

**M.Sc. Previous Year**

**Botany, MB-02**

**CYTOLOGY, GENETICS  
AND CYTOGENETICS**



**मध्यप्रदेश भोज (मुक्त) विश्वविद्यालय – भोपाल**

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## INTRODUCTION

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Cell biology is the study of cell structure including their physiology, structure, and life cycle that revolves around the concept that the cell is the fundamental unit of life. Some organisms have only one cell, while others are organized into cooperative groups with huge numbers of cells. Fundamentals of cell biology include the processes of mitosis and meiosis. Through these processes cells replicate genetic material in preparation for cell division, endowing future cells with instructions for life.

Plant breeding is the science of changing the traits of plants in order to produce desired characteristics. It can be accomplished through many different techniques ranging from simply selecting plants with desirable characteristics for propagation, to methods that make use of knowledge of genetics and chromosomes, to more complex molecular techniques. Genes in a plant determine what type of qualitative or quantitative traits it will have. Plant breeders strive to create a specific outcome of plants and potentially new plant varieties.

Plant breeding has been practiced for thousands of years, since near the beginning of human civilization. It is practiced worldwide by individuals, such as gardeners and farmers, and by professional plant breeders employed by organizations, such as government institutions, universities, crop-specific industry associations or research centers. International development agencies believe that breeding new crops is important for ensuring food security by developing new varieties that are higher yielding, disease resistant, drought tolerant or regionally adapted to different environments and growing conditions.

This book, *Cytology, Genetics and Cytogenetics*, is divided into four blocks that are further divided into fourteen units which will help to understand the basic concepts cells, structure of prokaryotic and eukaryotic cells, structure and function of various cell organelles (nucleus, endoplasmic reticulum, Golgi complex, mitochondria, chloroplast and lysosomes), nucleus and nuclear transport, functions and structure of cytoskeleton networks (microfilaments, intermediate filaments and microtubules), biological membrane, structure of lipid bilayer, transport of ions and molecules across the membranes. protein sorting in mitochondria, chloroplast, endoplasmic reticulum and nucleus, protein processing and trafficking from endoplasmic reticulum to Golgi, cell division and cell cycle, basic account on Mendelian genetics and gene interaction, linkage, crossing over, male sterility, polyploidy and its types and origin, basic account on mutation and population genetics, objectives of plant breeding, genetic variability and its role in plant breeding, breeding methods in pollinated, vegetatively propagated and apomictic plants. inbreeding depression theories, mutation breeding and breeding for disease resistance and stress tolerance.

## NOTES

*Introduction*

**NOTES**

The book follows the self-instruction mode or the SIM format wherein each unit begins with an 'Introduction' to the topic followed by an outline of the 'Objectives'. The content is presented in a simple and structured form interspersed with 'Check Your Progress' questions and answers for better understanding. A list of 'Key Words' along with a 'Summary' and a set of 'Self Assessment Questions and Exercises' is provided at the end of the each unit for effective recapitulation.

*Self - Learning  
Material*

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## BLOCK - I

### THE CELL

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*The Structure of  
Prokaryotic and  
Eukaryotic Cell*

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# UNIT 1 THE STRUCTURE OF PROKARYOTIC AND EUKARYOTIC CELL

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## NOTES

### Structure

- 1.0 Introduction
- 1.1 Objectives
- 1.2 Cells: An Introduction and History
- 1.3 Structure of Prokaryotic Cell
- 1.4 Structure of Eukaryotic Cell
- 1.5 Difference Between Prokaryotic and Eukaryotic Cell
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## 1.0 INTRODUCTION

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The cell is the basic structural, functional, and biological unit of all known living organisms. A cell is the smallest unit of life. Cells are often called the ‘building blocks of life’. The study of cells is called cell biology. Cells consist of cytoplasm enclosed within a membrane, which contains many biomolecules such as proteins and nucleic acids. Organisms can be classified as unicellular or multicellular. While the number of cells in plants and animals varies from species to species, humans contain more than 10 trillion cells. Most plant and animal cells are visible only under a microscope, with dimensions between 1 - 100 micrometers.

Cells were discovered by Robert Hooke in 1665, who named them for their resemblance to cells inhabited by Christian monks in a monastery. Cell theory, first developed in 1839 by Matthias Jakob Schleiden and Theodor Schwann, states that all organisms are composed of one or more cells, that cells are the fundamental unit of structure and function in all living organisms, and that all cells come from pre-existing cells. Cells emerged on Earth at least 3.5 billion years ago.

Prokaryotes include bacteria and archaea, two of the three domains of life. Prokaryotic cells were the first form of life on Earth, characterised by having

## NOTES

vital biological processes including cell signaling. They are simpler and smaller than eukaryotic cells, and lack membrane-bound organelles such as a nucleus. The DNA of a prokaryotic cell consists of a single chromosome that is in direct contact with the cytoplasm. The nuclear region in the cytoplasm is called the nucleoid. Most prokaryotes are the smallest of all organisms ranging from 0.5 to 2.0  $\mu\text{m}$  in diameter.

Plants, animals, fungi, slime moulds, protozoa, and algae are all eukaryotic. These cells are about fifteen times wider than a typical prokaryote and can be as much as a thousand times greater in volume. The main distinguishing feature of eukaryotes as compared to prokaryotes is compartmentalization: the presence of membrane-bound organelles in which specific activities take place. Most important among these is a cell nucleus, an organelle that houses the cell's DNA.

In this unit, you will learn about the structure of prokaryotic and Eukaryotic cell, distinguish between prokaryotic and eukaryotic cell, difference between animal and plant cell in detail.

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### 1.1 OBJECTIVES

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After going through this unit, you will be able to:

- Understand the structure of prokaryotic cell
- Explain the structure of eukaryotic cell
- Distinguish between prokaryotic and eukaryotic cell
- Explain the structure of plant cell
- Describe in detail the structure and anatomy of animal cell
- Discuss the difference between animal and plant cell

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### 1.2 CELLS: AN INTRODUCTION AND HISTORY

---

Living beings are composed of cell or many cells. A cell is a mass of cytoplasm enclosed by plasma membrane and contain certain organelles besides nucleus. It is a unit of biological entity covered by semipermeable membrane and capable of medium free of other living systems.

Cell is an open system as it allows the entry and exit of matter and energy. It takes up matter for sustenance, growth and division. Organisms are built according to information encoded in a collection of genes. This vast information is packaged into a set of chromosomes that occupies the space of cell nucleus. Genes are more than storage lockers for information, they constitute the blueprint for reconstructing cellular structure. The molecular structure of genes allows for changes in genetic information that lead to variation among individuals which forms the basis of evolution. Exchange of matter and energy between a cell and its surrounding

environment is dynamic. By being able to regulate the entry and exit of materials into and out of it and by chemical changes, cell attains homeostasis in which internal levels of materials remain constant. Such an open, steady state system is in contrast to the closed, equilibrium system of non-living world which don't allow the movement of material into and out of them. All of the energy required by life on the earth surface arrives in the form of electromagnetic radiation. The energy of light is trapped by light absorbing pigments present in membranes of photosynthetic cells.

Cells expend an enormous amount of energy by breaking down and rebuilding the macromolecules and organelles of which they made of. The first culture of human cell was begun by George Gey of Johns Hopkins University in 1951. The cells are obtained from a malignant tumour and named HeLa cells. Every cell is enclosed on all sides by a distinct covering called plasma membrane. It keeps the cell contents in place and prevents their mixing up with extracellular materials. This enables the cell to maintain its highly organized structure and carry on reactions in a regulated manner.

In 1809, Lamarck stated that nobody can have life if its constituent parts are not cellular tissue. A French biologist **H.J Dutrochet** stated in 1824 that all organic tissue are globular cells of small size which appear to be united by simple adhesive forces. Thus all tissues, animal and plant organs are only cellular tissue variously modified. In 1831, a Scotch botanist **Robert Brown** discovered the nucleus in orchid root cell. It was found that cells were surrounded by some sort of limiting structure, cell membrane. A French zoologist **Dujardin** discovered in 1835 a semifluid living material in certain protozoans. He called this material sarcode. In 1840, a similar material was noted in plant cells by biologist Purkinje. He proposed the term protoplasm. Theodore Schwann stated that all animals were formed of cells. Schwann found that animal cells had nuclei and were enclosed by thin cell membrane instead of thick cell wall. He defined the cell as a membrane bound nucleus containing structure. He proposed cell hypothesis stating that all plant and animal bodies are composed of cells. The cells of protists fungi, plants and animals have in their cytoplasm many membrane bound compartments called organelles.

The first three organelles are surrounded by double membrane known as envelope. The part of cytoplasm excluding compartments are eukaryotic cells. The cell of bacteria and blue green algae don't contain membrane bound compartment called single compartment cell called prokaryotic cell. The lack of compartments show primitive nature of these organisms.

### **Statements of Cell Theory**

It states that:

- All living things are composed of minute units, the cells.
- A cell is a mass of protoplasm containing a nucleus and bounded by cell membrane and by cell wall.

### **NOTES**

- All cells are basically alike in structure and metabolic activities.
- The function of an organism is the result of the activities and interaction of its constituent cells.

## NOTES

### **Shortcomings of the Cell Theory**

- Bacteria and blue green algae don't have organized nucleus. Their genetic material is not enclosed by nuclear envelope and lies in the cytoplasm.
- Protozoans are not cellular. They are acellular.
- Certain fungi, have hyphae composed of a multinucleate mass of cytoplasm without division into cells.
- Some tissue have a good deal of nonliving material, the matrix, between the cells.

### **Cell Properties**

#### **Organization**

Organism is the sum of its component cells, and its activities are the sum of the activities of its cell. New function emerge with each increasing level of organization. Just as molecules have different properties from its constituent atoms, tissue have different function from its cells, organs have different activities from its component tissue, and organism have different properties from its organ system.

#### **Cellular Totipotency**

Every cell contains a complete set of genes in its nucleus. If the nucleus of fertilized egg of frog is removed and replaced with the nucleus from skin cell, normal development starts and perfect frog is produced. This experiment shows that the nuclei of all cells are totipotent, i.e., they have a complete genetic information.

#### **Autonomous Existence**

Each cell take up food material as micromolecules and synthesizes living matter. Each cell itself disposes of its own worn out components with the help of lysosomes, each cell has its own life span, and each cell respire and draws oxygen from the environment and oxidizes food molecules to liberate energy which stores as ATP molecules.

#### **Differentiation, Dedifferentiation and Redifferentiation**

The cells at certain sites in the body of multicellular organism remain undifferentiated and retain the power of mitotic divisions to provide for growth. Such cells are known as undifferentiated cells. At certain times, the specialized cells revert to actively dividing state for producing new cells. This process is known as dedifferentiation. A stem cutting of a plant, say rose when fixed in the soil, sprouts new roots by dedifferentiation and grows into a new plant. The new cells produced by the dedifferentiation once again differentiate into the cells of various tissue which

make up into new parts in place of lost ones. This change in cells is called redifferentiation.

### **Various Chemical Reactions**

Cells function like miniature chemical plants. The simplest bacterial cell is capable of different chemical transformations, which occur at any rate in the inanimate world. All chemical changes that take place in cells require enzymes that increase the rate at which reaction occurs. The sum total of all the reaction in cell represent cell metabolism.

### **Response to Stimuli**

Cells within a multicellular plant or animal respond to stimuli. Most cells are covered with receptors that interact with substance in highly specific ways. Cell possess receptors to hormones, extracellular materials and to substance on surface of other cell. A cell receptors provide pathways through which external agents can evoke specific response in target cells. Cell respond to stimuli by changing their metabolic activities.

### **Cells Evolve**

It is presumed that cells evolved from some type of precellular life form, which in turn evolve from non-living organic material. If we observe the features of bacterial cell living in intestinal tract, cell that is part of lining of intestinal tract, we can see the difference between the two cells. Both are evolved from common ancestral cell that lived more than 3 billion years ago. These structures are shared by two distantly related cells, such as their similar plasma membrane and ribosomes are present in ancestral cells.

### **Self Regulation**

Cell regulatory mechanism becomes evident when they break down, for example, the failure of cell to correct mistake when its duplicate its DNA result in debilitating mutation, transform the cell into a cancer cell.

### **Highly Complex and Organized**

The more complex a structure, greater the number of parts that must be in proper place, less tolerance of errors in nature and interaction of parts, the more regulation that must be exerted to maintain the system.

Every cell is enclosed by a distinct covering called plasma membrane which keeps the cell contents properly and prevent their mixing up with extracellular materials which enables the cell to maintain its highly organized structure and to carry on reaction in regulated manner. The exchange of materials allowed by cell membrane is in a selective and regulated manner which keeps the cell contents distinct from surrounding materials. The cells of bacteria and blue green algae do not contain membrane bound compartments in the cytoplasm. Such single

## **NOTES**

## NOTES

compartment cells are called prokaryotic. The lack of compartments shows the primitive nature of organism. The main advantage of compartments is that to enable the cell to keep separate the various kinds of chemical reaction occurring in it all the time. Cell is organized mass of protoplasm which is capable of performing all life activities and is separated from environment by protective and selectively permeable envelope. The envelope is made up of cell wall in (plants) and plasma membrane in (all types of cell). Plasma membrane is part of protoplasm while cell wall is product of unit mass of protoplasm. The unit mass of protoplasm contained in cell is called protoplast. Protoplast is differentiated into four parts plasma membrane, cytoplasm, nucleus and vacuoles.

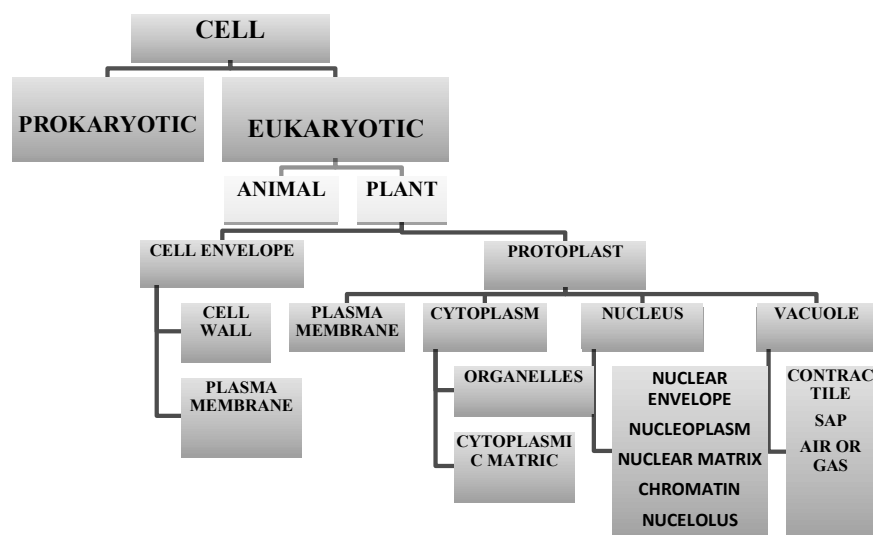


Fig. 1.1 Cell Classification

Two main type of cells present are prokaryotic and eukaryotic (Refer Figure 1.1). Cytoplasm is differentiated into cytoplasmic matrix, cell organelles and cell inclusions. Cytoplasmic matrix (hyaloplasma) is the fluid part of cytoplasm which exists in both sol and gel respectively called plasmasol and plasma gel. Water constitutes 90% of the matrix. Matrix is the crystallo-colloidal complex where some chemicals are present as true solution and others as colloidal solution, for example, minerals, sugars, amino acids, vitamins, proteins, enzymes, etc.

The autonomic vital movement that occurs in the cytoplasmic matrix also called as protoplasmic streaming or cyclosis. Reason for cyclosis is either sol-gel changes or movement by microfilaments through their molecular motors. The matrix is the seat of synthesis of no of biochemicals like fats, proteins, carbohydrates, coenzymes. Cell organelles exchange materials through cytoplasmic matrix. It helps in distribution of materials inside the cell.

### Characteristics that Distinguish Prokaryotic and Eukaryotic Cell

The eukaryotic cell certainly evolved from prokaryotic ancestors. Because of their common ancestry, both type of cell share an identical genetic language, similar set



of metabolic pathways and many common structural features. Both type of cells are bounded by plasma membrane that serve as selectively permeable barrier between the living and non-living world. Both type of cell is surrounded by a rigid, non-living cell wall that protects the delicate life form. The genetic material of a prokaryotic cell is present in a nucleoid. In contrast, eukaryotic cell possess a nucleus, region bounded by a complex membranous structure called nuclear envelope. The chromosomal DNA of eukaryotes unlike prokaryotes is tightly associated with proteins to form complex nucleoprotein material called chromatin. Eukaryotic cell contain array of membrane bound organelles. It includes mitochondria, where chemical energy is made available to fuel cellular activities, an endoplasmic reticulum where many of cell proteins and lipids are manufactured. Golgi complex where materials are sorted, modified and transported to specific cellular destination. Plant cell contain additional membranous organelles, i.e., chloroplast which is site of photosynthesis and large vacuole that occupy most of the cell volume. Both the eukaryotic and prokaryotic cells possess ribosomes which are non-membranous particles on which proteins of cell are manufactured. However one major difference between both type of cells are eukaryotic cell divide by complex process of mitosis in which duplicated chromosomes condense into compact structures that are segregated by microtubule containing apparatus called mitotic spindle which allows each daughter cell to receive an equivalent array of genetic material. In prokaryotes, there is no compaction of the chromosome and no spindle. The DNA is duplicated, two copies are separated by the growth of intervening cell membrane. The movement of prokaryotic cell may be accomplished by a thin protein filament called flagellum which protrudes from the cell and rotates.

## NOTES

### **A Comparison of Prokaryotic and Eukaryotic Cell**

Features held in common by the two types of cells

- Plasma membrane of similar construction.
- Genetic information encoded in DNA using identical genetic code.
- Same mechanism of photosynthesis ( between cyanobacteria and green plants).
- Same mechanism for synthesizing and inserting membrane proteins.
- Shared metabolic pathways ( for example, glycolysis and TCA cycle).
- Same mechanism for transcription and translation of genetic information including ribosomes.
- Same apparatus for conservation of chemical energy as ATP (located in plasma membrane of prokaryotes and mitochondrial membrane of eukaryotes).
- Protein digesting structure (Proteasomes) of similar construction.

## NOTES

### Features of Eukaryotic Cells not found in Prokaryotes

- Complex membranous cytoplasmic organelles (endoplasmic reticulum, Golgi complex, lysosomes, peroxisomes).
- Complex cytoskeletal system (microfilaments, intermediate filaments, microtubules) and associated motor proteins.
- Ability to ingest fluid and particulate matter by enclosure within plasma membrane vesicles.
- Cellulose containing cell wall.
- Cell division using a microtubule containing spindle that separates chromosomes.
- Presence of two copies of genes per cell.
- Presence of three different RNA synthesizing enzymes (RNA polymerases).
- Sexual reproduction requiring meiosis and fertilization.

#### Check Your Progress

1. What is a cell?
2. Why is a cell called as an open system?
3. What does molecular structure of genes do?
4. Give the organization of cell.
5. Why is cell regulation important?

### 1.3 STRUCTURE OF PROKARYOTIC CELL

A prokaryotic cell is a single envelope system. There is no membrane enveloping the genetic material. It is present in bacteria and blue green algae. Some organisms like mycoplasma, rickettsias and spirochetes are similar to bacteria and are prokaryotes (Refer Figure 1.2). The prokaryotes comes under kingdom Monera and Super kingdom *Prokaryota*. Prokaryotes are divided into two groups: the *Archaea* (Archaeobacteria) and the Bacteria (Eubacteria). Members of the *Archaea* are closely related to eukaryotes than they are to the other group of prokaryotes. *Archaea* include group of organism whose evolutionary ties to one another are revealed by similarities in the nucleotide sequence of their nucleic acid. These species live in extremely inhospitable environment known as extremophiles. It includes methanogens, halophiles, acidophiles and thermophiles, included in the latter group are hyperthermophiles which live in hydrothermal vents of ocean floor. The latest among this group is strain 121, because it is able to grow and divide in superheated water at temperature of 121 degree Celsius. All other prokaryotes comes under domain bacteria. This includes smallest known cell called mycoplasma, only prokaryotes lacking cell wall. The most complex prokaryotes are cyanobacteria, which contain errays of membranes serve as site of photosynthesis.

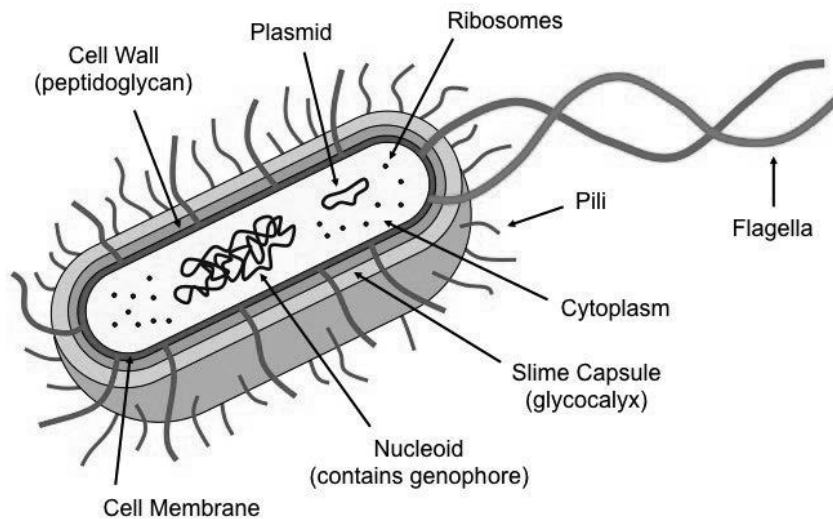


Fig 1.2 Prokaryotic Cell Structure under Electron Microscope

## NOTES

### Organization of prokaryotic cell

Prokaryotic cell mainly contains 3 components:

- Outer covering or cell membrane
- Cytoplasm
- Nucleoid

**Outer Covering:** It comprises three layers: inner plasma membrane, middle cell wall and outer slimy capsule.

- **Cell Membrane:** It is thin and flexible, composed of lipids and proteins. It controls the movement of molecules into and from the cell. It carries respiratory enzymes for energy reactions. In bacteria, small infolds of plasma membrane, i.e., mesosomes bear respiratory enzymes and is analogous to mitochondria of eukaryotic cell. In photosynthetic bacteria and in blue green algae, pigment and enzyme molecules that absorb and convert light into chemical energy are associated with plasma membrane and its infolds called photosynthetic lamellae. Photosynthetic lamellae are analogous to chloroplasts of eukaryotic cells. Plasma membrane of prokaryotic cell serve many function which are located in separate membrane bound organelles in eukaryotic cells. Plasma membrane is thought to play role in replication and division of nuclear material. The plasma membrane serve many function which are located in membrane bound organelles in eukaryotic cell. The infolds remains continuous with cell membrane. The prokaryotic cell is non compartmentalized.
- **Cell Wall:** The outer covering is surrounded by non-living, rigid cell wall. In gram positive bacteria, cell wall consists of thick layers. It consists of peptidoglycan and teichoic acid. Peptidoglycan consists of complex of

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oligosaccharide chains and short peptides called murein. The oligosaccharide is made of sugar derivatives acetylglucosamine and acetylmuramic acid joined together by  $\alpha$  1, 4 linkages. Teichoic acid consists of glycerol or ribitol subunits linked by phosphate groups. In gram negative bacteria, the cell wall consists of peptidoglycan, periplasmic space and outer membrane, the peptidoglycan layer lacks teichoic acid. The periplasmic space consists of proteins secreted by cell. The outer membrane consists of lipopolysaccharides. Blue green algae have cell wall structure similar to gram negative bacteria but still they are Gram positive in Gram negative bacteria, i.e., *E. coli*. The peptidoglycan layer is thin about 25Å lacks teichoic acid and lies next to the plasma membrane. The periplasmic space lies outside the peptidoglycan layer and contain proteins secreted by cell. The outer membrane consists of a lipid bilayer and lipopolysaccharides. It is permeable and contain porin proteins which line channels large enough to let chemicals pass.

- **Capsule:** It protects the cell against desiccation and virus attacks. It is present outside the cell wall in form of gelatinous coat.

**Cytoplasm:** It contains water, proteins, lipids, inorganic ions. It contains enzymes for various biosynthetic reactions and protein forming machinery containing ribosomes, all types of RNA. Ribosomes are the only organelle found in prokaryotic cell. They lie free in cytoplasm and form polyribosome at time of protein synthesis. Cytoplasm do not show streaming movements. It do not show cyclosis, phagocytosis, pinocytosis and exocytosis. They contain gas vacuoles in cytoplasm. They lack well developed organelles except ribosomes (which is 70 S type).

**Nucleoid (Genophore):** It consists of single chromosome that is coiled, double stranded, circular not associated with histones. Transcription and translation occur in the cytoplasm. There is no nucleolus. It consists of single chromosome which is coiled forming body. The chromosome is short, simple and attached to membrane. There is a single copy of chromosome. The DNA is double stranded and helical but circular and associated with proteins, replication of DNA is continuous throughout the cell cycle. Transcription and translation occur in the cytoplasm. Mitotic apparatus is not formed during cell division, there is no nucleolus.

**Plasmids:** They are small circular genetic material in addition to nucleiod. They generally code for proteins required by organism in resistance against antibiotics and toxic materials. They are not essential for the growth of the cell. Some encode proteins needed by organism to resist antibiotics.

