with the basis of Plant tissue culture, gene transfer, gene bank and nif genes. Plant tissue culture is the culturing of plant parts in the aseptic conditions. Tissue culture

holds tremendous potential in the conservation of endangered plants and obtaining the active constituents of the plants. Gene transfer is the uptake of foreign DNA or recombinant DNA by the cells. Various gene transfer techniques are broadly grouped into two broad categories viz. direct gene transfer and *Agrobacterium* mediated. The uptake of foreign DNA or the recombinant DNA by cells is called gene transfer or transformation. Gene banks are the repositories where biological material is collected, stored, catalogued and made available for redistribution. The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms.

Plant tissue culture is a collection of techniques used to sustain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is used to produce clones of plant in a method called micropropagation. Plant tissue culture relies on the fact that many plant cells have the ability to regenerate into a whole plant in a process called Totipotency. Single cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant on culture media given the required nutrients and hormones. The plant part obtained from a plant to be cultured is called explant while the main plant it is obtained from is called mother plant. Explant can be taken from different plant parts such as shoots, leaves, stems, flowers, roots, single undifferentiated cells etc.

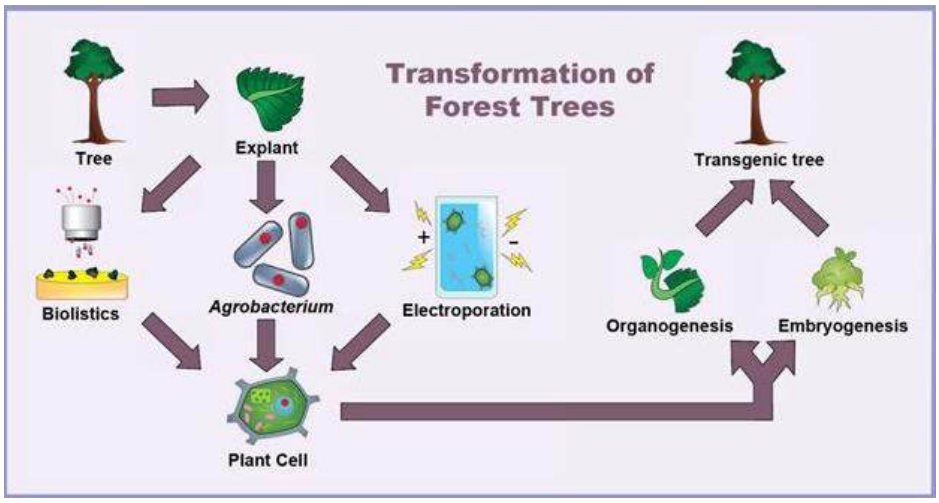

Fig.12.10 Diagram showing transformation of tree into transgenic tree by different methods of gene transfer

12.11 GLOSSARY

ABA: Abscisic acid is a plant hormone, functions in many plant developmental processes, including bud dormancy, and can be involved in stress responses.

Agar: (or agar-agar) is a jelly-like substance, obtained from algae. It is used for solidifying certain culture media, as a thickening agent for ice cream and other foods, as a substitute for gelatin, in adhesives, as an emulsifier, etc.

Agronomic: The science and technology of producing and using plants for food, fuel, fiber, and land reclamation. Agronomy has come to encompass work in the areas of plant genetics, plant physiology, meteorology, and soil science.

Amphiploids: A hybrid organism having a diploid set of chromosomes from each parental species.

Asepsis: The state of being free from disease-causing micro-organisms (such as pathogenic bacteria, viruses, pathogenic fungi, and parasites).

Auxins: A class of substances that in minute amounts regulate or modify the growth of plants, especially root formation, bud growth, and fruit and leaf drop.

Biolistics: Particle delivery system, originally designed for plant transformation, is a device for delivering exogenous DNA (transgenes) to cells.

Biotransformation: The series of chemical changes occurring in a compound, especially a drug, as a result of enzymatic or other activity by a living organism.