**Pili:** Short, rod like non motile structure made up of protein pilin. They are used for attachment to surfaces. Many bacteria in addition to flagella is short, rod like non motile processes called pili or fimbriae on the surface, formed of protein pilin. Pili is used for attachment to surfaces, some bacteria bear tubular sex pili.

**Flagella:** Many bacteria bear whip like locomotory organelle called flagella. It is locomotory organelle present in prokaryotes composed of spiral chains of subunits of protein named flagellin which is unrelated to actin or tubulin. A bacterial flagellum

is about 150Å thick and 10-15µm long. It has two main parts- filament and basal body.

**Filament:** It has the form of a fixed, spiral resembling a corkscrew. It is composed of intertwined spiral chains of subunits of protein named flagellin, it is unrelated to actin or tubulin.

**Basal Body:** It comprised of two regions: shaft and hook. The shaft bears a pair of rings in gram positive bacteria and two pairs of rings in gram negative bacteria. The inner ring is embedded in the plasma membrane and fixed to the shaft. The outer ring is attached to the cell wall but is free to slip on the shaft surface called S ring. The bacterial flagellum doesn't have any bounding membrane and grows at the tip. This is in contrast to the eukaryotic flagellum which is bounded by a cell membrane and grows at the base adjacent to the basal body.

## NOTES

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### 1.4 STRUCTURE OF EUKARYOTIC CELL

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#### Organisation

A eukaryotic cell is two envelope system. First membrane surrounds the cell while second envelope the nucleus and other organelles. It occurs in protists, fungi, plants and animals. The most complex cells are not found inside plants and animals but in single celled protists. Complex unicellular organism represent one evolutionary pathway. An alternate pathway leads to evolution of multicellular organism in which different activities are carried out by different specialized cells, which is formed by the process of differentiation. The pathway of differentiation followed by each embryonic cell depends upon the signals it receives from surrounding environment. Most eukaryotic cell possess single nucleus that contains only two copies of most genes (Refer Figure 1.3).

#### Size

Human cells range from 20 to 30µm. Nerve cells are the longest in size. Among the smallest are 2µm long *Plasmodium vivax*. The size of the cell is directly related to its function. The surface area of cell tells the capacity to exchange material with the environment. Cells and their organelles are defined in micrometers. There are many reasons for cell being small in size. The greater a cells cytoplasmic volume, longer it takes to synthesize the messages required by the cell. As cell increases in size, surface area / volume decreases. The ability of a cell to exchange substance with its environment is proportional to its surface area. If cell grows beyond a certain size, its surface would not be sufficient to take up the substance.

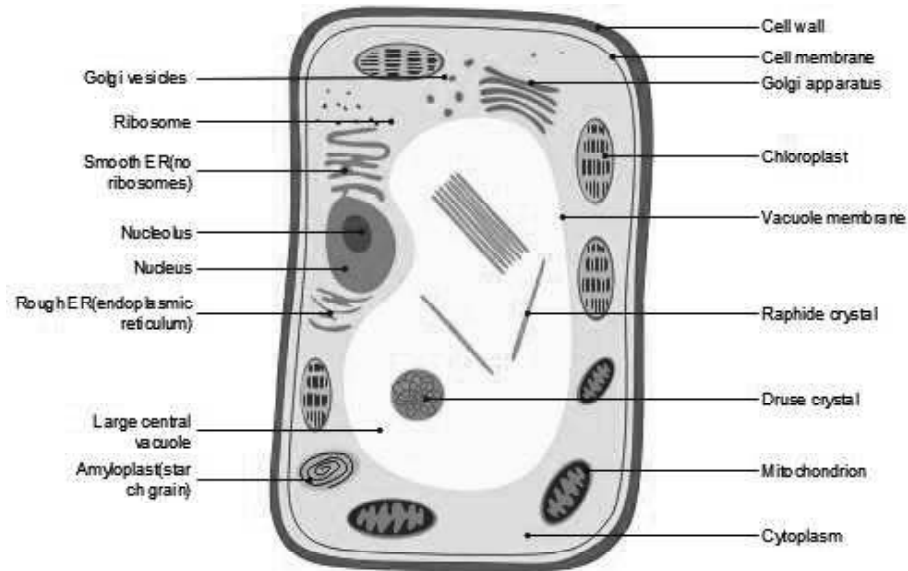
#### Shape

It may be spherical, polygonal, disc like, oval, spindle like, irregular. The shape is related to its function. Muscle and nerve cell are adapted to their function. Form of cell depends on its function, pressure, age and viscosity.

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### Number

Majority of organisms have numerous cells so are called multicellular organism while organism made up of single cell is unicellular. Though all multicellular organism begin life as single cell, i.e., zygote.



**Fig 1.3** Ultrastructure of a Eukaryotic Cell with Central Vacuole Cell Wall (Plant Cell)

### Physical Structure

It is composed of three layers:

- Cell membrane
- Cytoplasm
- Nucleus

### Cell Membrane

All eukaryotic cells are surrounded by elastic living covering called cell membrane. It is composed of lipid protein complex and lack respiratory enzymes. Certain protists, fungi and plant cells have rigid non-living covering called cell wall which protects and supports the cell.

### Cytoplasm

The part of cell between nucleus and plasma membrane is called cytoplasm. It consists of semifluid, translucent ground substance cytosol. Cytoplasm shows streaming movements and contain two structures, i.e., organelles and inclusions. Each organelle has its own function which is regulated by the control centre of the cell called nucleus.

Inclusions are non-living or deutoplasmic structures that includes stored organic materials (starch grains, aleurone layers, fat droplets and glycogen granules, pigment granules and inorganic crystals.

**Nucleus:** The genetic material is enclosed by distinct envelope. It is double stranded, helical and linear complexed with histones and non-histones. Replication of DNA occurs during S phase of cell cycle. Transcription occurs in nucleus and translation in cytoplasm. One or more rounded bodies called nucleoli is present in the nucleus. Nucleus controls all the activities of the cell and cell devoid of it dies.

**Plasmids:** There are no plasmids present in eukaryotic cells. Cilia and flagella have 9+2 plan of microtubules, composed of proteins tubulin. The cilia and flagella move the fluid around them and act as locomotory organelle.

## NOTES

### Check Your Progress

6. Who coined the term cell membrane?
7. What is the difference between protoplasm and protoplast?
8. What is a prokaryotic cell?
9. What does Archaea include?
10. What is photosynthetic lamellae?

## 1.5 DIFFERENCE BETWEEN PROKARYOTIC AND EUKARYOTIC CELL

Prokaryotic Cells	Eukaryotic Cells
• DNA is naked and circular, not associated with histones	• DNA is linear and associated with histone proteins to form nucleosomes
• Ribosomes are 70 S type	• Ribosomes are 80 S and 70 S in organelles
• Single envelope organization is present	• Two envelope organization is present
• An organized nucleus is absent, instead nucleoid is present	• An organized nucleus is present
• A spindle apparatus is not formed	• A spindle apparatus is generally present
• There is no change in the amount of DNA throughout the life cycle	• There is regular change in amount of DNA in the life cycle
• Transcription and Translation occur in cytoplasm	• Transcription occurs inside nucleus and translation in cytoplasm

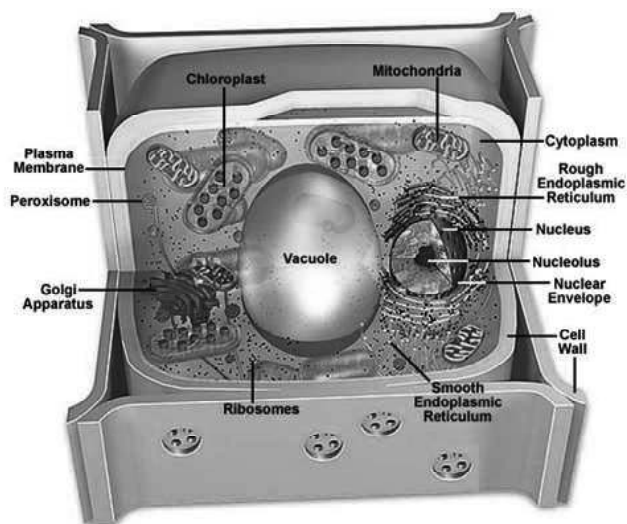
## NOTES

• Protein present in flagella is flagellin	• Flagella has tubulin protein
• Pilli and plasmids are found in organisms which help in conjugation	• Both pili and plasmids are absent in eukaryotes
• Flagella if present , are single stranded	• Flagella if present are 11 stranded
• Sap vacuoles are absent, Gas vacuoles may be present	• Sap vacuoles are present. Gas vacuoles are absent
• Examples: Bacteria, Blue green algae, mycoplasma	• Examples: Algae (Except Blue green algae), Fungi, plants and animals

## 1.6 PLANT AND ANIMAL CELL: STRUCTURE, ANATOMY AND DIFFERENCE

### 1.6.1 Plant Cell

Plant cells are the basic unit of life in organisms of the kingdom Plantae. They are eukaryotic cells, which have a true nucleus along with specialized structures called organelles that carry out different functions. Animals, fungi, and protists also have eukaryotic cells, while bacteria and archaea have simpler prokaryotic cells. Plant cells are differentiated from the cells of other organisms by their cell walls, chloroplasts, and central vacuole. Plant cells are eukaryotic cells that vary in several fundamental factors from other eukaryotic organisms. Both plant and animal cells contain nucleus along with similar organelles. One of the distinctive aspects of a plant cell is the presence of a cell wall outside the cell membrane. They are rectangular and comparatively larger than animal cells.



**Fig. 1.4** Anatomy of Plant Cell



## **Anatomy of Plant Cell**

Just like an organ in an animal, plant cells have various components known as cell organelles that perform various tasks and function to sustain itself (Refer Figure 1.4). These organelles include:

### **Cell Wall**

It is a rigid layer which is composed of cellulose, glycoproteins, lignin, pectin, and hemicellulose found on the outside of the plant cell that gives it strength and also maintains high turgidity. It is located outside the cell membrane. It comprises proteins, polysaccharides, and cellulose. The primary function of the cell wall is to protect and provide structural support to the cell. The plant cell wall is also involved in protecting the cell against mechanical stress and to provide form and structure to the cell. It also filters the molecules passing into and outside the cell. The composition of the plant cell wall differentiates it from the cell walls of other organisms. For example, fungi cell walls contain chitin, and bacterial cell walls contain peptidoglycan, and these substances are not found in plants. A main difference between plant and animal cells is that plant cells have a cell wall while animal cells do not. Plant cells have a primary cell wall, which is a flexible layer formed on the outside of a growing plant cell, and a secondary cell wall, a tough, thick layer formed inside the primary plant cell wall when the cell is mature.

The formation of the cell wall is guided by microtubules. It consists of three layers, namely, primary, secondary and the middle lamella. The primary cell wall is formed by cellulose laid down by enzymes.

### **Cell Membrane**

It is the semi-permeable membrane that is present within the cell wall. It is composed of a thin layer of protein and fat. The cell membrane plays an important role in regulating the entry and exit of specific substances within the cell. For instance, cell membrane keeps toxins from entering inside, while nutrients and essential minerals are transported across.

### **Nucleus**

The nucleus is a highly specialized, membrane-bound structure that is present only in eukaryotic cells. It serves as the information processing and administrative center of the cell. This organelle has two major functions: it stores the cell's hereditary material, or DNA, and it coordinates the cell's activities, which include growth, intermediary metabolism, protein synthesis, and reproduction (cell division).

- **Nucleolus:** It manufactures cell's protein-producing structures and ribosomes.
- **Nucleopore:** Nuclear membrane is perforated with holes called nucleopore that allows proteins and nucleic acids.

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### **Plastids**

They are membrane-bound organelles that have their own DNA. They are necessary to store starch, to carry out the process of photosynthesis. It is also used in the synthesis of many molecules which form the cellular building blocks.

### **Leucoplasts**

They are found in non-photosynthetic tissues of plants. They are used for the storage of protein, lipid, and starch.

### **Chloroplasts**

Chloroplasts are found only in plant and algae cells. These organelles carry out the process of photosynthesis, which turns water, carbon dioxide, and light energy into nutrients. They are oval-shaped and have two membranes: an outer membrane, which forms the external surface of the chloroplast, and an inner membrane that lies just beneath. Between the outer and inner membrane is a thin intermembrane space about 10-20 nanometers wide. Within the other membrane, there is another space called the stroma, which is where chloroplasts are contained.

Chloroplasts themselves contain many flattened disks called thylakoids, and these have a high concentration of chlorophyll and carotenoids, which capture light energy. The molecule chlorophyll also gives plants their green colour. Thylakoids are stacked on top of one another in vascular plants in stacks called grana.

### **Chromoplasts**

They are heterogeneous, coloured plastids organelle which is responsible for pigment synthesis and for storage in photosynthetic eukaryotic organisms. Chromoplasts have red, orange and yellow coloured pigments which provide colour to all ripen fruits and flowers.

### **Central Vacuole**

It occupies around thirty per cent of the cell's volume in a mature plant cell. Tonoplast is a membrane that surrounds central vacuole. The vital function of central vacuole apart from storage is to sustain turgid pressure against the cell wall. The central vacuole consists of cell sap. It is a mixture of salts, enzymes, and other substances.

### **Vacuoles**

Plant cells are unique in that they have a large central vacuole. A vacuole is a small sphere of membrane within the cell that can contain fluid, ions, and other molecules. Vacuoles are basically large vesicles. They can be found in the cells of many different organisms, but plant cells characteristically have a large vacuole that can take up anywhere from 30-80 percent of the cell.

The central vacuole of a plant cell helps maintain its turgor pressure, which is the pressure of the contents of the cell pushing against the cell wall. A plant thrives best when its cells have high turgidity, and this occurs when the central

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vacuole is full of water. If turgor pressure in the plants decreases, the plants begin to wilt. Plant cells fare best in hypotonic solutions, where there is more water in the environment than in the cell; under these conditions, water rushes into the cell by osmosis, and turgidity is high. Animal cells, on the other hand, can lyse if too much water rushes in; they fare better in isotonic solutions, where the concentration of solutes in the cell and in the environment is equal and net movement of water in and out of the cell is the same.

### **Endoplasmic Reticulum**

The endoplasmic reticulum is a network of sacs that manufactures, processes, and transports chemical compounds for use inside and outside of the cell. It is connected to the double-layered nuclear envelope, providing a pipeline between the nucleus and the cytoplasm. In plants, the endoplasmic reticulum also connects between cells via the plasmodesmata.

### **Microfilaments**

Microfilaments are solid rods made of globular proteins called actin. These filaments are primarily structural in function and are an important component of the cytoskeleton.

### **Microtubules**

These straight, hollow cylinders are found throughout the cytoplasm of all eukaryotic cells (prokaryotes don't have them) and carry out a variety of functions, ranging from transport to structural support.

### **Golgi Apparatus**

The Golgi apparatus is the distribution and shipping department for the cell's chemical products. It modifies proteins and fats built in the endoplasmic reticulum and prepares them for export as outside of the cell.

### **Peroxisomes**

Microbodies are a diverse group of organelles that are found in the cytoplasm, roughly spherical and bound by a single membrane. There are several types of microbodies but peroxisomes are the most common.

### **Plasmodesmata**

Plasmodesmata are small tubes that connect plant cells to each other, providing living bridges between cells.

### **Plasma Membrane**

All living cells have a plasma membrane that encloses their contents. In prokaryotes and plants, the membrane is the inner layer of protection surrounded by a rigid cell wall. These membranes also regulate the passage of molecules in and out of the cells.

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### **Ribosomes**

They are the smallest membrane-bound organelle which comprises RNA and protein. They are the sites for protein synthesis, hence they are also referred to as the protein factories of the cell. All living cells contain ribosomes, tiny organelles composed of approximately 60 percent RNA and 40 percent protein. In eukaryotes, ribosomes are made of four strands of RNA. In prokaryotes, they consist of three strands of RNA.

### **Other Organelles**

Plant cells have many other organelles that are essentially the same as organelles in other types of eukaryotic cells, such as animal cells. The nucleus contains a cell's deoxyribonucleic acid (DNA), its genetic material. DNA contains instructions for making proteins, which controls all of the body's activities. The nucleus also regulates the growth and division of the cell. Proteins are synthesized in ribosomes, modified in the endoplasmic reticulum, and folded, sorted, and packaged into vesicles in the Golgi apparatus.

Cytosol is the liquid contained within cells. It is mostly made of water, and also contains ions like potassium, proteins, and small molecules. Cytosol and all the organelles within it, except for the nucleus, are called the cytoplasm. The cytoskeleton is a network of filaments and tubules found throughout the cytoplasm of the cell. It has many functions; it gives the cell shape, provides strength, stabilizes tissues, anchors organelles within the cell, and has a role in cell signaling. The cell membrane, a double phospholipid layer, surrounds the entire cell.

### **Mitochondria**

Mitochondria are also found in plant cells. They produce ATP through cellular respiration. They are the double-membraned organelles found in the cytoplasm of all eukaryotic cells. They provide energy by breaking down carbohydrate and sugar molecules, hence they are also referred to as the 'Powerhouse of the cell'. Photosynthesis in the chloroplasts provides the nutrients that mitochondria break down for use in cellular respiration. Interestingly, both chloroplasts and mitochondria are thought to have formed from bacteria being engulfed by other cells in an endosymbiotic (mutually beneficial) relationship, and they did so independently of each other.

### **Lysosome**

Lysosomes are called as suicidal bags as they hold digestive enzymes in an enclosed membrane. They perform the function of cellular waste disposal by digesting worn-out organelles, food particles and foreign bodies in the cell.

### **Functions of Plant Cells**

Plant cells are the basic building block of plant life, and they carry out all of the functions necessary for survival. Photosynthesis, the making of food from light

energy, carbon dioxide, and water, occurs in the chloroplasts of the cell. The energy molecule adenosine triphosphate (ATP) is produced through cellular respiration in the mitochondria. There are five types of plant cells, each with different functions:

- Parenchyma cells are the majority of cells in a plant. They are found in leaves and carry out photosynthesis and cellular respiration, along with other metabolic processes. They also store substances like starches and proteins and have a role in plant wound repair.
- Collenchyma cells provide support to growing parts of a plant. They are elongated, have thick cell walls, and can grow and change shape as a plant grows.
- Sclerenchyma cells are hard cells that are the main supporting cells in the areas of a plant that have ceased growing. Sclerenchyma cells are dead and have very thick cell walls.
- Xylem cells transport mostly water and a few nutrients throughout a plant, from the roots to the stem and leaves.
- Phloem cells transport nutrients made during photosynthesis to all parts of a plant. They transport sap, which is a watery solution high in sugars.

### **Leaf Tissue Organization**

The plant body is divided into several organs: roots, stems, and leaves. The leaves are the primary photosynthetic organs of plants, serving as key sites where energy from light is converted into chemical energy. Similar to the other organs of a plant, a leaf is comprised of three basic tissue systems, including the dermal, vascular, and ground tissue systems. These three motifs are continuous throughout an entire plant, but their properties vary significantly based upon the organ type in which they are located. All three tissue systems are discussed in this section.

### **1.6.2 Animal Cell**

An animal cell is any cell found in an organism from the kingdom Animalia. Animal cells may be different sizes and shapes and may carry out a wide range of actions which tend to be specialized depending on the type of animal cell. It differs from plant or fungi cells. Like plant and fungi cells, an animal cell is eukaryotic, but animal cells lack the cell wall structure found in plant and fungi cell types. Animal cells also do not contain chloroplasts as plant cells do, as animal cells are heterotrophic and do not perform photosynthesis. Animal cells are surrounded by a cell membrane and contain organelles which perform various functions required to keep the cell alive and operating normally.

Animal cells are typical of the eukaryotic cell, enclosed by a plasma membrane and containing a membrane-bound nucleus and organelles. Unlike the eukaryotic cells of plants and fungi, animal cells do not have a cell wall. This

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feature was lost in the distant past by the single-celled organisms that gave rise to the kingdom Animalia. Most cells, both animal and plant, range in size between 1 and 100 micrometers and are thus visible only with the aid of a microscope.

The lack of a rigid cell wall allowed animals to develop a greater diversity of cell types, tissues, and organs. Specialized cells that formed nerves and muscles—tissues impossible for plants to evolve—gave these organisms mobility. The ability to move about by the use of specialized muscle tissues is a hallmark of the animal world, though a few animals, primarily sponges, do not possess differentiated tissues. Notably, protozoans locomote, but it is only via non-muscular means, in effect, using cilia, flagella, and pseudopodia.

The animal kingdom is unique among eukaryotic organisms because most animal tissues are bound together in an extracellular matrix by a triple helix of protein known as collagen. Plant and fungal cells are bound together in tissues or aggregations by other molecules, such as pectin. The fact that no other organisms utilize collagen in this manner is one of the indications that all animals arose from a common unicellular ancestor. Bones, shells, spicules, and other hardened structures are formed when the collagen-containing extracellular matrix between animal cells becomes calcified.

Animals are a large and incredibly diverse group of organisms. Making up about three-quarters of the species on Earth, they run the gamut from corals and jellyfish to ants, whales, elephants, and, of course, humans. Being mobile has given animals, which are capable of sensing and responding to their environment, the flexibility to adopt many different modes of feeding, defense, and reproduction. Unlike plants, however, animals are unable to manufacture their own food, and therefore, are always directly or indirectly dependent on plant life.

Most animal cells are diploid, meaning that their chromosomes exist in homologous pairs. Different chromosomal ploidies are also, however, known to occasionally occur. The proliferation of animal cells occurs in a variety of ways. In instances of sexual reproduction, the cellular process of meiosis is first necessary so that haploid daughter cells, or gametes, can be produced. Two haploid cells then fuse to form a diploid zygote, which develops into a new organism as its cells divide and multiply.

The earliest fossil evidence of animals dates from the Vendian Period (650 to 544 million years ago), with coelenterate-type creatures that left traces of their soft bodies in shallow-water sediments. The first mass extinction ended that period, but during the Cambrian Period which followed, an explosion of new forms began the evolutionary radiation that produced most of the major groups, or phyla, known today. Vertebrates (animals with backbones) are not known to have occurred until the early Ordovician Period (505 to 438 million years ago) (Refer Figure 1.5).

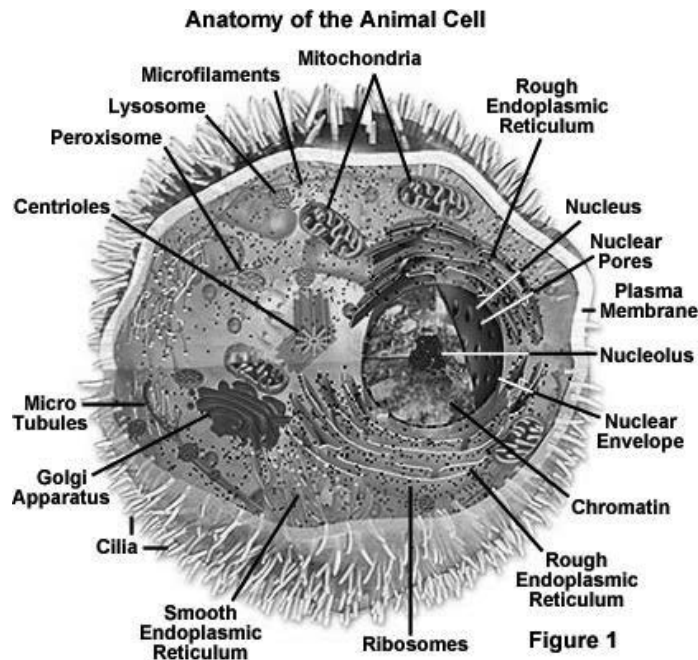


Fig. 1.5 Anatomy of Animal Cell

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### Parts of an Animal Cell

Depending on the type of the animal cell in question, some cellular components listed below may not be found in every animal cell. However, the components listed below are typical components found in most animal cells. There are 12 main components of an animal cell:

### Cell Membrane

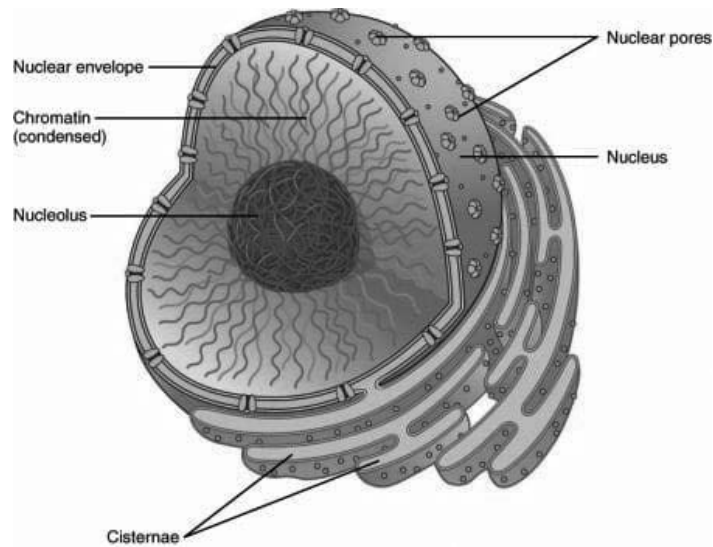
The cell membrane is the outer edge of the cell and forms the boundary between the inside of the cell with all of its organelles and the extracellular matrix. The cell membrane is composed of a lipid bilayer, which forms spontaneously in an aqueous environment as the hydrophobic tails of the lipids press together while the hydrophilic head groups of the lipids form a protective boundary to keep water out of the centre of the membrane.

Embedded within the cell membrane are all sorts of macromolecules such as glycoproteins, which act as recognition sites or aid in stability, and channel proteins, which allow certain materials in and out of the cell. The cell membrane is semi-permeable, which means that only certain molecules are allowed to pass through the membrane easily. Other molecules must use the channels in the membrane to gain access to the cell. The selective permeability of the cell membrane allows the cell to regulate itself and maintain homeostasis.

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### Nucleus

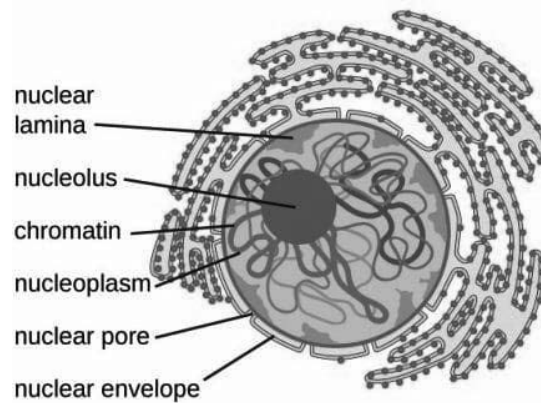
The nucleus has two main functions: it contains all of the deoxyribonucleic acid (DNA) of the cell, and it directs the activities of the cell. The DNA molecules found in each cell are the blueprints for proteins, which perform extensive and varied functions within living organisms. In order for the long strands of DNA to fit within the nucleus of the cell, the DNA molecules are wound around histones (a type of protein) to form chromosomes. The primary activities of the cell that are controlled by the nucleus are growth, division, and protein synthesis (Refer Figure 1.6).



*Fig. 1.6 Nucleus*

### The Nucleolus

The nucleolus is a small area within the nucleus where ribosomes are made. (Refer Figure 1.7)



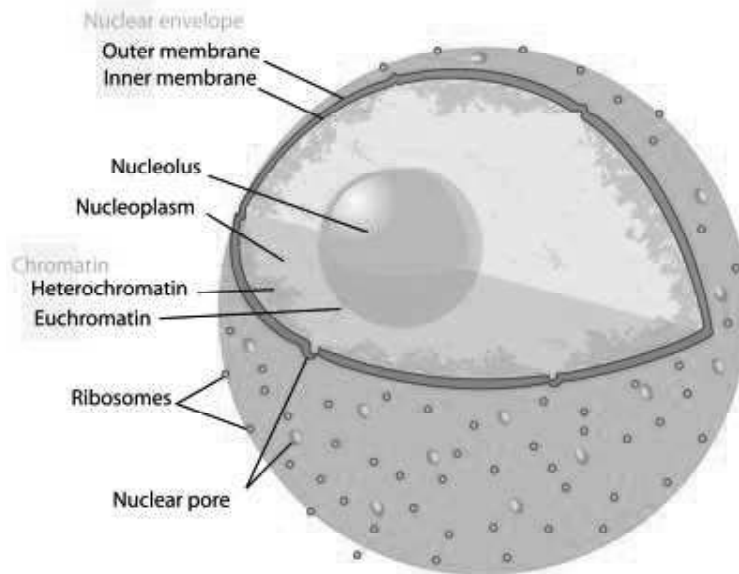
*Fig. 1.7 Nucleolus*



## Nuclear Membrane

The nuclear membrane is similar to the cell membrane, except that it surrounds the nucleus within the cell, and performs less of a regulatory function. The nuclear membrane is porous and allows RNA and proteins to pass in and out of the nucleus. The nuclear membrane is an important feature of eukaryotic cells; eukaryotic cells contain a true nucleus, and the nuclear membrane is the structure that defines the boundaries of the nucleus (Refer Figure 1.8).

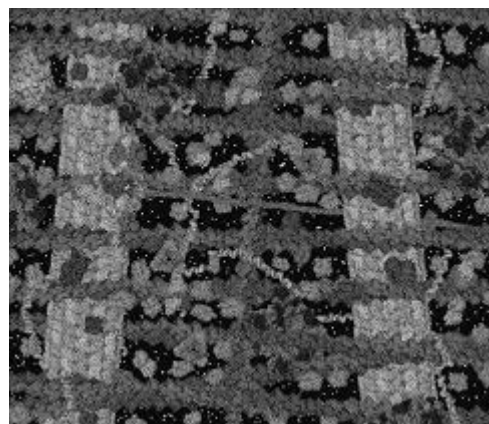
## NOTES



*Fig. 1.8 Nuclear Membrane*

## Cytoplasm/Cytosol

The cytosol is a thick, gel-like fluid that fills the space inside of a cell, and in which the organelles are suspended. The name of the total contents of the cell, minus the nucleus, is the cytoplasm, the cytosol plus the suspended organelles (Refer Figure 1.9).



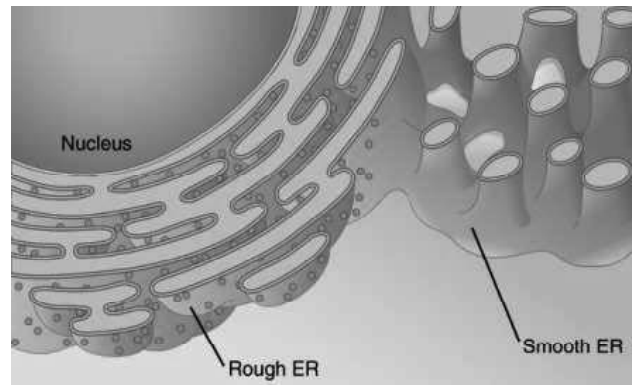
*Fig. 1.9 Cytoplasm/Cytosol*

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### Endoplasmic Reticulum

Endoplasmic reticulum is composed of interconnected membranous channels called cisternae and is connected to the nuclear membrane. The endoplasmic reticulum functions in transportation and modification of molecules.

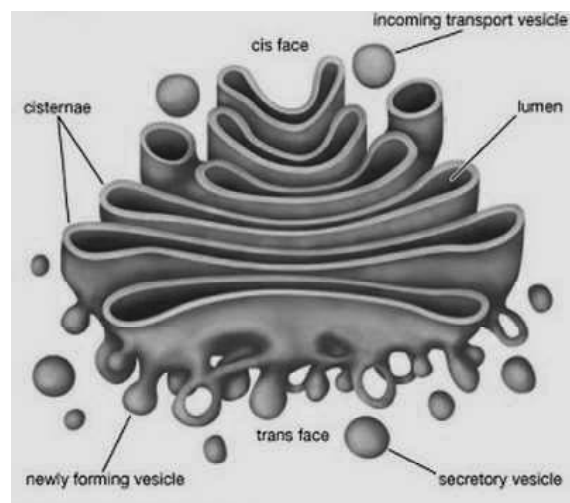
Endoplasmic reticulum may be rough or smooth; rough endoplasmic reticulum has ribosomes bound to its surface, and smooth endoplasmic reticulum does not. The rough endoplasmic reticulum modifies and transports the proteins made by the attached ribosomes for use or further modification. The smooth endoplasmic reticulum modifies lipids and steroids (Refer Figure 1.10).



*Fig. 1.10 Endoplasmic Reticulum*

### Golgi Apparatus

The Golgi apparatus is also made of cisternae that are not interconnected. The Golgi functions in packaging and shipping. It takes molecules produced by the cell, such as proteins and lipids, modifies them if necessary (such as folding for proteins), and packs them into vesicles so that they can be shipped around or outside of the cell (Refer Figure 1.11).



*Fig. 1.11 Golgi Apparatus*

## Ribosomes

Ribosomes are organelles made of ribonucleic acid (RNA) and protein and are either attached to the endoplasmic reticulum or suspended in the cytosol. Ribosomes facilitate protein synthesis.

## Mitochondria

Mitochondria are large organelles that have both an inner and outer membrane, as well as their own mitochondrial DNA. Mitochondria are the site of cellular respiration in cells, where oxygen and glucose are converted into adenosine triphosphate (ATP), which cells use for energy (Refer Figure 1.12).



Fig. 1.12 Mitochondria

## Centrioles/Centrosomes

Centrosomes contain centrioles; the centrioles are small gatherings of microtubules that help with cell division during mitosis. The centrosomes organize and synthesize microtubules.

## Cytoskeleton

The cytoskeleton is composed of a network of filaments and tubules that allows the organelles of the cell to remain in place and gives the cell strength and shape. The cytoskeleton can also play a role in transport within the cell (Refer Figure 1.13).

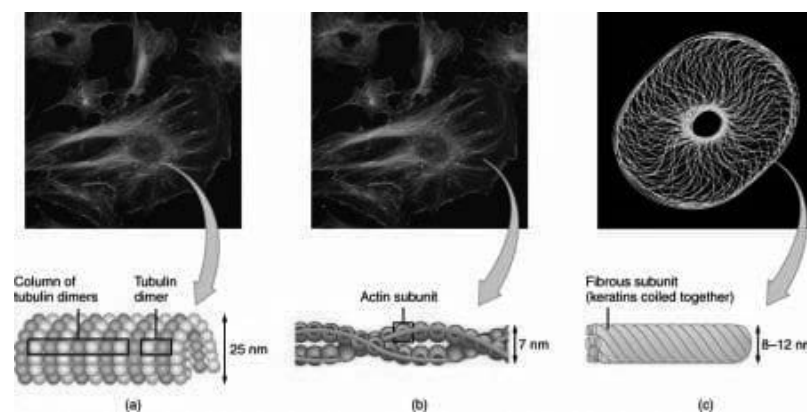


Fig. 1.13 Cytoskeleton

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### Vacuoles

Vacuoles are small storage pockets formed of a single membrane layer containing gas (such as oxygen or carbon dioxide) or fluid (such as water) found within cells (Refer Figure 1.14).

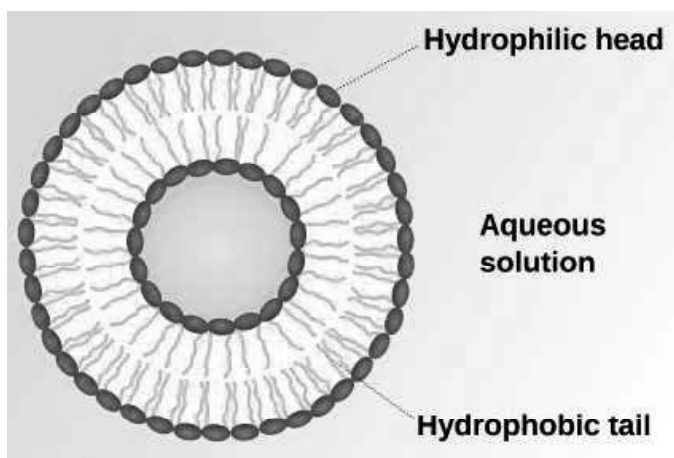


Fig. 1.14 Vacuoles

### Vesicles

Vesicles are similar to vacuoles but are part of the transportation system of the cell. Specialized vesicles can also be involved in cellular metabolism.

- **Lysosomes:** Lysosomes are specialized vesicles in which protein enzymes are contained. The lysosomes break down macromolecules into their components for further use by the cell.
- **Peroxisomes:** Peroxisomes are common in animal cells and perform oxidative digestion.

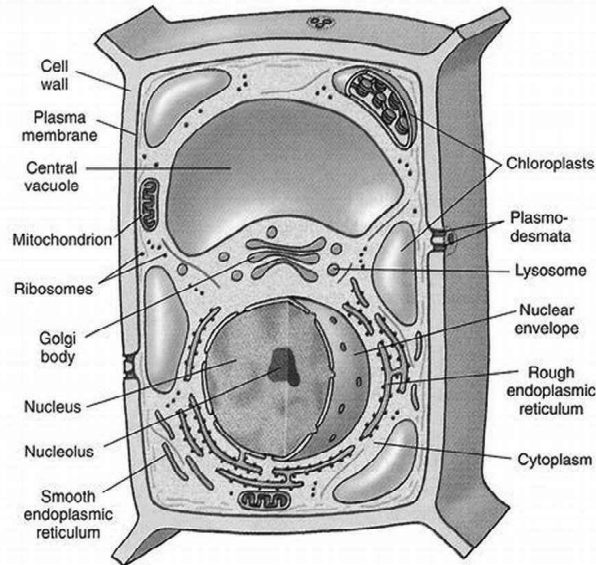
### Difference between Plant and Animal Cell

Below is the table that explains the difference between a plant and animal cell. Figure 1.15 and 1.16 illustrates the structure of plant cell and animal cell.

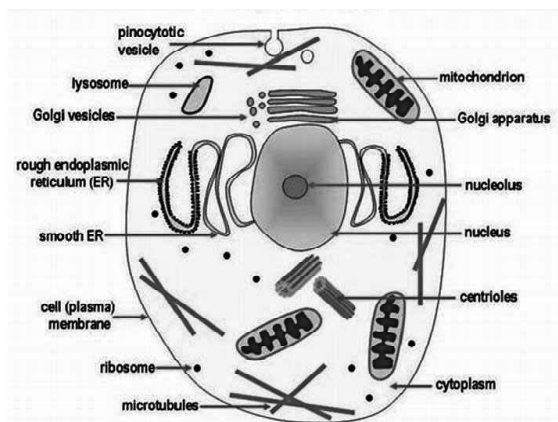
PLANT CELL	ANIMAL CELL
<ul style="list-style-type: none"> <li>• A cell wall is present on the outside to provide shape and rigidity</li> </ul>	<ul style="list-style-type: none"> <li>• A cell wall is absent</li> </ul>
<ul style="list-style-type: none"> <li>• Plastids are present</li> </ul>	<ul style="list-style-type: none"> <li>• Plastids are absent</li> </ul>
<ul style="list-style-type: none"> <li>• Food reserve is starch</li> </ul>	<ul style="list-style-type: none"> <li>• Food reserve is glycogen</li> </ul>
<ul style="list-style-type: none"> <li>• Golgi apparatus consists of units called dictyosomes</li> </ul>	<ul style="list-style-type: none"> <li>• Golgi apparatus consists of single complex called Golgi body</li> </ul>
<ul style="list-style-type: none"> <li>• A mature plant cell possess large central vacuole</li> </ul>	<ul style="list-style-type: none"> <li>• A central vacuole is absent but small sap vacuoles may be present</li> </ul>

<ul style="list-style-type: none"> <li>Centrioles are absent except in lower forms</li> </ul>	<ul style="list-style-type: none"> <li>Centrioles are present</li> </ul>
<ul style="list-style-type: none"> <li>Spindle apparatus is anastral</li> </ul>	<ul style="list-style-type: none"> <li>Spindle apparatus is astral</li> </ul>
<ul style="list-style-type: none"> <li>Lysosomes are less common and their function is performed by vacuoles</li> </ul>	<ul style="list-style-type: none"> <li>Lysosomes are abundant</li> </ul>
<ul style="list-style-type: none"> <li>It does not take part in phagocytosis</li> </ul>	<ul style="list-style-type: none"> <li>It may take part in phagocytosis</li> </ul>
<ul style="list-style-type: none"> <li>Cells are fused in the region of middle lamella</li> </ul>	<ul style="list-style-type: none"> <li>Cells are fused by means of junctions</li> </ul>
<ul style="list-style-type: none"> <li>Plant cells don't burst if placed in hypotonic solution</li> </ul>	<ul style="list-style-type: none"> <li>Animal cells placed in hypotonic solution will burst</li> </ul>
<ul style="list-style-type: none"> <li>Glyoxysomes may be present in fat rich tissues</li> </ul>	<ul style="list-style-type: none"> <li>Glyoxysomes are absent</li> </ul>
<ul style="list-style-type: none"> <li>Cytokinesis occurs by cell plate method</li> </ul>	<ul style="list-style-type: none"> <li>Cytokinesis occurs by cleavage</li> </ul>

## NOTES



*Fig 1.15 Generalized Ultrastructure of Plant Cell with Cell Wall*



*Fig 1.16 Ultrastructure of Animal Cell under Electron Microscope*

## NOTES

### Check Your Progress

11. Where does transcription and translation occurs in prokaryotic and eukaryotic cells?
12. Lysosomes are mostly present in plant cell or animal cells?
13. Which type of DNA does prokaryotic and eukaryotic cells possess?
14. Which type of ribosomes are present in does prokaryotic and eukaryotic cells?
15. Is cell envelope present in prokaryotic and eukaryotic cells?
16. Is cell wall present in animal and plant cell?
17. In which cell plastids are present?
18. What type of Golgi apparatus is present in animal and plant cell?

## 1.7 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. A cell is a mass of cytoplasm enclosed by plasma membrane and contain certain organelles besides nucleus.
2. Cell is an open system as it allows the entry and exit of matter and energy.
3. The molecular structure of genes allows for changes in genetic information that lead to variation among individuals which forms the basis of evolution.
4. Organism is the sum of its component cells, and its activities are the sum of the activities of its cell. New function emerge with each increasing level of organization. Just as molecules have different properties from its constituent atoms, tissue have different function from its cells, organs have different activities from its component tissue, and organism have different properties from its organ system.
5. Cell regulatory mechanism becomes evident when they break down, for example, the failure of cell to correct mistake when its duplicate its DNA result in debilitating mutation, transform the cell into a cancer cell.
6. Nägeli and Crammer coined the term cell membrane in 1855.
7. Protoplasm is the living portion of the cell that includes cytoplasm, nucleus and all the organelles, portion containing plasma membrane, cytoplasm, nucleus and vacuoles except cell wall is called protoplast.
8. A prokaryotic cell is a single envelope system. There is no membrane enveloping the genetic material. It is present in bacteria and blue green algae.
9. Archaea include group of organism whose evolutionary ties to one another are revealed by similarities in the nucleotide sequence of their nucleic acid.

These species live in extremely inhospitable environment known as extremophiles. It includes methanogens, halophiles, acidophiles and thermophiles, included in the latter group are hyperthermophiles which live in hydrothermal vents of ocean floor.

10. In photosynthetic bacteria and in blue green algae, pigment and enzyme molecules that absorb and convert light into chemical energy are associated with plasma membrane and its infolds called photosynthetic lamellae.
11. Both transcription and translation occurs in cytoplasm in prokaryotic cell while in eukaryotic cell, transcription occurs in nucleus and translation in cytoplasm
12. Lysosomes are abundant in animal tissue while in plant tissue it is present less common as its function is performed by vacuoles in plant cells.
13. In prokaryotic cells the DNA is naked and circular, not associated with histones whereas in eukaryotic cell's DNA is linear and associated with histone proteins to form nucleosomes.
14. In prokaryotic cells the ribosomes are 70 S type, whereas in eukaryotic cells ribosomes are 80 S and 70 S in organelles.
15. In prokaryotic cells single envelope organization is present, whereas in eukaryotic cells two envelope organization is present.
16. In plant cell, cell wall is present on the outside to provide shape and rigidity, whereas in animal cells cell wall is absent.
17. Plastids are present in plant cell and absent in animal cells.
18. In plant cells Golgi apparatus comprises of units called dictyosomes whereas in animal cells Golgi apparatus consists of single complex called Golgi body.

## NOTES

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## 1.8 SUMMARY

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- Robert Hooke discovered cell in 1665 when he observed small compartments in a slice of cork under his microscope.
- Cell is the smallest unit of structure and function of living beings and is made of mass of protoplasm surrounded by selectively permeable membrane.
- Plasma membrane is part of protoplasm while cell wall is a product of unit mass of protoplasm. A plant cell is formed of two parts cell wall and protoplast. Protoplast is differentiated into four parts plasma membrane, cytoplasm, nucleus and vacuoles
- Nemiah Grew in 1682 found that all plant tissues contain cells and started cell concept.
- Leeuwenhoek in 1683 observed free cells of bacteria, protozoa, RBCs and sperms. Living substance was named sarcode by Dujardin in 1836 and Protoplasm by Purkinje in 1839.

## NOTES

- Living beings are composed of cell or many cells. A cell is a mass of cytoplasm enclosed by plasma membrane and contain certain organelles besides nucleus. It is a unit of biological entity covered by semipermeable membrane and capable of medium free of other living systems.
- Cell is an open system as it allows the entry and exit of matter and energy. It takes up matter for sustenance, growth and division. Organisms are built according to information encoded in a collection of genes. This vast information is packaged into a set of chromosomes that occupies the space of cell nucleus.
- Genes are more than storage lockers for information, they constitute the blueprint for reconstructing cellular structure. The molecular structure of genes allows for changes in genetic information that lead to variation among individuals which forms the basis of evolution.
- Exchange of matter and energy between a cell and its surrounding environment is dynamic. By being able to regulate the entry and exit of materials into and out of it and by chemical changes, cell attains homeostasis in which internal levels of materials remain constant.
- All of the energy required by life on the earth surface arrives in the form of electromagnetic radiation. The energy of light is trapped by light absorbing pigments present in membranes of photosynthetic cells.
- The part of cytoplasm excluding compartments are eukaryotic cells. The cell of bacteria and blue green algae don't contain membrane bound compartment called single compartment cell called prokaryotic cell. The lack of compartments show primitive nature of these organisms.
- Organism is the sum of its component cells, and its activities are the sum of the activities of its cell. New function emerge with each increasing level of organization. Just as molecules have different properties from its constituent atoms, tissue have different function from its cells, organs have different activities from its component tissue, and organism have different properties from its organ system
- Every cell contains a complete set of genes in its nucleus. If the nucleus of fertilized egg of frog is removed and replaced with the nucleus from skin cell, normal development starts and perfect frog is produced. This experiment shows that the nuclei of all cells are totipotent, i.e., they have a complete genetic information.
- Cells function like miniature chemical plants. The simplest bacterial cell is capable of different chemical transformations, which occur at any rate in the inanimate world.
- All chemical changes that take place in cells require enzymes that increase the rate at which reaction occurs. The sum total of all the reaction in cell represent cell metabolism.



- Cells within a multicellular plant or animal respond to stimuli. Most cells are covered with receptors that interact with substance in highly specific ways. Cell possess receptors to hormones, extracellular materials and to substance on surface of other cell.
- A cell receptors provide pathways through which external agents can evoke specific response in target cells. Cell respond to stimuli by changing their metabolic activities.
- Both type of cells are bounded by plasma membrane that serve as selectively permeable barrier between the living and non-living world. Both type of cell is surrounded by a rigid, non-living cell wall that protects the delicate life form.
- The genetic material of a prokaryotic cell is present in a nucleoid. In contrast, eukaryotic cell possess a nucleus, region bounded by a complex membranous structure called nuclear envelope.
- The chromosomal DNA of eukaryotes unlike prokaryotes is tightly associated with proteins to form complex nucleoprotein material called chromatin.
- Eukaryotic cell contain array of membrane bound organelles. It include mitochondria, where chemical energy is made available to fuel cellular activities, an endoplasmic reticulum where many of cell proteins and lipids are manufactured.
- Plant cells have a primary cell wall, which is a flexible layer formed on the outside of a growing plant cell, and a secondary cell wall, a tough, thick layer formed inside the primary plant cell wall when the cell is mature.
- The formation of the cell wall is guided by microtubules. It consists of three layers, namely, primary, secondary and the middle lamella. The primary cell wall is formed by cellulose laid down by enzymes.
- Cell membrane of plant cell is the semi-permeable membrane that is present within the cell wall. It is composed of a thin layer of protein and fat. The cell membrane plays an important role in regulating the entry and exit of specific substances within the cell. For instance, cell membrane keeps toxins from entering inside, while nutrients and essential minerals are transported across.
- Animal cells are typical of the eukaryotic cell, enclosed by a plasma membrane and containing a membrane-bound nucleus and organelles. Unlike the eukaryotic cells of plants and fungi, animal cells do not have a cell wall.
- The lack of a rigid cell wall allowed animals to develop a greater diversity of cell types, tissues, and organs. Specialized cells that formed nerves and muscles—tissues impossible for plants to evolve—gave these organisms mobility.
- The animal kingdom is unique among eukaryotic organisms because most animal tissues are bound together in an extracellular matrix by a triple helix of protein known as collagen.

## NOTES

## NOTES

- Animals are a large and incredibly diverse group of organisms. Making up about three-quarters of the species on Earth, they run the gamut from corals and jellyfish to ants, whales, elephants, and, of course, humans.
- The earliest fossil evidence of animals dates from the Vendian Period (650 to 544 million years ago), with coelenterate-type creatures that left traces of their soft bodies in shallow-water sediments.
- Depending on the type of the animal cell in question, some cellular components listed below may not be found in every animal cell. However, the components listed below are typical components found in most animal cells.
- The cell membrane of an animal cell is the outer edge of the cell and forms the boundary between the inside of the cell with all of its organelles and the extracellular matrix.
- Embedded within the cell membrane are all sorts of macromolecules such as glycoproteins, which act as recognition sites or aid in stability, and channel proteins, which allow certain materials in and out of the cell. The cell membrane is semi-permeable, which means that only certain molecules are allowed to pass through the membrane easily.
- The nucleus of animal cell has two main functions: it contains all of the deoxyribonucleic acid (DNA) of the cell, and it directs the activities of the cell.
- The nuclear membrane is similar to the cell membrane, except that it surrounds the nucleus within the cell, and performs less of a regulatory function. The nuclear membrane is porous and allows RNA and proteins to pass in and out of the nucleus.
- Endoplasmic reticulum is composed of interconnected membranous channels called cisternae and is connected to the nuclear membrane. The endoplasmic reticulum functions in transportation and modification of molecules.
- Endoplasmic reticulum may be rough or smooth; rough endoplasmic reticulum has ribosomes bound to its surface, and smooth endoplasmic reticulum does not. The rough endoplasmic reticulum modifies and transports the proteins made by the attached ribosomes for use or further modification. The smooth endoplasmic reticulum modifies lipids and steroids.