Brassinosteroids: A group of some 40 different steroids that are synthesized by plants and are potent hormones affecting many aspects of plant growth. It was first isolated from the pollen of *Brassica napus*, hence the name.

Callus: Plant callus (plural calluses or calli) is a growing mass of unorganized plant parenchyma cells. In living plants, callus cells are those cells that cover a plant wound.

Chromatography: A physical method of separation that distributes components to separate between two phases, one stationary (stationary phase), the other (the mobile phase) moving in a definite direction.

Clone: A cell, cell product, or organism that is genetically identical to the unit or individual from which it was derived.

Crown gall: A disease of peaches, apples, roses, grapes, etc., characterized by the formation of galls on the roots or stems usually at or below ground level, caused by a bacterium, Agrobacterium tumefaciens.

Cryobank: A place of storage that uses very low temperatures to preserve semen or transplantable tissues.

Cryopreservation: The storage of blood or living tissues at extremely cold temperatures, (often -196 °C).

Cytokinins: A class of plant growth substances (Phytohormones) that promote cell division, or cytokinesis, in plant roots and shoots. They are involved primarily in cell growth and differentiation, but also affect apical dominance, axillary bud growth, and leaf senescence.

Diazotrophs: Bacteria and archaea that fix atmospheric nitrogen gas into a more usable form such as ammonia. A diazotroph is an organism that is able to grow without external sources of fixed nitrogen.

Electroporation: (or electropermeabilization), is a microbiology technique in which an electrical field is applied to cells in order to increase the permeability of the cell membrane, allowing chemicals, drugs, or DNA to be introduced into the cell.

Embryogenesis: The process by which the embryo forms and develops.

Explant: Small pieces of plant parts or tissues that are aseptically cut and used to initiate a culture in a nutrient medium.

Flavonoids: A diverse group of phytonutrients (plant chemicals) found in almost all fruits and vegetables. Along with carotenoids, they are responsible for the vivid colors in fruits and vegetables.

Gene bank: A type of biorepository which preserve genetic material. For plants, this could be by freezing cuttings from the plant, or stocking the seeds (e.g. in a seed bank).

Germplasm: The protoplasm of the germ cells containing the chromosomes.

Gibberellins: Plant hormones that regulate growth and influence various developmental processes, including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction, and leaf and fruit senescence.

Glycaemia: The concentration of sugar or glucose in the blood.

Imbibition: A special type of diffusion when water is absorbed by solids-colloids causing an enormous increase in volume.

***In vitro*:** (of a biological process) made to occur in a laboratory vessel or other controlled experimental environment rather than within a living organism or natural setting.

Insulinaemia: The (normal) presence of insulin in the bloodstream.

Intergeneric: Existing between or obtained from different genera.

Kinetin: A type of cytokinin, a class of plant hormone that promotes cell division.

Liposomes: A microscopic artificial sac composed of fatty substances and used in experimental research of the cell.

Meristem: A tissue in plants made of dividing cells. They are in parts of the plant where growth can take place.

Morphogenesis: The development of structural features of an organism or part.

Mutant: A new type of organism produced as the result of mutation.

Mycoprotein: A form of single-cell protein, also known as fungal protein. It's a completely meat-free form of high quality protein and is also a good source of dietary fibre.

Mycotoxins: A toxin produced by a fungus.

Nif gene: Are genes encoding enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms.

Nod factor: Signaling molecules produced by bacteria known as rhizobia during the initiation of nodules on the root of legumes.

Nodulation: The formation or presence of nodules.

Organogenesis: The production and development of the organs of an animal or plant.

PEG: Polyethylene glycol is a polyether compound with many applications from industrial manufacturing to medicine.

Phylogenetic: The study of the evolutionary history and relationships among individuals or groups of organisms (e.g. species, or populations).

Phytopathogen: Any organism that is pathogenic to plants.

Plasmid: A genetic structure in a cell that can replicate independently of the chromosomes, typically a small circular DNA strand in the cytoplasm of a bacterium or protozoan. Plasmids are much used in the laboratory manipulation of genes.