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## 1.9 KEY WORDS

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- **Cell:** Smallest structural and functional unit of any organism.
- **Prokaryotic cell:** Cells where genetic material DNA is not organized into nucleus.
- **Eukaryotic cell:** Cells having true well organised nucleus.

- **Protoplasm:** The living contents of the cell.
- **Cytoplasm:** Jelly like semifluid bulk mass of protoplasm bounded on outside by plasma membrane.
- **Cytoplasmic streaming or cyclosis:** It is an autonomic vital movement that occurs continually in the cytoplasmic matrix of eukaryotic cells.
- **Transcription:** It is kind of gene expression in which a particular segment of DNA is copied into RNA by enzyme RNA polymerase.
- **Translation:** Process in which mRNA is decoded in a ribosome to produce a specific amino acid or polypeptide.
- **Plasmids:** Small circular DNA strand in a cell that can replicate independently of the chromosomes.
- **Nucleoid:** Naked irregular structure present in prokaryotic cell, also called as genophore.

## NOTES

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### 1.10 SELF ASSESSMENT QUESTIONS AND EXERCISES

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#### Short Answer Questions

1. How do prokaryotic and eukaryotic cells resemble?
2. Write a note on plasmids.
3. Brief a note on the following:
  - Cell Wall
  - Cell Membrane
  - Cytoplasm
  - Pili
  - Flagella
4. Describe the structure of plant cell.
5. How do nucleoid and nucleus differ?
6. Describe the organization of prokaryotic cells.
7. Brief a note on plant cell.

#### Long Answer Questions

1. Give the salient features of eukaryotic cells?
2. Elaborate a note on cell structure and history.
3. Explain the organisation of prokaryotic cell.
4. Discuss in detail about the structure of prokaryotic cell.

## NOTES

5. Explain in detail the structure of eukaryotic cell.
6. List all the major points that make a prokaryotic cell different from a eukaryotic cell.
7. Explain in detail the structure of plant cell.
8. Write a detailed note on animal cell explaining about its structure and anatomy.
9. Distinguish between animal and plant cell.

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## 1.11 FURTHER READINGS

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## UNIT 2 STRUCTURE AND FUNCTIONS OF DIFFERENT CELL ORGANELLES

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### NOTES

#### Structure

- 2.0 Introduction
- 2.1 Objectives
- 2.2 Nucleus
- 2.3 Endoplasmic Reticulum
- 2.4 Golgi Complex
- 2.5 Mitochondria
- 2.6 Chloroplast
- 2.7 Lysosome
- 2.8 Answers to Check Your Progress Questions
- 2.9 Summary
- 2.10 Key Words
- 2.11 Self Assessment Questions and Exercises
- 2.12 Further Readings

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### 2.0 INTRODUCTION

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The nucleus is an organelle found in eukaryotic cells. The nuclear membrane is fully enclosed and enclosed inside it is the majority the cell's genetic material. This material is organized as DNA molecules, along with a variety of proteins, to form chromosomes. Eukaryotic cells have intracellular membranes around organelles and vacuoles called as cell organelles or organoids. These are highly organized subcellular protoplasmic structures that have shape, composition and definite function which can be carried out by them even outside cytoplasm provided they are supplied with substance which is normally provided by the cell. Nucleus is extra cytoplasmic organelle while others are cytoplasmic organelles. A few examples include endoplasmic reticulum, Golgi apparatus, lysosomes, mitochondria, plastids, microbodies, cytoskeletal structures, etc. Centrioles, cilia and flagella occur in primitive plant cells. The membranes compartmentalize the cell. Neither the cell nor the compartments in it are totally isolated from surrounding medium. The membranes allow continuous flow of selected material across them as required from time to time. This helps the cell and organelles to have content different from those of surrounding medium. The prokaryotic cells lack intracellular membranes.

In this unit, you will learn about the structure and function of a nucleus, endoplasmic reticulum, Golgi complex, mitochondria, chloroplast and lysosomes in detail.

## 2.1 OBJECTIVES

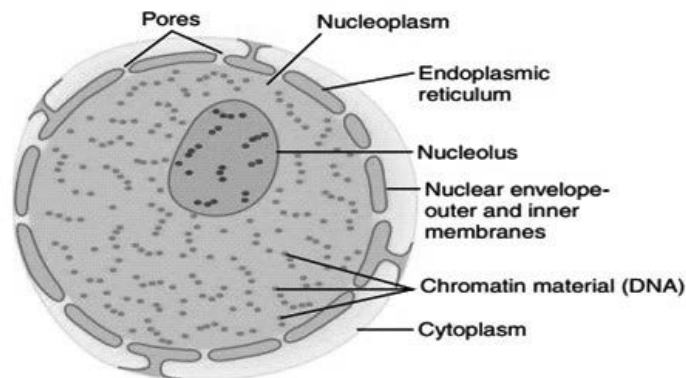
### NOTES

After going through this unit, you will be able to:

- Understand eukaryotic cells and their functions
- Discuss the structure and functioning of a nucleus
- Explain the function of endoplasmic reticulum
- Analyse the process of photosynthesis in Chloroplasts
- Explain the structure and functioning of a lysosome

## 2.2 NUCLEUS

Nucleus is a specialized double membrane bound protoplasmic structure which carries all the genetic information for controlling cell metabolism and transmitting the information to next generation. Nucleus is the largest cell organelle, and it was first studied by Robert brown in orchid root cell. It is present in all the eukaryotic cells except mature sieve cells of higher plants, RBCs of mammals, and blood platelets. Experimental evidence of role of nucleus in transmission of hereditary information comes from the work of Boveri on sea urchins. Nucleus is generally the most conspicuous organelle of a eukaryotic cell. It is noticeable with light microscope but its fine structure can be revealed by electron microscope. Nucleus was observed by a Dutch microscopist, Anton van Leewenhoek, in the RBCs of fishes. A Scotch botanist Robert Brown was the first to describe the nucleus in orchid cells in 1831. Nucleus is present in all eukaryotic cells, but certain mature cells don't have nucleus. The mammalian red blood cells lose their nuclei at maturity and survive for few months only. The food conducting phloem cells called sieve tubes also lose their nuclei at maturity but remain functional for several years. Hence it was known that the nucleus is essential for the survival of cell. The cells deprived of nuclei cannot divide and differentiate. Prokaryotic cells don't have an organized nucleus having nuclear envelope. They have one or more nucleoid each having circular DNA molecule without membrane (Refer Figure 2.1).



**Fig. 2.1** Structure of a Nucleus

**Number:** Commonly cells are uninucleate. The protozoan Paramecium is binucleate one for controlling metabolic activity and other for possessing hereditary information. Multinucleate or polynucleate condition is found in cells of bone marrow (upto 100 nuclei), latex vessels. Multinucleate animal cells are called syncytial cells while in plants it is known as coenocyte cells.

**Position:** Nucleus is found in peripheral position in plant cell due to development of large central vacuole, also in adipose cells. In glandular cells, it is present towards the base. Nucleus is generally found in the region of maximum metabolic activity in the cytoplasm.

**Shape:** It is generally oval in plant cells, elongated in muscle cells, kidney shaped in paramecium, variously lobed in basophil, neutrophil, monocyte and lymphocyte of WBCs.

**Chemical Composition:** DNA – 10-12%, RNA- 5%, Lipids-3%, Basic proteins-15%, acid proteins, neutral proteins and enzymes -65%. Minerals like Calcium, magnesium, sodium in traces.

**Ultrastructure:** Interphase nucleus is differentiated into 5 parts, namely- nuclear envelope, nucleoplasm, nuclear matrix, chromatin and nucleolus.

### 1. Nuclear Envelope (Karyotheca)

It separates the nucleus from the cytoplasm. The separation of a cell genetic material from surrounding cytoplasm is the single most important feature that distinguishes eukaryotes from prokaryotes.

- It is made up of two membranes both made of lipoprotein. Inner membrane is smooth and provides sites for attachment to the chromatin fibers.
- Outer membrane may be smooth or may bear ribosomes.
- The two membranes are separated by electron transparent perinuclear space which is 100-700 Å in width. The inner surface of the nuclear envelope is bound by integral membrane protein to a thin filamentous meshwork called nuclear lamina. It provides support to the nuclear envelope; serve as a site for attachment for chromatin fibers.
- Outer membrane is connected to endoplasmic reticulum due to which perinuclear space contains fluid similar to the one present in spaces of Endoplasmic reticulum.

### Functions

Here below are the functions of a nuclear envelope:

- It maintains the shape of nucleus.
- It protects the genetic material from enzymes and other biochemicals present in the cytoplasm.

## NOTES

- Inner membrane provides attachment sites to telomeres of elongated chromosomes.
- Outer membrane is the seat of attachment of microfilaments and microtubules.

## NOTES

### 2. Nuclear Matrix

- Network of fibrils of acid proteins which function as scaffold for chromatin.
- It provides sites for attachment to chromatin.
- Just below nuclear envelope, matrix forms dense layer called nuclear lamina.
- Nuclear lamina performs three functions i.e. provide mechanical strength to envelope, provide components for nuclear pore complex formation and attachment sites to parts of chromatin.

### 3. Nucleoplasm, Nucleosol, Karyolymph; (Strasburger, 1882)

- It is transparent, jelly like colloidal complex.
- It is similar in composition to cytosol as it contains nucleosides, various enzymes (DNA polymerase, RNA polymerase and nucleoside phosphorylase).
- Proteins present in nucleoplasm are essential for spindle formation.

### 4. Nucleolus

- Discovered by Fontana (1781). It is darkly staining, round, irregular structure attached to chromatin at region called nucleolar organizer region.
- Up to 1600 nucleoli have been reported in the oocytes of *Xenopus*.
- Cells having small nucleoli produce little protein synthesis.
- Covering membrane is absent in them though calcium is essential for maintaining its configuration.

### Functions

- Essential for spindle formation during nuclear division.
- The proteins are associated with rRNA to produce ribosomes.
- Transcription and early processing of tRNA occur in nucleolus.
- Principal site for synthesis of ribosomal RNAs.

### 5. Chromatin

- It is a DNA-protein hereditary complex named due to its ability to get stained with basic dyes.
- Chromatin is differentiated into two parts: Euchromatin and heterochromatin.
- Euchromatin: It forms majority of chromatin.



- It shows normal cycle of crossing over, replication and gene activation.
- It is influenced by changes in pH temperature, hormones and chemicals.
- Heterochromatin is condensed, granular part which shows late replication, high condensation, little gene activity and crossing over.
- Larger heterochromatin granules are called chromocentres or false nucleoli.
- Heterochromatin is of two types: constitutive and facultative.
- Constitutive heterochromatin is present in all the cells, it contains DNA having repeated sequences and provides strength to delicate regions.
- Facultative heterochromatin develops at a particular stage of life, it is meant for inactivating genes not required in a set of cells.
- One X chromosome of female becomes heterochromatic during embryogenesis.
- Heterochromatin is generally found in areas having fewer genes like centromere, satellite and telomere.

## NOTES

### Functions

The functions of chromatin are listed below:

- It is carrier of hereditary information.
- It gives rise to chromosomes passed to the next generation during reproduction.
- It controls biosynthetic activity of the cell through the formation of mRNAs.

### Functions of Nucleus

Herein below are the functions of a nucleus:

- It controls metabolism of cell and other activities through formation of RNAs (mRNA, rRNA, tRNA) which controls synthesis of enzymes.
- It contains hereditary information called chromatin which is a DNA-protein complex made of fibers that condense to form chromosomes.
- It possesses all the genetic information required for growth and development of organism, metabolism and behavior.
- It directs cell differentiation by allowing only particular sets of genes to function.
- The nucleolus part of nucleus forms the ribosomes.
- Nucleus directs the synthesis of structural proteins and chemicals required for growth and maintenance.
- All the variations caused by changes in genetic material are present in nucleus.

## 2.3 ENDOPLASMIC RETICULUM

### NOTES

Discovered by **Porter and Thomson in 1945**. It is a three dimensional, interconnected system of membrane lined channels that run through the cytoplasm. ER is connected with plasma membrane (cortical ER) as well as nuclear envelope (perinuclear ER). The RER is composed of network of flattened sacs and is continuous with the outer membrane of nuclear envelope which bears ribosomes on its cytosolic surface. In contrast, the membranous elements of SER are tubular and form interconnecting system through the cytoplasm ER forms 50-90% of membrane system of cell which increases the internal surface 40 times compared to external surface. It is noticeable only with electron microscope. In 1945, Porter, Claude and Fullman noted with the help of electron microscope a delicate membranous network in the cytoplasm. It was called endoplasmic reticulum by Keith Porter in 1953.

**Location:** ER is extensive in metabolically active cells, i.e., pancreas, liver. Simple in storage cells (tubules in adipose tissue) reduced in spermatocytes, absent in eggs, RBCs, prokaryotic cells.

**Structure:** It is the largest membrane in a cell. It represents 30-60% of total membrane in a cell. It comprises three types of elements: cisternae, tubules and vesicles

**Cisternae:** Flattened, sac like, un-branched, lie parallel to but interconnected with one another. They are found in bundles where they lie parallel to one another. It is involved in synthetic activity. They bear ribosomes on the surface

**Tubules:** Irregular, branching elements often free of ribosomes. Tube like extensions connected with cisternae and vesicles to form reticular system.

**Vesicles:** Oval, vacuole like which occur isolated in cytoplasmic matrix and free of ribosomes. They are also called as microsomes.

All the elements freely communicate with one another and contains fluid called endoplasmic matrix in their lumina. The membrane bounding cisternae, tubules and vesicles of ER is similar to the cell membrane (Refer Figure 2.2).

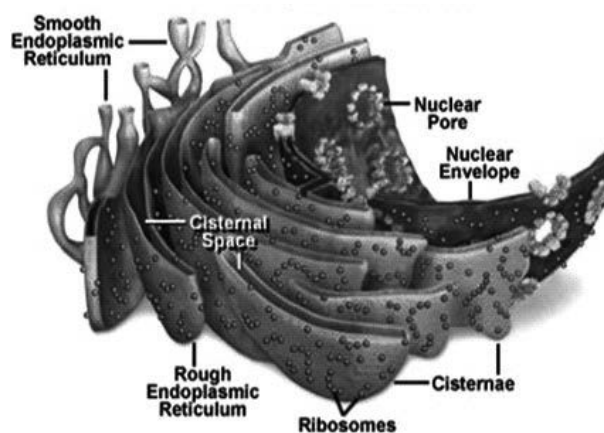


Fig 2.2 Endoplasmic Reticulum

**Ultrastructure:** The membranes are composed of two layers of phospholipid sandwiched by two layers of proteins. As many as 30-40 enzymes are present along with ER membranes for various synthetic activities. The membranes have high protein to lipid ratio.

**Types:** It is of two types, smooth and rough. In liver both types of Endoplasmic reticulum is found. In others, only one type is found. Sarcoplasmic reticulum of muscle cell form plexus around myofibrils. A third type called annulate ER is also found sometimes. All the three types are continuous with one another, nucleus and plasma membrane.

**Smooth Endoplasmic Reticulum:** It is well developed in skeletal muscle, adipose cell, spermatocytes, leucocytes, glycogen storing liver cells, cells that synthesize and secrete steroids. Smooth ER of muscle cells is called sarcoplasmic reticulum. It releases and reabsorbs calcium. SER of retinal pigment cells form tightly packed vesicles and tubules called as myeloid bodies.

**Rough Endoplasmic Reticulum:** It has rough membranes due to presence of ribosomes on its outer surface, also called granular endoplasmic reticulum. As it is basophilic, area could be stained so is called ergastoplasm. It is highly developed in cells that synthesize and secrete proteins. These include liver cells, pancreatic cells, salivary gland cells, cartilage cells, plasma cells and endocrine cells that secrete peptide hormones. The rough ER is more sparsely distributed in plant cells. The membrane bears number of gated channels or translocons in area of attached ribosome to pass polypeptide into channel of ER for transport. Rough ER is sparsely distributed in plant cells compared to animal cells.

**Transitional Endoplasmic Reticulum:** It is part of smooth endoplasmic reticulum that lies towards the face of Golgi apparatus. It is also called as ER-Golgi intermediate compartment also called as transition vesicles.

**Annulate Endoplasmic Reticulum:** It is believed to be formed by pratrusion from the nuclear envelope. It has storage sites of huge nuclear pore complexes. It takes part in synthesis of new nuclear envelope after telophase.

### Functions

The functions of a rough endoplasmic reticulum are discussed below:

- The RER provides a large surface for attachment of ribosomes.
- RER offers extensive surface on which protein synthesis can be carried on by ribosomes. The newly formed protein enters the ER membranes, becoming part of membrane structure or pass into the ER lumen. The proteins becoming a part of ER membrane move from ER via membranes of other cell organelles, namely Golgi apparatus, to become permanent plasma membrane proteins. The proteins entering ER lumen are packed for export. RER provides a large surface area to ribosomes.
- The proteins in ER lumen are processed and enclosed in spherical membrane bound vesicle which pinches off from the ER. Some remain in cytoplasm as

### NOTES

## NOTES

storage vesicles; others migrate to plasma membrane and expel their content by exocytosis. The process of directing proteins to their final destination is called protein sorting or protein trafficking. There is a coordinated system of protein synthesis, transport and secretion in the cell. It synthesizes membrane proteins, serum proteins and other proteins

- It provide enzyme precursor for formation of lysosomes by Golgi complex.
- Proteins synthesized by ribosomes enter the channels of RER both as intracellular and extracellular transport
- It contain single receptor protein or ribophorins for providing attachment to ribosomes

### Smooth Endoplasmic Reticulum (SER)

The SER provides surface for the synthesis of fatty acids, phospholipids, glycolipids, steroids and visual pigments. A few features and functions of SER are listed below.

- Sequestering calcium ions with in cytoplasm of skeletal and cardiac muscle cells. The regulated release of  $\text{Ca}^{2+}$  from the SER triggers contraction.
- Detoxification in a liver of many organic compounds leads to proliferation of SER in liver cells. It is carried out by a system of oxygen transferring enzyme like cytochrome P450 family. The harmless compound benzopyrene is formed when meat is charred on a grill is converted.
- SER carries enzymes for glycogen metabolism in liver cells. Granules of glycogen are attached in larger numbers to outside of SER membranes in liver cells. When body needs energy, glycogen is hydrolysed under hormonal control by enzyme phosphorylase to glucose 1 phosphate, which is converted to glucose 6 phosphate in the cytoplasm. Glucose 6 phosphate cannot leave the liver cell as membranes are impermeable to it. The enzyme glucose 6 phosphatase of SER membrane catalyzes the dephosphorylation of glucose 6 phosphate to glucose and transfers the glucose formed into the SER lumen. From here glucose enters the blood which carries it to needy cell for use in energy release.
- The SER produces Golgi apparatus, lysosomes, microbodies (peroxisomes, glyoxisomes, etc.) and vacuoles. The protein shift from RER through SER to Golgi apparatus for further processing.
- The sarcoplasmic reticulum in skeletal muscle cells releases  $\text{Ca}^{2+}$  ions to cause contraction and absorbs  $\text{Ca}^{2+}$  ions to bring about relaxation.
- The SER has enzymes that bring about detoxification in the liver i.e. converts harmful materials such as pesticides, carcinogens into harmless ones for excretion by the cell.
- The SER membranes carry out the initial reaction in the oxidation of fats. Synthesis of fats inside the adipose tissue.
- Formation of visual pigments from vitamin A.

- Synthesis of ascorbic acid
- Synthesis of glycogen and glycogenolysis in liver cells
- It contains cytochrome P<sub>450</sub> and related enzymes that take part in detoxification of toxins. These enzymes change lipid soluble toxins to water soluble state so that it can be excreted out of the body
- SER produces Golgi apparatus, lysosomes, microbodies and vacuoles
- SER membrane carry out the reaction in oxidation of fats

## NOTES

### Check Your Progress

1. How many enzymes are present along with the ER membranes for synthetic activities?
2. What does the SER provide?
3. What is a nucleus?
4. Into how many parts is the interphase nucleus differentiated?

## 2.4 GOLGI COMPLEX

The Golgi apparatus also called Golgi complex is system of membranes which takes part in membrane transformation, secretion and production of biochemicals which is noticeable with both light and electron microscope. Golgi apparatus was discovered by Italian Scientist Camillo Golgi in 1898 in nerve cells of barn owl and cat by metallic impregnation method. It is also named as Golgisome, Golgi membranes, Golgi body. Though Golgi complex remained a centre of controversy for decades between those who believed that organelle existed in living cells and thought it as artificial structure formed during microscopy preparation. It consists of flattened disc like, membranous cisternae and tubules. The Golgi stacks in mammalian cell are interconnected by membranous tubules and form large ribbon like complex situated next to the cell nucleus (Refer Figure 2.3 and 2.4).

**Location:** It is present in all eukaryotic cell except mammalian RBCs, sperm cells of bryophytes and pteridophytes and sieve tubes of plants. In secretory and absorptive cells, Golgi lies between the nucleus and cell surface. In invertebrate and plant cells, Golgi complex consists of isolated units called dictyosomes or Golgi stacks. Dictyosomes are capable of changing position with the help of ATP dependent motors.

The cytoplasm containing Golgi complex has no organelles and glycogen granules and called as zone of exclusion. In enucleated Amoeba, the Golgi complex becomes reduced and disappears, but redevelops after re-nucleation of the organism. This shows that nucleus is necessary for maintaining a healthy Golgi complex.

## NOTES

**Structure:** Golgi apparatus varies in size and form in different cell types but has similar organization for any kind of cells, for example, it is well developed in secretory and nerve cells, but small in muscle cells.

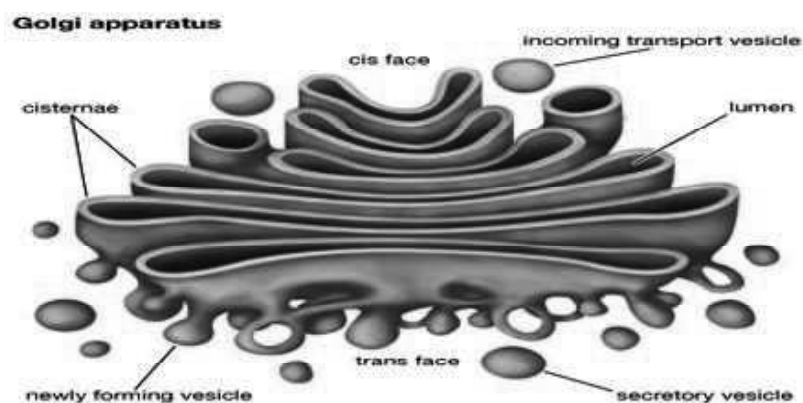
Usually a compact Golgi apparatus is made up of four parts- cisternae, tubules, vesicles and vacuoles. Tubules, vacuoles and vesicles are more on the outer side of apparatus.

**Cisternae:** Membrane lined flat which occur in stack of 4-8 in lower organisms. The cisternae are curved with convex forming, cis face towards endoplasmic reticulum and concave maturing face, trans face towards plasma membrane. Cisternae in between cis and trans ones is medial cisternae. Much of processing and elaboration of biochemicals occur in medial cisternae.

**Tubules:** Short branched hollow filament which form complicated network. They are quite active in elaboration of secretory products.

**Vesicles:** Small sacs of 70-80 nm diameter which develop as protrusion from tubules, cisternae and ER. All of them are coated with proteins. Protein covering of coated vesicles is of two types- clathrin and cytosolic coat protein. Clathrin coated vesicles take part in transport of storage proteins. They take part in endocytosis by forming receptor over cell membrane. Some secretory vesicles pass to plasma membrane and release content to the outside.

**Golgian Vacuoles:** They function as lysosome precursors. They are expanded part of cisternae which gets modified to form vacuoles.



*Fig 2.3 Golgi Apparatus*

**Chemical Composition:** Protein content is 60-75% while lipid content is 20-35%. Important enzymes present are adenosine diphosphatase, ATPase, CTPase, Glucose 6 phosphatase, Cytochrome c reductase, thiamine pyrophosphatase. The membranes of the Golgi apparatus resemble the cell membrane in molecular structure. They consist of a phospholipid bilayer sandwiched by two protein monolayers. A variety of enzymes are associated with the Golgi membranes. These include ATPase, thiamine pyrophosphatase, glycosyl transferase, glucose 6 phosphatase, etc. The cis and trans regions of the Golgi complex are different in their protein and lipid composition. The cis region resembles the ER in chemistry

and trans region resembles plasma membrane. This shows that molecular change occurs in the membranes of the Golgi complex.

### **Origin**

The Golgi apparatus originates from the smooth endoplasmic reticulum.

### **Functions**

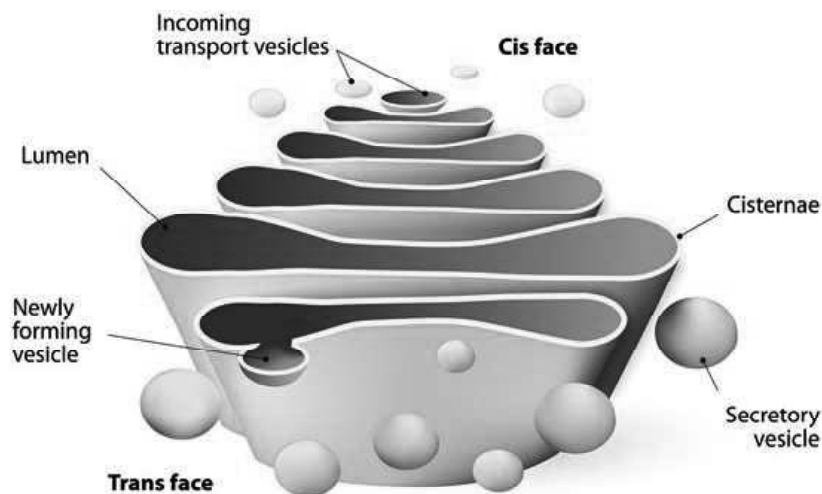
Herein below are the functions of Golgi apparatus listed in detail.

- Golgi apparatus synthesizes mucopolysaccharides from sugars.
- Golgi apparatus brings about membrane transformation, that is converting one type of membrane (i.e., that of ER) into other types (i.e., selectively permeable plasma membrane, differentiated membrane of lysosome).
- Golgi apparatus links the sugars with proteins coming from rough ER to form glycoproteins. N linked glycoproteins synthesized in the lumen of RER are passed into the lumen of Golgi apparatus. Here certain sugars (i.e. mannose) are removed while others are added. Glycosylation of OH groups of certain amino acids also take place so that each protein become specific and carries marker which specifies its ultimate destination. Glycoproteins are passed out to cell wall and other places through vesicles. Here they control biosynthetic activities. Mucoproteins are components of mucus and matrix of solidified connective tissue of animals (cartilage and bone).
- Golgi complex give rise to lysosomes by budding.
- The production of hormones by endocrine glands is mediated through Golgi apparatus.
- In chick embryo, the retinal pigment has been synthesized by Golgi apparatus
- Acrosome is an important constituent of tip of animal sperms which help in digesting away the covering sheath of the egg. After the formation of acrosome the rest of Golgi complex degenerates so that mature sperm is devoid of the apparatus.
- The Golgi complex to store cell secretion such as proteins and lipids.
- It gives rise to nematocysts in coelenterates.
- It brings about membrane transformation, i.e., changing one type of membrane to another.
- A variety of enzymes are localized in Golgi complex to help in biochemical reactions.
- The Golgi apparatus produces yolk and cortical in eggs. Formation of yolk is called vitellogenesis.
- In some algae, cellulose plates for cell wall are synthesized in Golgi complex. In plant cell, Golgi complex synthesize pectin and carbohydrates necessary for cell wall formation and produce secretion such as mucilage.

### **NOTES**

## NOTES

- The formation of root hair from their mother cells take place through agency of Golgi apparatus.
- Most of the complex carbohydrates are synthesized inside Golgi apparatus for example, hemicellulose, mucopolysaccharide, pectic compound. Therefore also called as carbohydrate factory.



*Fig 2.4 Representation of a Golgi Apparatus and its Sub Components*

## 2.5 MITOCHONDRIA

The name Mitochondria was given by Benda in 1898. It was first seen in 1880 by Kolliker who isolated them from insect muscle cells. Mitochondria is known by variety of names such as parabasal bodies, chondriosomes, and plasmosomes.

Cell organelles of aerobic eukaryotes which take part in oxidative phosphorylation and Krebs cycle of aerobic respiration therefore called as power house of cell. Michaelis in 1900, found mitochondria to be respiratory organelle as it can oxidize Janus Green B. Its ultrastructure can be studied under electron microscope. Mitochondria is absent in prokaryotes and anaerobic eukaryotes. They are secondarily lost in RBC of mammals. Their number varies from one in some algae (*Chlorella*), 25 in sperm cell, 300-400 in kidney cell, 30,000 in some oocytes and 5 lacs in flight muscle cells. Cell of dormant seeds have few mitochondria. In general green plant cell contain less number of mitochondria as compared to non-green plant cells and animal cells. The position of mitochondria in a cell depends upon the requirement of energy and amino acids (Refer Figure 2.5).

**Shape and Size:** Mitochondria differ in shape. These can either be spherical, cylindrical, tubular or filamentous. In *Chlorella* the single mitochondria is tubular and branched. The shape is controlled by physiological condition of the cells.



**Structure:** Both the inner and outer membrane resemble plasma membrane in molecular structure. Mitochondrial envelope is asymmetrical to both structure and function.

**Membranes:** Outer membrane is smooth and permeable to small molecules having channels formed by protein porin. The outer membrane consists of 50% lipid. It contains enzymes but is poor in proteins.

Inner membrane is selectively permeable and permeable to only some metabolites. It is rich in double phospholipid called cardiolipin (having four fatty acids) which make membrane impermeable to ions. It contains no enzymes and carrier proteins. Protein content of the inner membrane is highest for any membrane. It regulates the entry of material into and out of mitochondria. Protein content of the inner membrane is the highest for any membrane being 70-75% of the total component. The inner membrane is infolded variously to form involutions called cristae which are meant for increasing the physiologically active area of inner membrane. The cristae are arranged like baffles at right angle to the longitudinal axis of the mitochondria. They are tubular (most plant cells) or plate like (most animal cells). A crista encloses a space that is in continuation of the outer chamber. The density of cristae indicates the intensity of respiration. The inner membrane as well as cristae possess small tennis racket like particles called elementary particles or oxysomes. Each elementary particle functions as ATP synthetase. It is differentiated into three parts- head, stalk and base.

**Cristae:** Cristae extend inwards to varying degrees. They are arranged in characteristic ways in different cells. They run at right angles to the long axis of mitochondria. In protozoans, insect flight muscle cell cristae are tubular. The active cells have many cristae whereas inactive cells have few. Heart and muscle cells have 3 times as many cristae as in liver, mitochondria

**Matrix:** The space between cristae is called inner chamber, filled with gel like material termed mitochondrial matrix. It contains proteins mainly in the form of enzymes concerned with energy producing activity, DNA is circular, active in transcription and has more guanine and cytosine. Ribosomes are 70 S in size. All the three RNAs are present in mitochondrial matrix.

**Oxysomes:** Inner membrane bears minute spaced particles known as elementary particles or oxysomes. An oxysome consists of 3 parts- rounded head piece joined by short stalk located in inner membrane. The oxysome complex represents ATPase or ATP synthetase which is concerned with ATP formation.

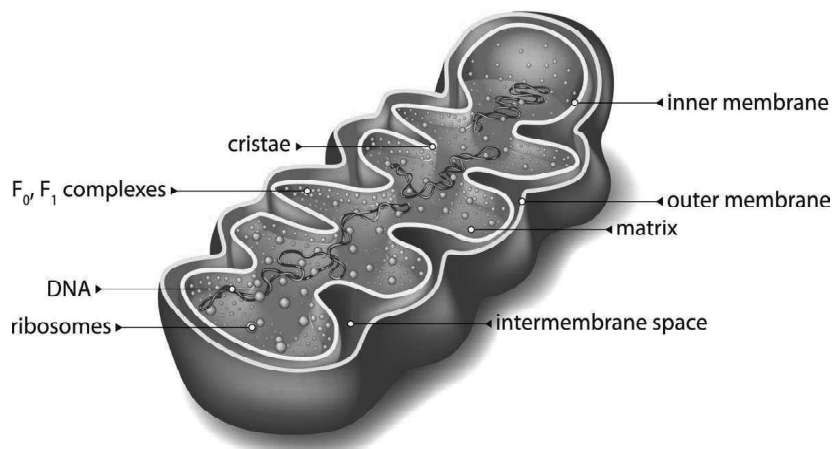
**Outer Chamber:** It is the space that lies between the outer and inner membrane of the mitochondrial envelope. It extends into the spaces of the cristae. The chamber contains a fluid having few enzymes.

**Inner Chamber:** It contains a semi fluid matrix. pH of matrix is higher than cytoplasm. The matrix has protein particles, ribosomes, RNA, DNA, amino acid synthesis and fatty acid metabolism, crystals of calcium phosphate. DNA is naked, circular.

## NOTES

**Chemical Composition:** Proteins- 65-70%, Lipids- 25-30% (mostly phospholipid) such as cephalin and lecithin. About 60 different enzymes are found to exist in the mitochondria.

## NOTES



*Fig. 2.5 Diagrammatic Representation of Mitochondria*

## Functions

The functions of Mitochondria are listed below in detail:

- They provide intermediates for synthesis of biochemicals like chlorophyll, cytochromes, steroids, alkaloids, etc.
- Synthesis of many amino acids occurs in mitochondria. The first formed amino acid is glutamic acid and aspartic. They are synthesized from  $\alpha$  ketoglutaric acid and oxaloacetic acid respectively.
- Mitochondria may store and release calcium when required. They contain  $K^+$ ,  $Mg^{2+}$  and phosphate in cells.
- Organism receives mitochondria from mother and takes part in maternal inheritance.
- They are mini biochemical factories where food is oxidized to carbon dioxide and water. They undergo oxidation and form energy rich ATP. ATP performs various energy requiring processes like muscle contraction, nerve impulse conduction, cell division, movement. Because of formation of ATP the mitochondria are called power house of cell.

### Check Your Progress

5. What is the Golgi apparatus?
6. Where does the Golgi apparatus originate from?
7. Is mitochondrial envelope symmetrical to structure and function?
8. Who gave the term mitochondria?

## 2.6 CHLOROPLAST

Plastids are the semiautonomous organelles having DNA and double membrane envelope which synthesizes various types of organic compounds like fatty acids, amino acids, purines, pyrimidines etc., found in plant cells and certain protists. Plastids develop from colorless precursors called proplastids. Proplastids are small spherical, colorless structures which occur in meristematic cells. It is covered by double membrane envelope. Its internal membrane is shown to develop lamellae. Lamellae occur free in the interior. They have some starch and possess circular nucleoid and are of two types: leucoplasts and chromoplasts. Leucoplasts are colorless, while chromoplast is coloured and occurs in cells exposed to sunlight. Chromoplast with green pigment or chlorophyll is known as chloroplast (Refer Figure 2.6).

The chloroplast are greenish plastids that possess photosynthetic pigments, chlorophylls, carotenoids and take part in the synthesis of food from inorganic raw material in the presence of sunlight. Chloroplast of algae other than green ones are called chromatophores. Chloroplast is the most common type of plastids as they provide food to all organism through photosynthesis. Like mitochondria, the chloroplasts don't have fixed position and shift from place to place. Bacteria, blue green algae, protist, fungi and animals lack chloroplast. All plastids develop from small rounded pigmented bodies called proplastids. The proplastid grows and in presence of light, its lamellae develop to form mature chloroplast.

**Shape:** They are spherical, lens shaped, disc like. In lower plants, they may be cup shaped (as *Chlamydomonas*), ribbon like in *spirogyra*, spiral in *spirogyra*, girdle shaped (as *Ulothrix*).

**Movements :** Chloroplasts change their position due to either cyclists or direction and intensity of illumination. The movement of chloroplasts in response to light is called phototactic movements. Under strong light the chloroplasts come to lie one behind the other with their edges towards the light. This minimizes the light absorption. This is called parastrophe. In moderate light, chloroplast arrange themselves towards the illuminated side of the cells with their flat sides facing the sun called epistrophe. In dark arrangement of chloroplast is known as apostrophe. The movement is due to blue light photoreceptor called phototropin.

**Size:** The chloroplasts of shade plants are larger than those of sun plants. The chloroplasts of higher plants are of 5-10 $\mu$ m long.

**Number:** Higher plants have 20-35 chloroplasts per cell, in some cases about 500 or more.

**Structure:** It is a vesicle bounded by an envelope of two unit membrane and filled with fluid matrix.

**Membranes:** Each membrane is about 50-70Å thick. The two membranes are separated by narrow fluid filled inter membrane space. The membrane resemble

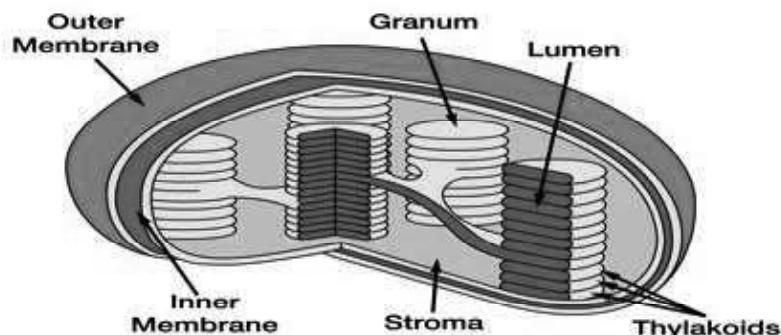
### NOTES

## NOTES

plasma membrane in structure. The outer membrane is smooth and freely permeable and contains protein channels called porin. The inner membrane has selectively permeability and rich in proteins and contains permeases. These regulate the movement of metabolite into and out of chloroplast. Inner mitochondrial membrane is in folded to provide large surface area known as cristae.

**Lamellae:** Lamellae often take the form of flattened ovoid sac thylakoid which lie closely packed one on another forming grana. A thylakoid encloses a space called as locus bounded by single membrane. The thylakoids are interconnected by branching tubules termed as frets. Thylakoids occur singly in red algae, in pairs in cryptophytes. Thylakoid membrane contains rounded particles called quantosomes. A quantosome contain 250 molecules of chlorophyll, amount necessary for photosynthesis

**Matrix:** It is colorless ground substance called stroma. The chloroplast stroma contains proteins, lipids, small circular, double helical DNA molecule, RNA molecule. Proteins in matrix are enzymes meant for dark reaction of photosynthesis



*Fig 2.6 Diagram of a Chloroplast*

### Photosynthesis in Chloroplasts

Photosynthesis takes place in two set of reaction, namely light reaction and dark reaction. These are explained below:

**Light Reaction:** The chlorophyll molecules trap the radiant energy of sunlight. This energy is used to remove electrons and protons from water to form oxygen. Electrons are transferred through thylakoid membrane to electron acceptor  $\text{NADP}^+$ . The movement of electrons is coupled to transport of protons across membrane from stroma to thylakoid lumen. The protons move down concentration gradient from lumen to stroma which leads to synthesis of ATP from ADP. The process is called photophosphorylation.

**Dark Reaction:** These reaction occur in the absence of light. Using the energy of ATP and NADPH generated by light, energy poor carbon dioxide is converted into energy rich 6 carbon sugar. Process is called carbon dioxide fixation.

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## 2.7 LYSOSOME

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Membrane bound secretion vesicles containing enzymes for intracellular digestion. They are the important products of the secretory pathway in the cells. They are specialized membrane bound secretion vesicles containing enzymes for intracellular digestion. It was reported by the Belgian cytologist and biochemist Christian de Duve in 1955. His findings were based on biochemical studies. In 1956, Novikoff observed lysosomes with the help of electron microscope. It occurs in all animal cells. Some mammalian red blood corpuscles lack lysosomes. They occur in protist, fungi and plants. Lysosomes are small vesicles bounded by single membrane and contain hydrolytic enzymes in form of granules of 5-8 nm. About 60 types of enzymes occur in them. The important enzymes discovered are acid phosphatases, sulphatases, proteases, nucleases, lipases, glycosidases are called acid hydrolases as they function in acidic medium with pH4-5. Acidic conditions are maintained inside by pumping of protons by ATP dependent pumps.

Lysosomes are called suicide bags because of presence of large no of digestive enzymes. In plants and fungi their function is taken over by vacuoles. In animals, they are abundant in macrophages, Kuffer cells etc. They are abundant in white blood corpuscles and secretory cells of pancreas, spleen, liver and kidneys. Lysosomes are evenly distributed in the cytoplasmic matrix. Certain meristematic cells of roots of plants have irregular lysosomes. A lysosome is a tiny sac bounded by a single unit membrane of lipoprotein. It contain dense granular material, which contain hydrolytic enzymes. The common enzymes in lysosomes are proteases, nucleases, glycosidases, lipases, sulphatases and phosphatases which hydolyse proteins, nucleic acids, polysaccharides, lipids, organic sulphate and phosphate respectively. However not all enzymes are found in one lysosome.

There are many kinds of lysosomes containing different set of enzymes. Thus they are heterogeneous organelles. They store the hydrolyzing enzymes of the cell. Their membrane prevent the enzyme from escaping into the cytoplasm. The materials needeing hydrolysis must enter the lysosome so their enzyme remain isolated from cytoplasm. In injured and dead cells, the lysosome membrane ruptures, releasing the enzymes that lyse the weakened cells. In intact cells, compounds like cholesterol and cortisone prevent rupturing of lysosome membrane.

**Structure:** Lysosomes show polymorphism due to various origin and function. They are of four types, i.e., primary, secondary, autophagic and residual bodies. Now terms primary and secondary have become obsolete. They are of three types- Heterophagic lysosomes, autophagic lysosomes and residual bodies.

- 1. Heterophagic Lysosome (Digestive Vacuoles):** Lysosomes with extracellular material for digestion. The ingested matter is enclosed in membrane lined phagosome. Phagosome fuses with endosome to produce digestive vacuole. Fusion of lysosome with other membrane bound vesicles is highly selective. Lysosome fuses only with vesicles containing materials to

### NOTES

## NOTES

be digested. The membranes of lysosomes and vesicles having material have some sort of recognition system. Lysosome may fail to fuse with phagosome. The internal materials are acted upon by acid hydrolases. The solubilised products of digestion are passed out into cytosol through diffusion.

- 2. Autophagic Lysosomes (Autophagosomes):** A cell may digest its own organelles such as mitochondria and ER. This process is called autophagy or autolysis. Primary lysosome fuse together about the unwanted organelles forming large sac known as autophagic vacuole. The enzymes of lysosome digest the organelles thus enclosed. The products of digestion enter the cytoplasm through lysosome membrane and reprocessed into new molecules. Autophagic vacuoles develop in liver cells to digest cell components in a starving animal. Self-eating of degenerate intracellular structure. Destruction of various organelle in RBCs occur through autophagy. Autophagy of larger structure is called macrophagy. Autolysis is self-destruction of cell, tissue or organ with the help of lysosome. It occurs in diseased, ageing and dead cells.
- 3. Residual Body (Tertiary Lysosomes):** Those lysosome in which only indigestible materials have been left. In secondary lysosome, the enzymes digest the incoming materials. The products of digestion pass through lysosome membrane into matrix for use as a source of nutrition. Indigestible matter, such as bacterial cell walls that are resistant to hydrolases remains in secondary lysosome. A secondary lysosome left with indigestible matter is known as residual body. The residual body meets the cell membrane and residue is released by exocytosis in protozoans. Residual bodies are stored in cells in vertebrates and play role in ageing process. The residual bodies are result of enzymes in lysosomes and cause certain diseases. It is pass outwardly and fuse with plasma membrane to throw the debris into external environment by ephagy. Residual bodies remain inside the cell which leads to pathological disease (storage disease) like hepatitis, Tay sach's disease, polynephritis, hurler's disease (Refer Figure 2.7 and 2.8).

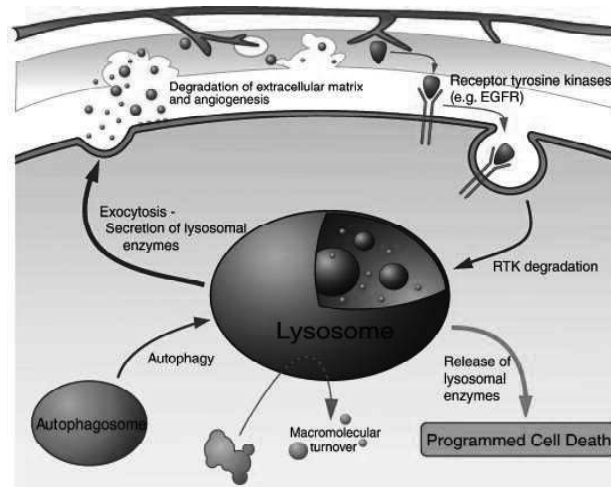


Fig 2.7 Diagrammatic Representation of a Lysosome

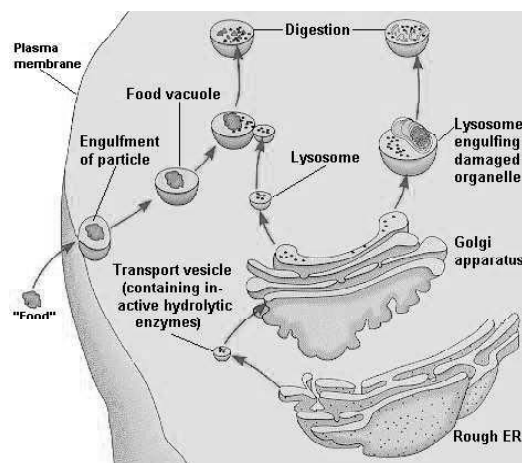
Lysosomes are considered to be arise from the Golgi complex or smooth endoplasmic reticulum. They originate as membranous vesicles containing enzymes that are stored in Golgi complex and received from the rough endoplasmic reticulum.

### Functions

Here below are the functions of lysosomes:

- Lysosomes devour foreign substance, toxic bacteria and other microbes and take part in natural defense of the body.
- Lysosome perform intracellular scavenging by removing old useless organelles.
- Lysosomes provide enzymes required for breaking membrane of eggs.
- The residual bodies don't undergo exocytosis, instead remain inside the cell and cause disease, i.e., polynephritis, hepatitis.
- They cause breakdown of ageing and dead cells.
- In metamorphosis, certain embryonic parts like tail, gills are digested through lysosomes.
- They are essential for cell division by overcoming agents that represses mitotic cycle.
- They remove carcinogens by engulfing them.
- Active hormone thyroxine is formed by hydrolysis of thyroglobulin by lysosomes.

### NOTES



**Fig 2.8** Diagrammatic Representation of Digestion with Lysosomes

### Check Your Progress

9. What are plastids?
10. Where do plastids develop from?
11. What makes chloroplasts change their position?
12. Why are lysosomes called suicide bags?

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## 2.8 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

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### NOTES

1. As many as 30-40 enzymes are present along with ER membranes for various synthetic activities.
2. The SER provides surface for the synthesis of fatty acids, phospholipids, glycolipids, steroids and visual pigments.
3. Nucleus is a specialized double membrane bound protoplasmic structure which carries all the genetic information for controlling cell metabolism and transmitting the information to next generation.
4. Interphase nucleus is differentiated into 5 parts, namely- nuclear envelope, nucleoplasm, nuclear matrix, chromatin and nucleolus.
5. The Golgi apparatus is system of membranes which takes part in membrane transformation, secretion and production of biochemical.
6. The Golgi apparatus originates from the smooth endoplasmic reticulum.
7. No, mitochondrial envelope is asymmetrical to both structure and function.
8. The name mitochondria was given by Benda in 1898.
9. Plastids are the semiautonomous organelles having DNA and double membrane envelope.
10. Plastids develop from small rounded pigmented bodies called proplastids.
11. Chloroplasts change their position due to either cyclists or direction and intensity of illumination.
12. Lysosomes are called suicide bags because of presence of large no of digestive enzymes.

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## 2.9 SUMMARY

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- Nucleus is a specialized double membrane bound protoplasmic structure which carries all the genetic information for controlling cell metabolism and transmitting the information to next generation.
- Nucleus is present in all eukaryotic cells, but certain mature cells don't have nucleus. The mammalian red blood cells lose their nuclei at maturity and survive for few months only. The food conducting phloem cells called sieve tubes also lose their nuclei at maturity but remain functional for several years.
- Experimental evidence of role of nucleus in transmission of hereditary information comes from the work of Boveri on sea urchins. Nucleus is generally the most conspicuous organelle of a eukaryotic cell.
- Commonly cells are uninucleate. The protozoan Paramecium is binucleate one for controlling metabolic activity and other for possessing hereditary



information. Multinucleate or polynucleate condition is found in cells of bone marrow (upto 100 nuclei), latex vessels.

- Nucleus is found in peripheral position in plant cell due to development of large central vacuole, also in adipose cells.
- Interphase nucleus is differentiated into 5 parts, namely- nuclear envelope, nucleoplasm, nuclear matrix, chromatin and nucleolus.
- The separation of a cell genetic material from surrounding cytoplasm is the single most important feature that distinguishes eukaryotes from prokaryotes.
- It is DNA protein hereditary complex named due to its ability to get stained with basic dyes.
- Chromatin is differentiated into two parts: Euchromatin and heterochromatin.
- Constitutive heterochromatin is present in all the cells, it contain DNA having repeated sequences and provide strength to delicate regions.
- Facultative heterochromatin develops at particular stage of life, it is meant for inactivating genes not required in a set of cells.
- The RER is composed of network of flattened sacs and is continuous with the outer membrane of nuclear envelope which bears ribosomes on its cytosolic surface.
- It is noticeable only with electron microscope. In 1945, Porter, Claude and Fullman noted with the help of electron microscope a delicate membranous network in the cytoplasm.
- ER is extensive in metabolically active cells, i.e., pancreas, liver.
- Flattened, sac like, un-branched, lie parallel to but interconnected with one another.
- All the elements freely communicate with one another and contains fluid called endoplasmic matrix in their lumina.
- The membranes are composed of two layers of phospholipid sandwiched by two layers of proteins.
- Smooth ER of muscle cells is called sarcoplasmic reticulum. It releases and reabsorbs calcium.
- The rough ER is more sparsely distributed in plant cells. The membrane bears number of gated channels or translocons in area of attached ribosome to pass polypeptide into channel of ER for transport.
- RER offers extensive surface on which protein synthesis can be carried on by ribosomes. The newly formed protein enters the ER membranes, becoming part of membrane structure or pass into the ER lumen.
- The proteins in ER lumen are processed and enclosed in spherical membrane bound vesicle which pinches off from the ER.