Plasmolysis: Contraction of the protoplast of a plant cell as a result of loss of water from the cell.

Polyamines: An organic compound having two or more primary amino groups. They play multiple roles in cell growth, survival and proliferation.

Proliferation: The growth or production of cells by multiplication of parts.

Protoplast: The contents of a cell within the cell membrane, considered as a fundamental entity.

Rhizosphere: The area of soil that surrounds the roots of a plant and is altered by the plant's root growth, nutrients, respiration, etc.

Somatic cells: One of the cells that take part in the formation of the body, becoming differentiated into the various tissues, organs, etc.

Spermidine: Polyamine compound found in ribosomes and living tissues, and having various metabolic functions within organisms. It was originally isolated from semen.

Symbiosis: The living together of two dissimilar organisms, as in mutualism, commensalism, amensalism, or parasitism.

Terpenoid: Any of a class of hydrocarbons that consist of terpenes attached to an oxygen-containing group. Terpenoids are widely found in plants, and can form cyclic structures such as sterols.

Tissue culture: A method in which fragments of a tissue (plant or animal tissue) are introduced into a new, artificial environment, where they continue to function or grow.

Totipotency: The ability of a single cell to divide and produce all of the differentiated cells in an organism.

Transgene: A gene or genetic material that has been transferred naturally, or by any of a number of genetic engineering techniques from one organism to another.

Weedicides: The weed killers or pesticides that are used to kill unwanted plants.

12.12 SELF ASSESSMENT QUESTIONS

12.12.1 Objective type Questions:

1. Which of the following is the most commonly used carbon source in Plant tissue culture media?

(a) Agar (b) Sucrose

(c) Kinetin (d) EDTA

2. Higher concentration of which hormone promotes shoot induction in Plant tissue culture
(a) Sucrose (b) Inositol
(c) Auxins (d) Cytokinins

3. The culture of tissues and cells cultured in a liquid nutrient medium produce single cells and cell clumps, this is called
(a) Callus culture (b) Meristem culture
(c) Tissue culture (d) Suspension culture

4. Solidifying agent used in the plant tissue cultures
(a) Agar (b) Sucrose
(c) Inositol (d) Glycine

5. Which of the following form the most successful plant transformation system
(a) Particles Bombardment method (b) Direct gene transfer
(c) Agrobacterium mediated gene transfer (d) Electroporation

6. Golden rice developed as the transgenic plant is rich in
(a) Vitamin C (b) Vitamin D
(c) Vitamin A (d) Vitamin E

7. Bacillus thuringiensis is a
(a) Bacteria (b) Fungi
(c) Mold (d) Cotton

8. Primary enzyme encoded by nif genes is
(a) Protease complex *(b) Nitrate complex*
(c) Nitrogenase complex *(d) Nitrite complex*

9. Which of the following is a free-living anaerobic nitrogen-fixing bacterium
(a) *Rhodobacter capsulatus* (b) *Klebsiella pneumonia*
(c) *Rhodospirillum rubrum* (d) *Bacillus thuringiensis*

10. Which vector is mostly used in crop improvement?
(a) Plasmid (b) Cosmid
(c) Phasmid (d) Agrobacterium

11. Which of the following plant cell will show Totipotency?
(a) Xylem vessels (b) Sieve tube
(c) Meristem (d) Cork cells

12. Somaclonal variations are the ones
    (a) Caused by mutagens (b) Produce during tissue culture
    (c) Caused by gamma rays (d) Induced during sexual embryogeny

13. A medium which is composed of chemically defined compound is called
    (a) Natural media (b) Synthetic media
    (c) Artificial media (d) None of these

14. Growth hormone producing apical dominance is
    (a) Auxin (b) Gibberellins
    (c) Ethylene (d) Cytokinin

15. Part of plant used for culturing is called
    (a) Scion (b) Explant
    (c) Stock (d) Callus

16. The most widely used chemical for protoplast fusion, is
    (a) Mannitol (b) Sorbitol
    (c) Mannol (d) Polyethyleneglycol (PEG)