## NOTES

## NOTES

- The process of directing proteins to their final destination is called protein sorting or protein trafficking.
- Proteins synthesized by ribosomes enter the channels of RER both as intracellular and extracellular transport.
- Sequestering calcium ions within cytoplasm of skeletal and cardiac muscle cells. The regulated release of  $\text{Ca}^{2+}$  from the SER triggers contraction.
- Detoxification in a liver of many organic compounds leads to proliferation of SER in liver cells. It is carried out by a system of oxygen transferring enzyme like cytochrome P450 family.
- The SER has enzymes that bring about detoxification in the liver i.e. converts harmful materials such as pesticides, carcinogens into harmless ones for excretion by the cell.
- The Golgi apparatus also called Golgi complex is system of membranes which takes part in membrane transformation, secretion and production of biochemicals which is noticeable with both light and electron microscope.
- It is present in all eukaryotic cell except mammalian RBCs, sperm cells of bryophytes and pteridophytes and sieve tubes of plants.
- Dictyosomes are capable of changing position with the help of ATP dependent motors.
- The cytoplasm containing Golgi complex has no organelles and glycogen granules and called as zone of exclusion.
- Golgi apparatus varies in size and form in different cell types but has similar organization for any kind of cells. E.g. it is well developed in secretory and nerve cells, but small in muscle cells.
- Membrane lined flat which occur in stack of 4-8 in lower organisms.
- Small sacs of 70-80 nm diameter which develop as protusion from tubules, cisternae and ER.
- Protein content is 60-75% while lipid content is 20-35%. Important enzymes present are adenosine diphosphatase, ATPase, CTPase, Glucose 6 phosphatase, Cytochrome c reductase, thiamine pyrophosphatase.
- Golgi apparatus brings about membrane transformation, that is converting one type of membrane (i.e., that of ER) into other types (i.e. selectively permeable plasma membrane, differentiated membrane of lysosome).
- N linked glycoproteins synthesized in the lumen of RER are passed into the lumen of Golgi apparatus.
- The Golgi complex to store cell secretion such as proteins and lipids.
- The name Mitochondria was given by Benda in 1898.

- Cell organelles of aerobic eukaryotes which take part in oxidative phosphorylation and Krebs cycle of aerobic respiration therefore called as power house of cell.
- Mitochondria differ in shape. These can either be spherical, cylindrical, tubular or filamentous.
- Both the inner and outer membrane resemble plasma membrane in molecular structure.
- Inner membrane is selectively permeable and permeable to only some metabolites. It is rich in double phospholipid called cardiolipin (having four fatty acids) which make membrane impermeable to ions.
- The cristae are arranged like baffles at right angle to the longitudinal axis of the mitochondria.
- Cristae extend inwards to varying degrees. They are arranged in characteristic ways in different cells.
- The space between cristae is called inner chamber, filled with gel like material termed mitochondrial matrix.
- Inner membrane bears minute spaced particles known as elementary particles or oxysomes.
- Synthesis of many amino acids occur in mitochondria. The first formed amino acid are glutamic acid and aspartic.
- Plastids are the semiautonomous organelles having DNA and double membrane envelope which synthesizes various types of organic compounds like fatty acids, amino acids, purines, pyrimidines etc., found in plant cells and certain protists.
- Proplastids are small spherical, colorless structures which occur in meristematic cells.
- Leucoplasts are colorless, while chromoplast is coloured and occurs in cells exposed to sunlight. Chromoplast with green pigment or chlorophyll is known as chloroplast.
- Membrane bound secretion vesicles containing enzymes for intracellular digestion.

## NOTES

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### 2.10 KEY WORDS

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- **Plastids:** These are membrane bound organelle found in plant cells, algae and some eukaryotes. They are the sites of manufacture and storage of chemical compounds.
- **Porins:** These are barrel proteins that cross cellular membrane and act as a pore, through which molecules can diffuse.

## NOTES

- **Autolysis:** This is the self-destruction of cell, tissue or organ with the help of lysosome. It occurs in ageing, dead and diseased cells
- **Macrophagy:** It refers to the self-eating of degenerate intracellular structure mainly large sized structures.
- **Oxysomes:** It is the inner membrane of mitochondria that possesses tennis like particles called oxysomes which function as ATP synthetase.
- **Vitellogenesis:** It is a Golgi apparatus that functions as centre around which yolk is deposited in animal oocytes and is called vitellogenesis.
- **Phototactic movement:** It is referred to the movement of chloroplast in response to light.

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## 2.11 SELF ASSESSMENT QUESTIONS AND EXERCISES

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### Short Answer Questions

1. Briefly discuss the functions of ER.
2. Describe the structure of Golgi apparatus.
3. Which organelle is called suicide bag? Describe its origin and function.
4. Describe the ultrastructure of chloroplast.
5. Name and describe the types of Endoplasmic Reticulum.
6. Write a brief note on quantosomes?

### Long Answer Questions

1. What is a nucleus? What are the functions of a nucleus? Explain in detail with the help of a diagram.
2. Write a detailed note on the Golgi apparatus. Also name three types of elements that form the Golgi apparatus.
3. Which endoplasmic reticulum is connected with detoxification? Discuss.
4. From where do lysosomes arise? Give a detailed answer from your learning of the text. Also support your answer with a diagrammatic representation.
5. What is rough endoplasmic reticulum? How is it different from smooth endoplasmic reticulum? Explain in detail.
6. Write a detailed note on photosynthesis in chloroplasts.

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## 2.12 FURTHER READINGS

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- Rastogi, S. C. 2012. *Cell Biology*, 3rd Edition. New Delhi: New Age International (P) Ltd.
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## NOTES

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## UNIT 3 ARCHITECTURE OF NUCLEUS AND NUCLEAR TRANSPORT

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### Structure

- 3.0 Introduction
- 3.1 Objectives
- 3.2 Architecture of Nucleus: The Nuclear Envelope
- 3.3 Nuclear Transport: Structure of Nuclear Pore Complex and its Role in Nucleocytoplasmic Exchange
- 3.4 Answers to Check Your Progress Questions
- 3.5 Summary
- 3.6 Key Words
- 3.7 Self Assessment Questions and Exercises
- 3.8 Further Readings

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### 3.0 INTRODUCTION

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The nuclear envelope is a highly regulated membrane barrier that separates the nucleus from the cytoplasm in eukaryotic cells. It consists of several distinct components. The core of envelope consists of two cellular membranes arranged parallel to one another and separated by intermembrane space of 10-50 nm.

The replication and transcription of genetic material within nucleus require the participation of large number of proteins that are synthesized in cytoplasm and transported across the nuclear envelope. The m RNAs, t RNAs and ribosomal subunits that are manufactured in the nucleus must be transported through the nuclear envelope.

The study of nuclear transport has been a very active area of research, driven by the development of invitro system capable of importing proteins into the nucleus. Protein containing NLS move into the nucleus in many steps. Nuclear import begins as an NLS containing cargo protein binds to heterodimeric soluble NLS receptor called importin that resides in the cytoplasm

In this unit, you will learn about the architecture of a nucleus and nuclear transport. This unit will also teach you about chromosomes, chromatin and nucleosomes.

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### 3.1 OBJECTIVES

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After going through this unit, you will be able to:

- Understand the architecture of a nucleus

- Discuss the structure of Nuclear pore complex
- Explain the function of nuclear pore complex in nucleocytoplasmic exchange
- Analyse the control of gene expression in eukaryotes

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### **3.2 ARCHITECTURE OF NUCLEUS: THE NUCLEAR ENVELOPE**

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The contents of the nucleus are present as viscous, amorphous mass of material enclosed by complex nuclear envelope that forms a boundary between the nucleus and cytoplasm. Within the nucleus of a typical interphase cell are the chromosomes, present as highly extended nucleoprotein fibers, chromatin, the nuclear matrix, which is protein containing fibrillar network, one or more nucleoli, irregular shaped dense structure that function in synthesis of ribosomal RNA and assembly of ribosomes., the nucleoplasm, the fluid substance in which the solutes is dissolved.

The membranes of the envelope serve as a barrier that keeps ions, solutes, macromolecules from passing between the nucleus and cytoplasm. The two membranes are fused at sites forming circular pores that contain complex assemblies of proteins. The outer membrane is studded with ribosomes and is seen continuous with the membrane of the rough endoplasmic reticulum. The inner surface of envelope is lined by a dense filamentous meshwork called nuclear lamina. The nuclear lamina supports the nuclear envelope and serves as a site of attachments for chromatin fibers at nuclear periphery. The separation of a cell's genetic material from the surrounding cytoplasm is the single most important feature that distinguishes eukaryotes from prokaryotes making the appearance of nuclear envelope a landmark in biological evolution. The envelope consists of two membranes arranged parallel to one another. The membranes serve as a barrier that keeps ions, solutes and macromolecules from passing between the nucleus and cytoplasm. The two membranes are fused at sites forming pores that contain complex assembly of proteins. The outer membrane is studded with ribosomes and is continuous with the membrane of the rough endoplasmic reticulum. The space between the membranes is continuous with the ER lumen. The inner surface of envelope is bound by integral membrane proteins to filamentous meshwork called lamina. The filaments of lamina is composed of polypeptides called lamins. Lamins are members of superfamily of polypeptides that assemble into intermediate filaments of the cytoplasm. The integrity of intermediate filaments is regulated by phosphorylation and dephosphorylation. Mutations in one of the lamin genes (LMNA) are responsible for various human diseases like rare form of muscular dystrophy in which muscle cell contain fragile nuclei. Mutations in Lamin A is linked to disease called Hutchinson- Gilford progeria syndrome, that is characterized by premature ageing and death during teenage years from heart attack.

### **NOTES**

NOTES

**Check Your Progress**

1. What is the nuclear envelope?
2. How is the integrity of intermediate filaments regulated?

**3.3 NUCLEAR TRANSPORT: STRUCTURE OF NUCLEAR PORE COMPLEX AND ITS ROLE IN NUCLEOCYTOPLASMIC EXCHANGE**

The nuclear envelope is the barrier between the nucleus and cytoplasm and nuclear pores are the gateways across the barrier. Unlike the plasma membrane, which prevents passage of macromolecules between cytoplasm and extracellular space, envelope is a hub of activity for movement of RNAs and proteins in both directions between the nucleus and cytoplasm. The replication and transcription of genetic material within nucleus require the participation of large number of proteins that are synthesized in cytoplasm and transported across the nuclear envelope. The mRNAs, tRNAs and ribosomal subunits that are manufactured in the nucleus must be transported through the nuclear envelope. Some components such as snRNAs move in both the directions, they are synthesized in the nucleus, assembled into RNP particles in the cytoplasm and then shipped back to the nucleus where they function in mRNA processing.

Materials as large as gold particles and ribosomal subunits can penetrate nuclear pore, one assumes that these pores are open channels, but opposite is true. Nuclear pores contain a complex, basketlike apparatus called nuclear pore complex that appears to fill the pore. The nuclear pore complex contains 100 different proteins and mass 30 times that of a ribosome. When low molecular weight solutes are injected into the cytoplasm of a cell, they penetrate the pores by simple diffusion. The ability of larger molecules to pass from the cytoplasm into the nucleus correlates with their normal location within the cell. In 1982, Robert Laskey and his coworkers at the medical research council of England found that nucleoplasmin, one of the abundant nuclear proteins of oocytes, contain a stretch of amino acids near its C terminus that function as a nuclear localization signal. This sequence enables a protein to pass through the nuclear pores and enter the nucleus.

Conversely if nuclear localization signal is fused to a non-nuclear protein such as serum albumin, and injected into the cytoplasm, modified protein becomes concentrated in the nucleus. Thus targeting of proteins to the nucleus is similar to trafficking of proteins that are destined for segregation within a particular organelle such as mitochondria or peroxisome. The study of nuclear transport has been a very active area of research, driven by the development of invitro system capable of importing proteins into the nucleus. Protein containing NLS move into the nucleus in many steps. Nuclear import begins as an NLS containing cargo protein binds to heterodimeric soluble NLS receptor called importin that resides in the cytoplasm.



The transport receptor escort the protein cargo to outer surface of nucleus where it docks with cytoplasmic filaments that extend from outer ring of the NPC. The receptor cargo complex moves through the nuclear pore by hopping from one binding site on the NPC to the next. The translocation of protein cargo through the pore is accompanied by changes in conformation of transporter, the large plug like structure situated in the centre of nuclear pore complex. Following translocation, the receptor cargo complex, is bound by a GTP binding protein called Ran, which induces the dissociation of the complex. Like other GTP binding proteins, Ran exists in an active GTP bound form or inactive GDP bound form. Ran's role in regulating nucleocytoplasmic transport is based on mechanism in which cell maintains a high concentration of Ran-GTP in nucleus and a very low concentration of Ran-GTP in cytoplasm.

## NOTES

The steep gradient of Ran- GTP across the nuclear envelope depends on the compartments of accessory proteins. One of these accessory proteins is sequestered in the nucleus where it promotes the conversion of Ran- GDP to Ran- GTP, thus maintaining the high nuclear level of Ran- GTP. Another accessory protein resides in the cytoplasm where it promotes the hydrolysis of Ran- GTP to Ran- GDP, maintaining low cytoplasmic level of Ran- GTP. Thus the energy released by GTP hydrolysis is used to maintain the Ran- GTP gradient across the nuclear envelope. The imported cargo is released into the nucleoplasm, and one portion of the NLS receptor is shuttled back to the cytoplasm together with the bound Ran. Once inside the cytoplasm, the GTP molecule bound to Ran is hydrolyzed, releasing Ran-GDP from the importin  $\alpha$  subunit (Refer Figure 3.1). RanGDP is shuttled back to the nucleus, where it is converted back to the GTP bound state for additional rounds of activity.

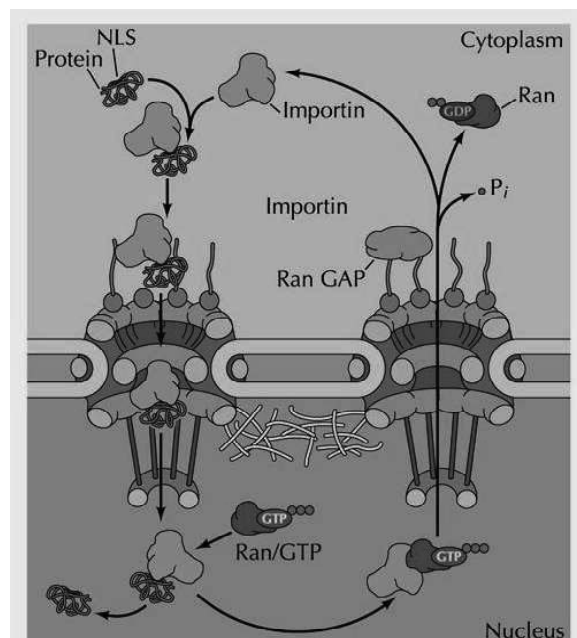


Fig 3.1 Ran-GTP in Cytoplasm and Nucleus

## NOTES

### The Nucleus as an Organized Organelle

The cytoplasm of a eukaryotic cell under the electron microscope shows the presence of diverse array of membranous organelle and cytoskeletal elements. With the development of new types of microscopic techniques, it become possible to localize specific DNA and RNA sequence within the nucleus.

Many important processes that occur within the nucleus includes transcription, RNA processing and replication are compartmentalized. Ribosomal RNA is synthesized and processed in a nucleolus but m RNAs are also synthesized and processed in restricted region of a nucleus. Rather than being spread uniformly throughout the nucleus, the processing machinery appears to be localized to 20-50 irregular regions called speckles. These speckle domains function as storage depots that supply splicing factors for use at nearby sites of transcription. The various components of the nucleus, including nucleoli and speckles are ordered within the compartment by a complex, interactive network of filaments that make up nuclear matrix.

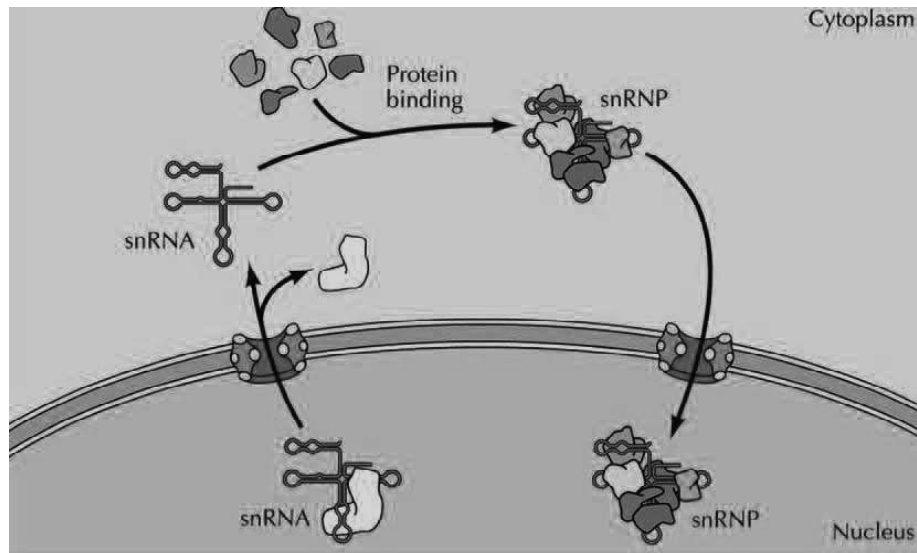
### The Nuclear Matrix

When nuclei are treated with non-ionic detergents and high salt which remove lipids and all of histone and non-histone proteins of the chromatin, DNA is seen as a halo surrounding a residual nuclear core.

If DNA fibres are digested with DNAase, the structure that possess the same shape as original nucleus but is composed of a network of thin protein containing fibrils. This insoluble fibrillar network is called nuclear matrix. The nuclear matrix serve as more than a skeleton to maintain the shape of the nucleus or a scaffold on which to organize loops of chromatin.

It serve to anchor much of machinery that is involved in various activities of the nucleus including transcription, RNA processing and replication. For example, if cells are incubated with radioactive RNA or DNA precursors for a brief period, nearly all the newly synthesized nucleic acid is found to be associated with the fibrils of nuclear matrix.

When nuclei are extracted with detergents and high salt, all of the newly transcribed pre m RNAs are associated with the nuclear matrix which remains within the nuclei. The newly synthesized RNAs are associated with stable elements of the nuclear matrix. As the pre m RNAs move away from the gene where they are synthesized, the introns are removed from the transcript by cell splicing machinery. The orientation of the track from nuclear interior towards nuclear periphery is consistent with a model in which nuclear matrix guides RNAs from their site of transcription to one or few nuclear pores.



**Fig 3.2** *snRNA, Protein Binding in Cytoplasm and Nucleus*

## NOTES

### Chromosomes and Chromatin

An average human cell contains about 6.4 billion base pairs of DNA divided among 46 chromosomes. Each unreplicated chromosome contains a single continuous DNA molecule, the larger the chromosome, longer the DNA it contains. Each base pair is about 0.34 nm in length, 6 billion base pairs constitute a DNA molecule fully 2m long.

### Nucleosomes

Chromosomes are composed of DNA and associated protein which together is called chromatin. The orderly packaging of eukaryotic DNA depends on histones, group of small proteins that possess high content of basic amino acids arginine and lysine. The amino acid sequences of histones, H3 and H4 has undergone little change over long period of time. Histones interact with backbone of DNA which is identical in all organisms. Nearly all amino acid in a histone molecule are engaged in interaction with another molecule either DNA or another histone. As a result very few amino acids can be replaced with other amino acid without affecting the function of a protein. Each nucleosome contains a nucleosome core particle consisting of 146 base pairs of supercoiled DNA wrapped twice around a disk shaped complex of eight histone molecules. The histone core of each nucleosome consists of two copies of each histones H2A, H2B, H3 and H4 assembled into octamer. The remaining type H1 reside outside the nucleosome particle. H1 histone is referred as linker histone as it binds to part of linker DNA that connects one nucleosome particle to the next. Together the H1 protein and histone octamer interact with 168 base pairs of DNA. DNA packaging has been advanced by dramatic portraits of nucleosome core particle obtained by X-ray crystallography. The eight histone molecules are organized into 4 heterodimers. Dimerization of histone is mediated

by C terminal domains consisting of alpha helix cylinders. In contrast, N terminal segment of each core histone takes the form of long flexible tail that extends through DNA helix and into the surrounding.

## NOTES

DNA and core histone are held together by types of non-covalent bonds including ionic bond between negatively charged phosphates of the DNA backbone and positively charged residues of the histones. In between these points of contact, two molecules are separated by space which provide access to DNA for transcription factors and other DNA binding proteins. With a nucleotide-nucleotide spacing of 0.34 nm, 200 base pairs of single 10 nm nucleosome would stretch nearly 70 nm. It is said that packaging ratio of DNA of nucleosomes is approximately 7:1.

### Higher Levels of Chromatin Structure

A DNA molecule wrapped around nucleosome core particle of 10 nm diameter is the lowest level of chromatin organization. The assembly of 30 nm fibre increases the DNA packing ratio an additional 6 fold. Maintenance of 30 nm fibre depends on the interaction between histone molecules of nucleosomes. Linker histones and core histones both been implicated in higher order packaging of chromatin. Structural studies indicate that the N terminal tail of an H4 histone from one nucleosome particle can reach out and make contact with both the linker DNA between nucleosome particle and H2A/ H2B dimer of adjacent particles. These types of interaction, are known to cause the folding of filament into a fibre. 30 nm chromatin fibre is gathered into a series of large supercoiled loops that are compacted into thicker fibres. (80-100nm). The DNA loops are tethered at their ends to proteins that are part of an organized nuclear scaffold. Included among these proteins is type II topoisomerase that regulates the degree of DNA supercoiling.

### Heterochromatin and Euchromatin

10 percent of the chromatin remains in a condensed compact form throughout the interphase. This compacted dense stained chromatin present at the periphery of the nucleus. Chromatin that remains compacted during interphase is called heterochromatin. Heterochromatin is divided into two classes: constitutive and facultative heterochromatin. Constitutive heterochromatin remains in compact state in all cells and represent DNA that is permanently silenced. The DNA of constitutive heterochromatin consists of repeated sequences and few genes. When genes that are active move into position adjacent to heterochromatin, they tend to get transcriptionally silenced, known as position effect. The spread of heterochromatin is blocked by specialized barrier sequences in the genome.

Facultative heterochromatin is chromatin that is inactivated during certain phases of organism life. The cells of male have tiny Y chromosome and larger X chromosome. Because the X and Y chromosomes have only few genes in common, males have a single copy of most genes that are carried on the sex chromosomes. Cells of female contain two X chromosome, only one of them is transcriptionally

active. The other X chromosome remains condensed as a heterochromatin clump called Barr body

### **Histone Code and Formation of Heterochromatin**

Cells contain remarkable array of enzymes that are able to add chemical groups to remove them from specific amino acid residues. The residues are subject to modification by methylation, acetylation and phosphorylation which leads to emergence of histone code, which states that activity of particular region of chromatin depends upon specific modification or combination of modification. Histone modification influence chromatin structure and function at two levels by affecting the degree of compaction, i.e., whether a chromatin is heterochromatic or euchromatic and that the specific gene will be transcribed. The lysine residue at the 9 position of the H3 histone in heterochromatic domains is methylated, whereas the same residue in euchromatic domains are unmethylated. Removal of acetyl groups from H3 and H4 histones is initial steps in conversion of euchromatin into heterochromatin.

### **NOTES**

### **Structure of Mitotic Chromosome**

When a chromosome undergoes compaction during prophase, it adopts a predictable shape by the length of DNA molecule. If the individual chromosomes are seen and ordered according to decreasing size, is called karyotype. Chromosome shown in karyotype is prepared using staining procedure that gives chromosomes cross banded appearance. The pattern of band is highly characteristic for each chromosome of a species. Karyotypes are prepared from culture of blood cell and used to screen for chromosomal abnormalities.

Each chromosome contains a single, double stranded DNA molecule. The tips of DNA molecule are composed of stretch of repeated sequences together with group of specialized proteins form cap at each end of the chromosome called telomere. Telomere contain sequence repeated from about 500 to 5000 times. Unlike most repeated sequences that vary from species to species, the same telomere sequence is found throughout the vertebrates. This similarity in sequence suggests that telomeres have conserved function in diverse organism. The protein that is bound to chromosomes plays a role in regulating telomere length. If telomere has such a huge impact on limiting the number of times a cell divide, one can expect telomeres to be major factor in human ageing. Studies have suggested that older person whose cells have shorter telomere are more likely to have cardiovascular disease. In Werner's syndrome, an inherited disease that cause patient to age more rapidly than normal is characterized by abnormal telomere maintenance. Women who are under stress as a result of caring for ill children have shorter telomere and reduced telomerase activity. Telomere shortening plays a key role in protecting humans from cancer by limiting the number of divisions of tumor cell.

## NOTES

### Centromere

Centromere contains a tandemly repeated, 171 base pair DNA sequence that extends for at least 500 kilobases. This stretch of DNA associates with specific proteins that separate it from other parts of chromosome. Centromeric chromatin binds specific proteins that serve as attachment site for microtubules that separate chromosome during cell division. The kinetochore assembles at centromere because of CENP-A at that site.

### Control of Gene Expression in Bacteria

In bacteria, the genes that encode the enzymes of metabolic pathway are clustered together on the chromosome in functional complex called an operon. A typical operon consists of structural genes, promoter region, operator region and regulatory gene.

### The Bacterial Operon

Structural gene code for the enzymes lie adjacent to one another and RNA polymerase moves from one gene to another transcribing all the genes into single mRNA. This mRNA is translated into various enzymes of metabolic pathways. Turning on one gene turns on all the enzymes producing genes of operon

The promoter is the site where the RNA polymerase binds to DNA prior to transcription. The operator resides adjacent to the promoter serve as binding site for protein called repressor. The repressor is an example of gene regulatory protein-protein that recognizes a specific sequence of base pairs within DNA and bonds to that sequence. The regulatory gene encodes repressor protein.

### Control of Gene Expression in Eukaryotes

Complex plants and animals are composed of many different types of cells and a genome containing tens of thousands of genes. Vertebrates are composed of hundreds of different cell types, each more complex than a bacterial cell. Frederick Steward and his colleague at Cornell University showed that a root cell isolated from mature plant could be induced to grow into fully developed plant. The average bacterial cell contains DNA to encode 3000 polypeptides of which one third are expressed. Contrary to this human cell contains enough DNA to encode million different polypeptides. Though vast majority of this DNA does not contain protein coding information, a mammalian cell manufactures many different polypeptides at any time. Many polypeptides, such as enzymes of glycolysis and electron carriers of respiratory chain are synthesized by all the cells of body. Regulation of gene expression occurs at three levels.

Transcriptional level control mechanism determines whether a gene can be transcribed. Processing level control mechanism determines the path by which primary mRNA transcript is processed into mRNA that can be translated into a polypeptide. Translational level control mechanism determines whether a particular mRNA is translated or not.

### Check Your Progress

3. What does an average human cell contain?
4. What are chromosomes composed of?
5. What does a typical operon consists of?

### NOTES

## 3.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. The nuclear envelope is a highly regulated membrane barrier that separates the nucleus from the cytoplasm in eukaryotic cells.
2. The integrity of intermediate filaments is regulated by phosphorylation and dephosphorylation.
3. An average human cell contains about 6.4 billion base pairs of DNA divided among 46 chromosomes.
4. Chromosomes are composed of DNA and associated protein which together is called chromatin.
5. A typical operon consists of structural genes, promoter region, operator region and regulatory gene.

## 3.5 SUMMARY

- The nuclear envelope is a highly regulated membrane barrier that separates the nucleus from the cytoplasm in eukaryotic cells. It consists of several distinct components.
- The membranes of the envelope serve as a barrier that keeps ions, solutes, macromolecules from passing between the nucleus and cytoplasm.
- The separation of a cell's genetic material from the surrounding cytoplasm is the single most important feature that distinguishes eukaryotes from prokaryotes making the appearance of nuclear envelope a landmark in biological evolution.
- The envelope consists of two membranes arranged parallel to one another. The membranes serve as a barrier that keeps ions, solutes and macromolecules from passing between the nucleus and cytoplasm.
- The filaments of lamina are composed of polypeptides called lamins.
- Lamins are members of superfamily of polypeptides that assemble into intermediate filaments of the cytoplasm.
- The integrity of intermediate filaments is regulated by phosphorylation and dephosphorylation.

## NOTES

- Mutations in one of the lamin genes (LMNA) are responsible for various human diseases like rare form of muscular dystrophy in which muscle cell contain fragile nuclei.
- The nuclear envelope is the barrier between the nucleus and cytoplasm and nuclear pores are the gateways across the barrier.
- The replication and transcription of genetic material within nucleus require the participation of large number of proteins that are synthesized in cytoplasm and transported across the nuclear envelope.
- Materials as large as gold particles and ribosomal subunits can penetrate nuclear pore, one assumes that these pores are open channels, but opposite is true.
- The nuclear pore complex contains 100 different proteins and mass 30 times that of a ribosome.
- Conversely if nuclear localization signal is fused to a non-nuclear protein such as serum albumin, and injected into the cytoplasm, modified protein becomes concentrated in the nucleus.
- The study of nuclear transport has been a very active area of research, driven by the development of invitro system capable of importing proteins into the nucleus.
- The translocation of protein cargo through the pore is accompanied by changes in conformation of transporter, the large plug like structure situated in the centre of nuclear pore complex.
- The cytoplasm of a eukaryotic cell under the electron microscope shows the presence of diverse array of membranous organelle and cytoskeletal elements.
- Many important processes that occur within the nucleus includes transcription, RNA processing and replication are compartmentalized.
- When nuclei are treated with non-ionic detergents and high salt which remove lipids and all of histone and non-histone proteins of the chromatin, DNA is seen as a halo surrounding a residual nuclear core.
- When nuclei are extracted with detergents and high salt, all of the newly transcribed pre m RNAs are associated with the nuclear matrix which remains within the nuclei.
- An average human cell contains about 6.4 billion base pairs of DNA divided among 46 chromosomes.
- Chromosomes are composed of DNA and associated protein which together is called chromatin.
- The remaining type H1 reside outside the nucleosome particle. H1 histone is referred as linker histone as it binds to part of linker DNA that connects one nucleosome particle to the next.



- DNA and core histone are held together by types of non-covalent bonds including ionic bond between negatively charged phosphates of the DNA backbone and positively charged residues of the histones.
- A DNA molecule wrapped around nucleosome core particle of 10 nm diameter is the lowest level of chromatin organization.
- The assembly of 30 nm fibre increases the DNA packing ratio an additional 6 fold.
- Facultative heterochromatin is chromatin that is inactivated during certain phases of organism life.
- Centromere contains a tandemly repeated, 171 base pair DNA sequence that extends for at least 500 kilobases.
- In bacteria, the genes that encode the enzymes of metabolic pathway are clustered together on the chromosome in functional complex called an operon.
- Structural gene code for the enzymes lie adjacent to one another and RNA polymerase moves from one gene to another transcribing all the genes into single mRNA.
- Frederick Steward and his colleague at Cornell University showed that a root cell isolated from mature plant could be induced to grow into fully developed plant.

## NOTES

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### 3.6 KEY WORDS

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- **Transcription:** It is the step of gene expression, in which a particular segment of DNA is copied into RNA by enzyme RNA polymerase
- **Translation:** It is the process in which ribosomes in the cytoplasm or ER synthesize proteins after the process of transcription of DNA to RNA in the nucleus
- **Operons:** It is the functioning unit of DNA containing cluster of genes under the control of a single promoter.
- **Heterochromatin:** This is a tightly packed form of DNA or condensed DNA which comes in varieties.
- **Euchromatin:** It is a chromosome material which doesn't stain strongly except during cell division. It represents major genes and is involved in transcription.
- **Histones:** These are highly alkaline protein present in eukaryotic cell nuclei that package and order DNA into units called nucleosomes. They are chief protein components of chromatin.
- **Nuclear lamina:** These are fibre like network present inside the nucleus of most cells. Mainly composed of intermediate filaments and membrane

## NOTES

associated proteins. Besides providing mechanical support, it regulates important cellular events like division and replication.

- **Nuclear matrix:** It is a network of fibres found throughout the nucleus and analogous to cell cytoskeleton.
- **Telomere:** These are cap like structure at the end of each strand of DNA that protect the chromosome. They protect genetic information during cell division.
- **Centromere:** These are specialized DNA sequence of chromosome that links a pair of sister chromatids. Spindle fibres are attached to the centromere.

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### 3.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

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#### Short Answer Questions

1. Write a short note on nuclear envelope.
2. What are lamins?
3. Write a brief note on nuclear matrix.
4. What are chromatins that remains compacted during interphase called? Briefly discuss the nature of chromatins.
5. Write a brief note on the structure of mitotic chromosome.
6. What do you mean by the bacterial operon?
7. What do you mean by histone code? Discuss its characteristics.

#### Long Answer Questions

1. What are chromosomes and chromatin? It is said that nearly all amino acid in a histone molecule are engaged in interaction with another molecule either DNA or another histone. Discuss the statement.
2. "DNA and core histone are held together by types of non-covalent bonds including ionic bond between negatively charged phosphates of the DNA backbone and positively charged residues of the histones." Explain the statement with the help of your learning of the text.
3. Write a note on the histone code and the formation of heterochromatin.
4. "When a chromosome undergoes compaction during prophase, it adopts a predictable shape by the length of DNA molecule." With the help of your learning of the text, discuss the structure and function of mitotic chromosome.
5. "The orderly packaging of eukaryotic DNA depends on histones." Discuss the packaging of eukaryotic DNA with respect to nucleosomes.

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### **3.8 FURTHER READING**

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### **NOTES**

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## UNIT 4 ARCHITECTURE AND FUNCTIONS OF CYTOSKELETON NETWORKS

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### Structure

- 4.0 Introduction
- 4.1 Objectives
- 4.2 Architecture and Functions of Cytoskeleton Networks
  - 4.2.1 Microfilaments: Architecture and Functions
  - 4.2.2 Intermediate Filaments: Architecture and Functions
  - 4.2.3 Microtubules: Architecture and Functions
- 4.3 Microfilaments and Microtubules
- 4.4 Answers to Check Your Progress Questions
- 4.5 Summary
- 4.6 Key Words
- 4.7 Self Assessment Questions and Exercises
- 4.8 Further Readings

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### 4.0 INTRODUCTION

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A cytoskeleton is present in the cytoplasm of all cells, including bacteria, and archaea. It is a complex, dynamic network of interlinking protein filaments that extend from the cell nucleus to the cell membrane. The cytoskeletal systems of different organisms are composed of similar proteins. In eukaryotes, the cytoskeletal matrix is a dynamic structure composed of three main proteins, which are capable of rapid growth or disassembly dependent on the cell's requirements at a certain period of time.

The structure, function and dynamic behavior of the cytoskeleton can be very different, depending on organism and cell type. Even within one cell the cytoskeleton can change through association with other proteins and the previous history of the network.

A multitude of functions can be performed by the cytoskeleton. Its primary function would arguably be to give the cell its shape and mechanical resistance to deformation, and through association with extracellular connective tissue and other cells it stabilizes entire tissues. The cytoskeleton can also contract, thereby deforming the cell and the cell's environment and allowing cells to migrate. Moreover, it is involved in many cell signaling pathways: in the uptake of extracellular material (endocytosis), segregates chromosomes during cellular division, is involved in cytokinesis (the division of a mother cell into two daughter cells), provides a

scaffold to organize the contents of the cell in space and for intracellular transport (for example, the movement of vesicles and organelles within the cell); and can be a template for the construction of a cell wall. Furthermore, it forms specialized structures, such as flagella, cilia, lamellipodia and podosomes.

In this unit, you will learn about cytoskeleton, architecture and functions of cytoskeleton networks of microfilaments, intermediate fibres, microtubules, difference between microfilaments and microtubules in detail.

## NOTES

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### 4.1 OBJECTIVES

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After going through this unit, you will be able to:

- Understand what cytoskeleton is
- Explain the architecture and functions of cytoskeleton networks of microfilaments, intermediate fibres and microtubules
- Distinguish between microfilaments and microtubules

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### 4.2 ARCHITECTURE AND FUNCTIONS OF CYTOSKELETON NETWORKS

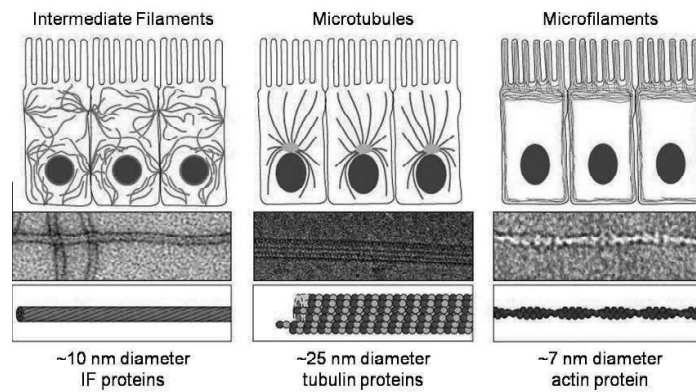
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The skeleton of a vertebrate is organ system that supports the soft tissues of the body and help in movements. The cell size increases during the course of evolution of eukaryotic cell, microtubules, microfilaments and intermediate filaments are necessary to support the extensive system of membranes. These organelles form the cytoskeleton of cell. All of them are linear, unbranched polymers of protein sub-units. Their proteins have low molecular weight and have capacity for rapid polymerization to cause assembly. The cytoskeletal organelles keep other organelles separated from one another to avoid interference in another activities. The microtubules and microfilaments are responsible for cellular movements. Microtubules are major component of cilia, flagella, centrioles and basal bodies. A living eukaryotic cell also possess a skeletal system which has analogous function. It is composed of three filamentous structures- microtubules, microfilaments and intermediate filaments which together form elaborate network. A three dimensional interconnected network of proteins that forms not only structural framework inside a eukaryotic cell is cytoskeleton.

There are three types of skeletal structures (Refer Figure 4.1):

- Microfilaments
- Intermediate Fibres
- Microtubules

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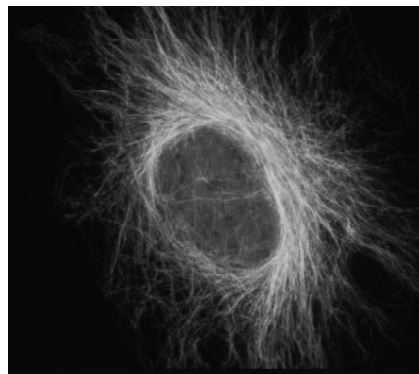


**Fig. 4.1** Three Types of Skeletal Structures: Microfilaments, Intermediate Fibres and Microtubules

### 4.2.1 Microfilaments: Architecture and Functions

Microfilaments are solid thinner structures composed of protein actin. Microtubules are rigid tubes composed of protein tubulin. Intermediate filaments are tough rope like fibres made of actin. They together are highly dynamic group of structures capable of rapid reorganization. Cytoskeleton function as a dynamic scaffold providing structural support that determine the shape and resist forces that deform it. An internal framework responsible for positioning the organelles which is performed by polarized epithelial cell. A network of tracks that direct the movement of materials and organelles within cell, for example, include delivery of mRNA to specific parts of cell, movement of carriers from ER to Golgi complex and transport of vesicles containing neurotransmitter down the length of nerve cell. Microtubules are the tracks over which peroxisomes are transported in mammalian cell. An essential component of cell division machinery.

Cytoskeletal elements make up apparatus responsible for separating chromosomes during mitosis and meiosis and splitting the parent cell into daughter cell. Cytoskeleton is most extensively studied subject in cell biology due to development of techniques that allow to pursue a coordinated morphological, biochemical and molecular approach (Refer Figure 4.2).



**Fig. 4.2** Cytoskeleton

The cytoskeleton function as machinery that moves materials and organelles within the cells, i.e., movement of transport vesicles from endoplasmic reticulum to the Golgi complex, and movement of vesicles containing neurotransmitters down the length of a nerve cell. It is a scaffold that provides structural support and helps maintain the shape of the cell. It is a site for anchoring mRNAs and facilitating their translation into polypeptides. Cytoskeletal elements make up the apparatus responsible for separating the chromosomes during mitosis and meiosis.

The microfilaments are visible only with electron microscope. They are ultramicroscopic, long, cylindrical rods which occur in eukaryotic cell. They are made up of actin constitute 10-15% protein. Cells are capable of remarkable motility. The neural crest cell in a vertebrate embryo leave the developing nervous system and migrate across the width of embryo forming products as pigment cells of skin, teeth and cartilage of jaws. Certain parts of cell can be motile.

Microfilaments are involved in intracellular motile processes such as movement of vesicles, phagocytosis and cytokinesis. Plant cells are thought to rely on microfilaments rather than microtubules for long distance transport of vesicles and organelles. It is found in all the eukaryotic cell (algae, fungi, plants and animals). They form extensive network in cytoplasm of non muscle cell known as myofilaments. They are permanent organelles in some cell types and appear and breakdown in others. They are solid, unbranched, rod like fibres of indefinite length. They are composed of globular protein actin but some filamentous protein myosin are also associated with it. A microfilament called as F actin, a polymer of dumbbell shaped subunits called G actin. An actin monomer has tightly bound ATP or ADP. Actin from organism as diverse as mammals can copolymerize showing actin has conserved many of its properties during evolution polymerised actin is in equilibrium with monomeric actin like tubulin of microtubules. Microfilaments are 8 nm in diameter and composed of globular subunits of protein actin. In the presence of ATP, actin monomers polymerize to form flexible, helical filament.

### **Microfilaments Assembly and Disassembly**

An actin monomer binds a molecule of ATP. Actin is ATPase just as tubulin is GTPase, and role of ATP in actin assembly is similar to GTP in microtubule assembly. The ATP associated with actin monomer is hydrolysed to ADP after it is incorporated into growing actin filament. As a result, bulk of actin consists of ADP- actin sub-units (Refer Figure 4.3).

Actin polymerization is demonstrated in in-vitro solutions containing ATP actin monomers. Cells maintain a dynamic equilibrium between monomeric and polymeric forms of actin just as with tubulins and microtubules. Change in local condition in particular part of cell can push the equilibrium towards either assembly or disassembly of microfilaments. Actin filaments play role in all of cells motile processes. The involvement of these filaments is demonstrated by treating the cells with drugs that disrupt microfilament based activities.

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Myosin is first isolated from skeletal muscle tissue and from wide variety of eukaryotic cell including protist, plants, non muscle cell of animals and cardiac and smooth muscle tissue. All myosin share a motor domain.

Myosins are divided into two groups:

- Conventional (Type II) Myosins , first seen in muscle tissue
- Unconventional Myosin

**Conventional ( Type II) Myosins:** Proteins of the myosin II class are primary motors for muscle contraction and is found in variety of non muscle cell. Type II are required for splitting a cell in two during cell division. The myosin II filaments that assemble in skeletal muscle cell are highly stable components of contractile apparatus

**Unconventional Myosins:** Several unconventional myosins are associated with various types of vesicles and organelles. Some vesicles contain microtubule based motors and microfilament based motors and two types of motors are physically linked to one another.

Microfilaments grow by addition of sub-units at both the ends. In animal cells microfilaments help in maintaining supporting framework for villi, pseudopodia and undulations. They take part in invaginating cell membrane during cleavage cytokinesis of animal cell. In some primitive organism, spindle apparatus is made up of microfilaments. Microfilaments along with myosin form constituent of myofibrils. They are contractile in nature. In pollen tubes, microfilaments guide the vesicles containing wall precursors towards the tip where growth of new wall is occurring, help in tip growth.

### Muscle Contractibility

Skeletal muscle cell have highly organized internal structure. A longitudinal section of muscle fibre reveals a cable made up of hundreds of thin cylindrical strand called myofibrils. Each myofibril consists of a repeating linear array of contractile units called sarcomeres. Each sarcomere exhibit a banding pattern which gives muscle fibre a striped appearance. All skeletal muscle operate by shortening . The units of shortening are sarcomeres whose combined decrease in length accounts for decrease in length of entire muscle.

In addition to actin, thin filaments of a skeletal muscle contain two proteins, tropomyosin and troponin. The tropomyosin is an elongated molecule that fit into the grooves within thin filament. Each rod shaped tropomyosin is associated with seven actin subunits along the thin filament. Troponin is a globular protein complex composed of three sub-units each having important role in overall function of the molecule. During contraction, each myosin head extend outward and binds to thin filament forming the cross bridges between two types of filaments



## Non-Muscle Motility

Skeletal muscle cells are an ideal system for study of contractility and movement because interacting contractile proteins are in high concentration and are part of defined cellular structure. The study of non-muscle motility is more challenging because critical components tend to be present in less ordered, more labile arrangements. They are restricted to a thin cortex beneath the plasma membrane. Purified actin can polymerize to form actin filaments, but such filaments cannot interact with one another to perform useful activities. The organization and behavior of actin filaments inside cells are determined by a variety of actin-binding proteins that affect assembly or disassembly of actin filaments, their physical properties, their interaction with one another and with other organelles.

Cell locomotion is required for many activities in higher vertebrates including tissue and organ development, formation of vessels, protection against infection. Cell locomotion contributes to the spread of cancerous tumours, for example, with a rounded white blood cell that receives a chemical signal coming from one direction. Once the stimulus is received at the plasma membrane, it triggers the localized polymerization of actin which leads to polarization of the cell and its movement towards the source of stimulus.

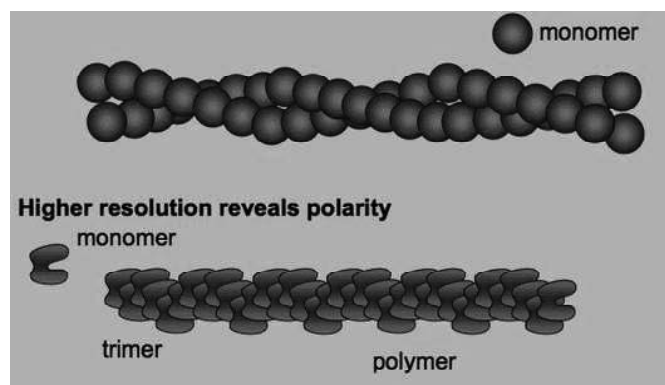


Fig. 4.3 Microfilaments: Twisted Pair of G-Actin Polymers

## Functions of Microfilaments

- The microfilaments bring about directed movement of particles and organelles in the cell.
- The microfilament bring about cleavage of cytoplasm to form two daughter cells.
- The microfilaments produce streaming movement of cytoplasm in both plant cell and animal cells. They have a layer of actin microfilaments between static cytoplasm and streaming cytoplasm causing unidirectional streaming.
- They form the part of cytoskeleton to support the cytosol and give form to cell processes. The actin-binding protein fimbrin and villin cross link the microfilaments to one another.

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- Microfilaments are responsible for changes in cell shape during development, division and motility.
- In invertebrates, contact of sperm with egg triggers the formation of finger like extension, acrosome process. The acrosomal process penetrates the jelly coat and vitelline membrane of egg enabling the sperm and egg plasma membrane to fuse.
- They also participate in gliding amoeboid locomotion shown by embryonic cells, leucocytes and macrophages.

### 4.2.2 Intermediate Filaments: Architecture and Functions

Intermediate filaments were discovered in muscle cell in 1968. They are found in animal cell in association with microtubules. They are absent in prokaryotes. They are rope like proteinaceous filaments of 8012 nm thick that occur singly, in bundles or network. They are most stable and least soluble of three cytoskeletal elements. They are intermediate in thickness. The protein monomers forming intermediate filaments are fibrous instead of globular. Two dimers align laterally but in antiparallel sequence to form tetrameric protofilament. Protofilaments get joined laterally and longitudinally to form intermediate filaments. It has rope like character with 8 protofilaments joined together. It occurs in cell and cell areas subject to mechanical stress. They don't occur in unicellular eukaryotes. Unlike microfilaments and microtubules, Ifs are heterogenous group of structures that are encoded by more than 65 different genes. The polypeptide sub-units of Ifs are divided into five major classes based on the type of cell where they are found as well as biochemical, genetic and immunologic criteria. Most of these polypeptides have similar arrangement of domains that allow them to form similar looking filaments. The polypeptides of Ifs contain central, rod shaped, helical domain of similar length and homologous amino acid sequence (Refer Figure 4.4).

**Structure:** They are long, unbranched filaments having diameter of 10 nm which is intermediate between microtubules and microfilaments. They are composed of protein sub-units which are extended molecules and form rope like polymers.

An IF sub-unit consists of 3 regions: head, central rod and tail. The central rod is 40 nm long and is formed by coiling  $\alpha$  helical section of two polypeptides around each other. Dimers join side by side forming tetramer. The tetramer join end to end and form protofilaments. Eight protofilaments are aligned lengthwise to form cylindrical thick filament. Intermediate filaments are insoluble under wide range of physiological conditions.

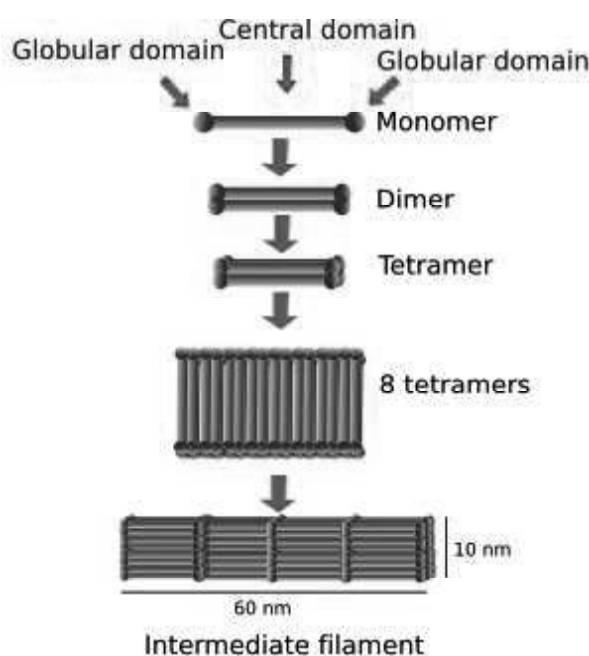
### Intermediate Filament Types

Intermediate filament is of five types of IF sub-unit proteins as seen in higher vertebrates:

- **Desmin:** Desmin containing IFs occur in muscle cells. They integrate various components of muscle cell into functional unit capable of shortening. These filaments may join Z lines to plasma membrane

- **Vimentin:** It occurs in mesenchyme cells such as fibroblast, endothelial cell, adipose cell and epithelial cell. They connect the nuclear envelope with plasma membrane. They serve to keep nucleus in place in the cell.
- **Neurofilaments:** They are found in axons of neurons in vertebrates. Neurofilaments and microtubules grow by addition of new subunits in cell body. Both remain closely associated and provide strength and rigidity to axons.
- **Cytokeratins:** It occurs in epithelial cells. Keratin filaments are joined to spot desmosomes, which interconnect adjacent cells. These junctions give strength and rigidity to the epithelium.
- **Glial Fibrillary Acidic Protein (GFAP):** This protein forms intermediate filaments in glial cells that surround neurons.