17. Who is the father of tissue culture?
    (a) Bonner (b) Haberlandt
    (c) Laibach (d) Gautheret

18. The production of secondary metabolites require the use of
    (a) Protoplast (b) Cell suspension
    (c) Meristem (d) Axillary buds

19. Totipotency means
    (a) Flowering in culture medium
    (b) Development of fruit from a flower in a culture
    (c) Development of an organ from a cell in culture medium
    (d) All of these

20. Haploid plants can be obtained from
    (a) Bud culture (b) Leaf culture
    (c) Root culture (d) Anther culture

21. In tissue culture medium, the embryoids formed from pollen grains is due to
    (a) Cellular Totipotency (b) Organogenesis

(c) Double fertilization (d) Test tube culture

22. In plant tissue culture, which of the following shows Totipotency?
(a) Meristem (b) Sieve tube
(c) Xylem vessel (d) Collenchyma

23. In plant tissue culture, the callus tissues can be regenerated into complete plantlets primarily by altering the concentration of
(a) Sugars (b) Vitamins
(c) Amino acids (d) Hormones

24. A major application of embryo culture is in
(a) Clonal propagation (b) Production of embryoids
(c) Overcoming hybridization barriers (d) Induction of Somaclonal variations

25. The problem of necrosis and gradual senescence while performing tissue culture can be overcome by
(a) Spraying auxins (b) Spraying cytokinins
(c) Suspension culture (d) Subculture

26. Somaclonal variation appears in plants
(a) Growing in polluted soil or water
(b) Exposed to gamma rays
(c) Raised in tissue culture
(d) Transformed by recombinant DNA technology

27. The ability of the component cells of callus to form a whole plant is known as
(a) Redifferentaition (b) Dedifferentiation
(c) Either (a) or (b) (d) None of these

28. An _____ is an excised piece of leaf or stem tissue used in micropropagation.
(a) Microshoot (b) Medium
(c) Extant (d) Scion

29. Protoplasts can be produced from suspension cultures, callus tissues or intact tissues by enzymatic treatment with
(a) Cellulolytic enzymes (b) Pectolytic enzymes
(c) Both Cellulolytic and Pectolytic enzymes (d) Proteolytic enzymes

30. Organogenesis is

(a) Formation of callus tissue
(b) Formation of root and shoots on callus tissue
(c) Both (a) and (b)
(d) Genesis of organs

12.12.1 Answers Key: 1. (b), 2. (d), 3. (d), 4. (a), 5. (c), 6. (c), 7. (a), 8. (c), 9. (b), 10. (d), 11. (c), 12. (b), 13. (b), 14. (a), 15. (b), 16. (d), 17. (b), 18. (b), 19. (c), 20. (d), 21. (a), 22. (a), 23. (d), 24. (c), 25. (b), 26. (c), 27. (a), 28. (c), 29. (c), 30. (b)

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12.14 SUGGESTED READING

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12.15 TERMINAL QUESTIONS

1. What is Plant Tissue Culture? What are the various tools used for plant tissue culture?
2. Write in detail about plant growth regulator?
3. What is culture media? Discuss culture techniques in plant tissue culture.
4. What is embryo culture and meristem culture? Also write their application.
5. Write a short note on:
   (a) Totipotency
   (b) Anther or pollen culture
   (c) Tissue & Cell culture
   (d) Electroporation
   (e) Particle Bombardment (Biolistics)
   (f) Nod factor
6. Give the practical applications of plant tissue.
7. What is gene transfer? Discuss various methods for gene transfer in plants.
8. What are transgenic plant? Describe the benefits of transgenic plants.
9. Write about *Agrobacterium* mediated gene transfer.
10. Give a detailed account Gene bank? Also write of its various types.
12. What are Nif gene? Discuss its role in nitrogen fixation.
13. Describe the Regulation of Nitrogen Fixation. What are the examples of nif gene structure and regulation in different diazotrophs?
14. Write about the Nod gene? Discuss the process of nodule formation in root.
15. Give a detailed account on Mycoprotein and their characteristics?