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*Fig. 4.4 Intermediate Filaments*

### Intermediate Filament Assembly and Disassembly

The unit of IF assembly is tetramer formed by two dimers that are aligned side by side in fashion with their N and C termini pointing in opposite direction. Because the dimer point in opposite direction, tetramer lacks polarity. Tetramers associate with one another side to side and end to end to form intermediates that assemble into final filament. Like the collagen fibres of extracellular matrix which is composed of staggered sub-units, Ifs are resistant to tensile forces.

Ifs tend to be less sensitive to chemical agents than other type of cytoskeletal elements and difficult to solubilize, because of insolubility, Ifs are permanent, unchanging structures. Epidermal cell contain pool of keratin sub-units like microtubule and microfilament subunits in dynamic equilibrium with polymerized

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form. Keratin filaments constitute the primary structural proteins of epithelial cell. Bundles of keratin containing Ifs form an elaborate cage like network around nucleus and radiate through cytoplasm. The cytoplasm of neurons contain loosely packed bundles of intermediate filaments whose long axes are parallel to that of nerve cell axon. In early stage of differentiation when axon is growing towards a target cell, it contain few neurofilaments but large number of supporting microtubules. Aggregation of NFs is seen in neurodegenerative disorders, ALS and Parkinsons disease. These NF aggregates block axonal transport leading to death of affected neurons. Studies revealed the importance of intermediate filaments in particular cell types. Desmin play a structural role in maintaining the alignment of myofibrils of muscle cell, and absence of these IFs make cell fragile. An inherited human disease named desmin related myopathy is caused by mutations in gene that encodes desmin. Person with this disorder suffer from skeletal muscle weakness, congestive heart failure

### Functions of Intermediate Filament

- They integrate the muscle cells components into a functional unit.
- It form a part of cytoskeleton that supports the fluid cytosol and maintains shape of the cell.
- They keep nucleus and other organelles in place.
- They prevent fusion of lipid droplets with one another.
- Nuclear matrix is formed of intermediate filament.
- Desmosomes are supported by intermediate filaments called tonofibrils.
- Keratin deposited in skin cell provide protection against abrasions.
- Intermediate filaments provide strength to axons and dendrons of nerve cells.

### 4.2.3 Microtubules: Architecture and Functions

Microtubules are unbranched hollow submicroscopic formed of protofilaments of protein tubulin which can undergo quick growth or dissolution by the assembly and disassembly of dimers. Colchicine prevents assembly of microtubules. It prevent spindle formation during cell division. Microtubules are found in cytoplasmic matrix of all eukaryotic cell from amoeba to higher plants. Microtubules also occur in cell organelles such as cilia, flagella, mitotic apparatus, sperm tail, basal body. Prokaryotic cell lack microtubules. The wall of microtubule is composed of globular proteins arranged in rows called protofilaments, aligned parallel to the long axis of the tubule. Each protofilament is formed from dimeric building blocks consisting of one  $\alpha$  tubulin and one  $\beta$  tubulin at other end. One end of microtubule is known as the plus end, the other as minus end.

Microtubule associated proteins: Microtubules contain additional proteins called Microtubule Associated Proteins (MAPs). The microtubule binding activity of various MAPs is controlled primarily by addition and removal of phosphate

group from particular amino acid residues. An high level of phosphorylation of one MAP has been implicated in development of several neurodegenerative disorders. The brain cells of people with these diseases contain strange tangled filaments consisting of molecules that are phosphorylated and unable to bind to microtubules. The neurofibrillary filament contribute to death of nerve cell. Person with inherited dementia called FTDP-17 carry mutation in gene indicating alteration of the protein.

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### **Microtubules as Structural Support and Organizers**

Microtubules are stiff enough to resist forces that compress and bend the fibre. This property enables them to provide mechanical support. The distribution of cytoplasmic microtubules in cell determine the shape of that cell. In cultured animal cell, microtubule extend in array from the area around the nucleus giving these cells round shape. In contrast, microtubules of columnar epithelial cell are oriented with their long axis parallel to long axis of cell. This suggests that microtubules help support the cells elongated shape. The role of microtubule as skeletal element is evident in highly elongate processes as cilia and flagella and axons of nerve cells. In plant cells, microtubules play an indirect role in maintaining cell shape through their influence on formation of cell wall. During interphase, most of microtubules are located beneath the plasma membrane forming cortical zone. The microtubules of cortex influence the movement of cellulose synthesizing enzyme located in the plasma membrane. The cellulose microfibrils of cell wall are assembled in an orientation that is parallel to underlying microtubules of cortex. The orientation of cellulose microtubules play role in determining the growth of cell and its shape. Newly synthesized cellulose microfibrils and coaligned microtubules are arranged perpendicular to the long axis of the cell. They are considered to play role in maintaining the internal organization of cell.

### **Microtubules as Agents of Intracellular Motility**

The transport of material from one membrane compartment to another depends on the presence of microtubules because of specific disruption of these cytoskeletal elements brings the movement to halt. The motor protein of cell convert chemical energy into mechanical energy which is used to move cargo transported by these proteins include ribonucleoprotein particles, vesicles, mitochondria, lysosome , chromosome and other cytoskeletal filaments. Kinesin and dynein move along microtubules whereas myosin move along microfilaments

### **Microtubule Organizing Centres**

The function of microtubule within a living cell depends on its location and orientation which makes it's understand why microtubule assembles in one place opposed to another. Centrosomes are not the only MTOCs in cells. The outer microtubules in cilia and flagella are generated from microtubules in structure called basal body which resides at base of cilia and flagella. Basal body is identical in structure to centriole, in fact basal bodies and centrioles can give rise to one another. Plant cells lack both centrosome and centrioles or any other type of MTOC.

## The Dynamic Properties of Microtubules

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All microtubules appear similar morphologically, there are marked differences in their stability. Microtubules are stabilized by the presence of bound MAPs and by certain post translational modification of tubulin subunits. Microtubules of the mitotic spindle are extremely labile that is sensitive to disassembly. Microtubules of mature neurons are less labile and those of centrioles, cilia and flagella are highly stable. Living cells are subject to variety of treatments that lead to the disassembly of cytoskeletal microtubules without disrupting other cellular structure. Disassembly can be induced by cold temperature, elevated  $\text{Ca}^{2+}$  concentration, variety of chemicals including colchicines, vincristine, vinblastine, podophyllotoxin. The microtubules of the cytoskeleton are subject to depolymerization and repolymerization as the requirements of cell change from one time to another.

The first approach to invitro assembly of microtubule was taken in 1972 by Richard Weisenberg of Temple University. Weisenberg found that microtubules could be disassembled and reassembled over and over by lowering and raising the temperature. GTP is required for microtubule assembly.

### Study of Microtubule Dynamics In-Vivo

Microtubule behavior could be explained by phenomenon called dynamic instability. It refers to the fact that growing and shrinking microtubules can coexist in the same region of the cell, and the given microtubule can switch back and forth between growing and shortening phase. It is a property of the plus end of microtubule, subunits are added to the plus end of microtubule during growth and lost from plus end during shrinkage.

**Structure:** They are hollow, unbranched cylinder about 200-270Å thick and many micrometers long. The wall of microtubule is composed of 13 parallel protofilaments that run its entire length. Microtubules assemble from pools of soluble tubulin dimer by polymerization. With exception of slime moulds and amoebae, microtubules occur widely in eukaryotic cells. They are present in cytoplasm and other structures like centrioles, basal bodies, cilia and flagella, spindle apparatus, nerve processes. They are differentiated into two groups- axonemal and cytoplasmic. Axonemal are stable microtubules present in basal bodies and appendages like cilia and flagella. Cytoplasmic microtubules form a dynamic network. They are polar with plus end and minus end. Microtubules show reorganization at the time of cell division. Microtubules are of indefinite length. The wall is formed of 13 laterally and helically arranged longitudinal strand called protofilaments. These strands are made of heterodimer subunits. Each heterodimer has two closely related globular protein called  $\alpha$  and  $\beta$  tubulins. It has three specific sites- GTP binding, colchicine binding and a non tubulin protein binding site.  $\text{Mg}^{2+}$  and GTP are essential for microtubule assembly. GTP hydrolysis lead to microtubule disassembly. No of proteins get associated with microtubule, they regulate microtubule structure, assembly and function. Proteins that bind along the sides of microtubules are microtubule associated proteins or MAPs (Refer Figure 4.5).

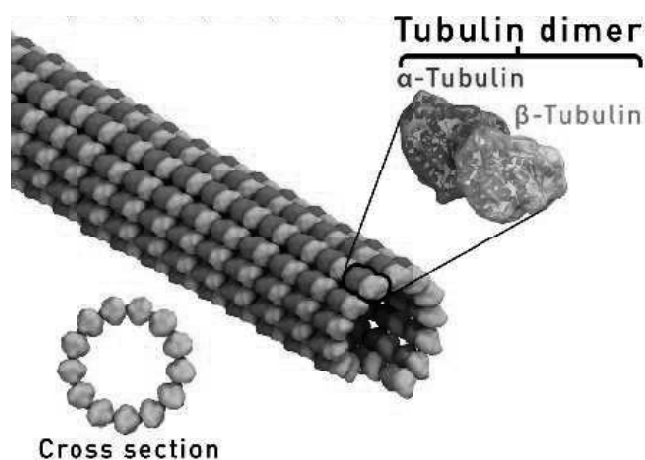


Fig.4.5 Microtubules

### Functions

- They are constituent of spindle fibres, centrioles, basal bodies, flagella and cilia.
- Microtubules function as cytoskeleton. They provide rigidity and shape to cell parts.
- In plant cells, the microtubules control orientation of cellulose microfibrils of the wall.
- They help in the movement of nuclei during division.
- Microtubules help in driving the food in the gullet.
- Being able to slide past one another, microtubules help in movement of cilia and flagella.
- Cell plate formation is found to be determined by a premitotic microtubular band.

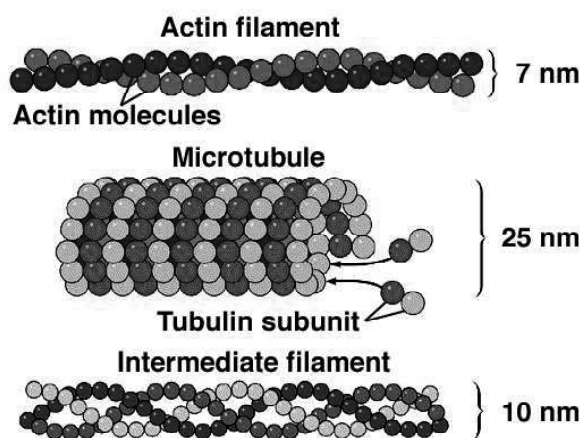


Fig. 4.6 Three Types of Cytoskeleton Filaments

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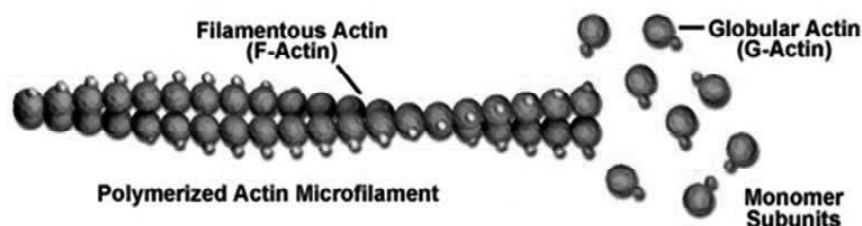
### Check Your Progress

1. What are microfilaments composed of?
2. What are microfilaments involved in?

## 4.3 MICROFILAMENTS AND MICROTUBULES

Microfilaments, also called actin filaments, are filaments in the cytoplasm of eukaryotic cells that form part of the cytoskeleton and are primarily composed of polymers of actin, but in cells are modified by and interact with numerous other proteins. Microfilaments are usually about 7 nm in diameter and composed of two strands of actin. Microfilament functions include cytokinesis, amoeboid movement and cell motility in general, changes in cell shape, endocytosis and exocytosis, cell contractility and mechanical stability. Microfilaments are flexible and relatively strong, resisting buckling by multi-piconewton compressive forces and filament fracture by nanonewton tensile forces. In inducing cell motility, one end of the actin filament elongates while the other end contracts, presumably by myosin II molecular motors. Additionally, they function as part of actomyosin-driven contractile molecular motors, wherein the thin filaments serve as tensile platforms for myosin's ATP-dependent pulling action in muscle contraction and pseudopod advancement. Microfilaments have a tough, flexible framework which helps the cell in movement (Refer Figure 4.7).

Common to all eukaryotic cells, these filaments are primarily structural in function and are an important component of the cytoskeleton, along with microtubules and often the intermediate filaments. Microfilaments range from 5 to 9 nanometers in diameter and are designed to bear large amounts of tension. In association with myosin, microfilaments help to generate the forces used in cellular contraction and basic cell movements. The filaments also enable a dividing cell to pinch off into two cells and are involved in amoeboid movements of certain types of cells.



*Fig. 4.7 Microfilament Structure and Assembly*

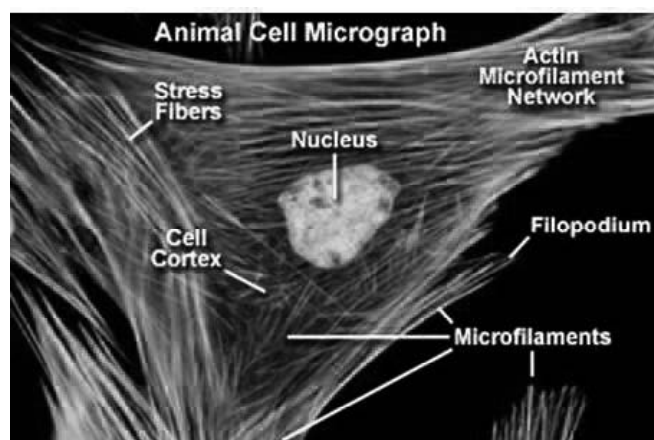
Microfilaments are solid rods made of a protein known as actin. When it is first produced by the cell, actin appears in a globular form (G-actin; Refer Figure 4.7). In microfilaments, however, which are also often referred to as actin filaments,



long polymerized chains of the molecules are intertwined in a helix, creating a filamentous form of the protein (F-Actin). All of the subunits that compose a microfilament are connected in such a way that they have the same orientation. Due to this fact, each microfilament exhibits polarity, the two ends of the filament being distinctly different. This polarity affects the growth rate of microfilaments, one end (termed the plus end) typically assembling and disassembling faster than the other (the minus end).

Unlike microtubules, which typically extend out from the centrosome of a cell, microfilaments are typically nucleated at the plasma membrane. Therefore, the periphery (edges) of a cell generally contains the highest concentration of microfilaments. A number of external factors and a group of special proteins influence microfilament characteristics, however, and enable them to make rapid changes if needed, even if the filaments must be completely disassembled in one region of the cell and reassembled somewhere else. When found directly beneath the plasma membrane, microfilaments are considered part of the cell cortex, which regulates the shape and movement of the cell's surface. Consequently, microfilaments play a key role in development of various cell surface projections as illustrated in Figure 4.8, including filopodia, lamellipodia, and stereocilia.

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*Fig.4.8 Animal Cell Micrograph*

Illustrated in Figure 4.8 is a fluorescence digital image of an Indian Muntjac deer skin fibroblast cell stained with fluorescent probes targeting the nucleus (blue) and the actin cytoskeletal network (green). Individually, microfilaments are relatively flexible. In the cells of living organisms, however, the actin filaments are usually organized into larger, much stronger structures by various accessory proteins. The exact structural form that a group of microfilaments assumes depends on their primary function and the particular proteins that bind them together. For instance, in the core of surface protrusions called microspikes, microfilaments are organized into tight parallel bundles by the bundling protein fimbrin. Bundles of the filaments are less tightly packed together, however, when they are bound by alpha-actinin or are associated with fibroblast stress fibers (the parallel green fibers in Figure

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4.8). Notably, the microfilament connections created by some cross-linking proteins result in a web-like network or gel form rather than filament bundles.

Over the course of evolutionary history of the cell, actin has remained relatively unchanged. This, along with the fact that all eukaryotic cells heavily depend upon the integrity of their actin filaments in order to be able to survive the many stresses they are faced with in their environment, makes actin an excellent target for organisms seeking to injure cells. Accordingly, many plants, which are unable to physically avoid predators that might want to eat them or harm them in some other way, produce toxins that affect cellular actin and microfilaments as a defensive mechanism. The death cap mushroom, for example, produces a substance called phalloidin that binds to and stabilizes actin filaments, which can be fatal to cells.

Microfilaments also provide some rigidity and shape to the cell. They can depolymerize (disassemble) and reform quickly, thus enabling a cell to change its shape and move. White blood cells (your body's infection-fighting cells) make good use of this ability. They can move to the site of an infection and engulf the pathogen.

### Organization

Actin filaments are assembled in two general types of structures: bundles and networks. Bundles can be composed of polar filament arrays, in which all barbed ends point to the same end of the bundle, or non-polar arrays, where the barbed ends point towards both ends. A class of actin-binding proteins, called cross-linking proteins, dictate the formation of these structures. Cross-linking proteins determine filament orientation and spacing in the bundles and networks. These structures are regulated by many other classes of actin-binding proteins, including motor proteins, branching proteins, severing proteins, polymerization promoters, and capping proteins.

### Associated Proteins

In non-muscle cells, actin filaments are formed proximal to membrane surfaces. Their formation and turnover are regulated by many proteins, including:

- Filament end-tracking protein (for example, formins, VASP, N-WASP)
- Filament-nucleator known as the Actin-Related Protein-2/3 (or Arp2/3) complex
- Filament cross-linkers (for example,  $\alpha$ -actinin, fascin, and fimbrin)
- Actin monomer-binding proteins profilin and thymosin  $\beta$ 4
- Filament barbed-end cappers such as Capping Protein and CapG, etc.
- Filament-severing proteins like gelsolin.
- Actin depolymerizing proteins such as ADF/cofilin.

The actin filament network in non-muscle cells is highly dynamic. The actin filament network is arranged with the barbed-end of each filament attached to the

cell's peripheral membrane by means of clamped-filament elongation motors, the above-mentioned 'actoclampins', formed from a filament barbed-end and a clamping protein (formins, VASP, Mena, WASP, and N-WASP).[9]The primary substrate for these elongation motors is profilin-actin-ATP complex which is directly transferred to elongating filament ends. The pointed-end of each filament is oriented toward the cell's interior. In the case of lamellipodial growth, the Arp2/3 complex generates a branched network, and in filopodia a parallel array of filaments is formed

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### **Actin Acts as a Track for Myosin Motor Motility**

Myosin motors are intracellular ATP-dependent enzymes that bind to and move along actin filaments. Various classes of myosin motors have very different behaviors, including exerting tension in the cell and transporting cargo vesicles.

### **Microtubules**

Microtubules are polymers of tubulin that form part of the cytoskeleton and provide structure and shape to the cytoplasm of eukaryotic cells, some bacteria and some archaea (like Asgard archaea). A microtubule can grow as long as 50 micrometres and are highly dynamic. The outer diameter of a microtubule is about 24 nm while the inner diameter is about 12 nm. They are formed by the polymerization of a dimer of two globular proteins, alpha and beta tubulin into protofilaments that can then associate laterally to form a hollow tube, the microtubule. The most common form of a microtubule consists of 13 protofilaments in the tubular arrangement.

Microtubules are very important in a number of cellular processes. They are involved in maintaining the structure of the cell and, together with microfilaments and intermediate filaments, they form the cytoskeleton. They also make up the internal structure of cilia and flagella. They provide platforms for intracellular transport and are involved in a variety of cellular processes, including the movement of secretory vesicles, organelles, and intracellular macromolecular assemblies (see entries for dynein and kinesin). They are also involved in cell division (by mitosis and meiosis) and are the major constituents of mitotic spindles, which are used to pull eukaryotic chromosomes apart.

Microtubules are nucleated and organized by Microtubule Organizing Centers (MTOCs), such as the centrosome found in the center of many animal cells or the basal bodies found in cilia and flagella, or the spindle pole bodies found in most fungi.

There are many proteins that bind to microtubules, including the motor proteins kinesin and dynein, severing proteins like katanin, and other proteins important for regulating microtubule dynamics. Recently an actin-like protein has been found in a gram-positive bacterium *Bacillus thuringiensis*, which forms a microtubule-like structure and is involved in plasmid segregation.

Microtubules have an outer diameter of 24 nm, a wall thickness of approximately 5 nm. The wall is a polymer composed of globular subunits (13),

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each of them consist of a single molecule of the protein tubulin. The subunits are arranged in longitudinal rows, termed protofilaments, that are aligned parallel to the long axis of the tubule.

The assembly of a microtubule occurs by the incorporation of dimeric building blocks. Each of these building blocks is an assembly unit that consists of two tubulin molecules, one  $\alpha$  and one  $\beta$  (heterodimer). The entire protofilament has an asymmetric structure with an  $\alpha$ -tubulin at one end and a  $\beta$ -tubulin at the other end. That is why, the polymer is a polarised structure with the one end distinguishable from the other end (the plus end and the minus end).

These straight, hollow cylinders are found throughout the cytoplasm of all eukaryotic cells (prokaryotes don't have them) and carry out a variety of functions, ranging from transport to structural support. Microtubules, which are about 25 nanometers in diameter, form part of the cytoskeleton that gives structure and shape to a cell, and also serve as conveyor belts moving other organelles throughout the cytoplasm. In addition, microtubules are the major components of cilia and flagella, and participate in the formation of spindle fibers during cell division (mitosis). The length of microtubules in the cell varies between 200 nanometers and 25 micrometers, depending upon the task of a particular microtubule and the state of the cell's life cycle (Refer Figure 4.9).

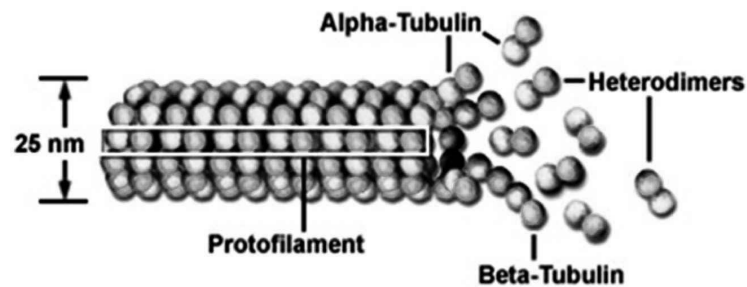


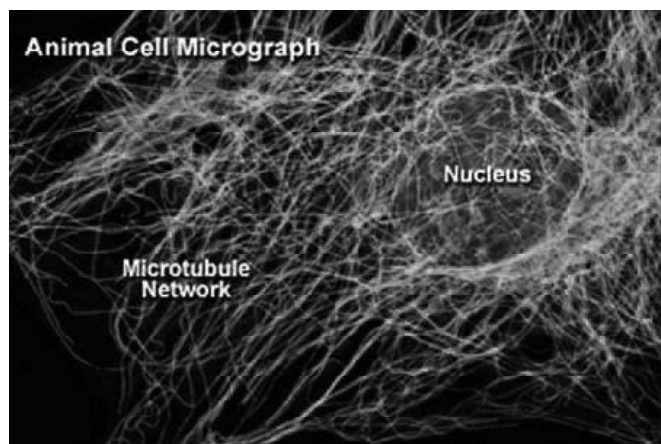
Fig.4.9 Microtubule Helical Structure

Microtubules are biopolymers that are composed of subunits made from an abundant globular cytoplasmic protein known as **tubulin**, as illustrated in Figure 4.9. Each subunit of the microtubule is made of two slightly different but closely related simpler units called ***alpha*-tubulin** and ***beta*-tubulin** that are bound very tightly together to form **heterodimers**. In a microtubule, the subunits are organized in such a way that they all point the same direction to form 13 parallel **protofilaments**. This organization gives the structure **polarity**, with only the ***alpha*-tubulin** proteins exposed at one end and only ***beta*-tubulin** proteins at the other.

By adding or removing globular tubulin proteins, the length of polymeric microtubules can be increased or decreased. Because the two ends of a microtubule are not the same, however, the rate at which growth or depolymerization occurs at each pole is different. The end of a polarized filament that grows and shrinks the fastest is known as the plus end and the opposing end is called the minus end. For

all microtubules, the minus end is the one with exposed **alpha**-tubulins. In an animal cell, it is this end that is located at the centriole-containing centrosome found near the nucleus, while the plus end, comprised of exposed **beta**-units, is projected out toward the cell's surface. Microtubules are continuously being assembled and disassembled so that tubulin monomers can be transported elsewhere to build microtubules when needed.

## NOTES



*Fig. 4.10 Animal Cell Micrograph*

Presented in Figure 4.10 is a digital image of the microtubule network found in an embryonic mouse cell as seen through a fluorescence optical microscope. The extensive intertwined network is labeled with primary antibodies to alpha-tubulin, which are then stained with secondary antibodies containing a green fluorescent dye. The nucleus was counterstained with a red dye to note its location in relation to the microtubule network. Fluorescence microscopy is an important tool that scientists use to examine the structure and function of internal cellular organelles.

In addition to their structural support role, microtubules also serve as a highway system along which organelles can be transported with the aid of motor proteins. For instance, the microtubule network interconnects the Golgi apparatus with the plasma membrane to guide secretory vesicles for export, and also transports mitochondria back and forth in the cytoplasm. Another example is the translocation of vesicles containing neurotransmitters by microtubules to the tips of nerve cell axons. The motor proteins involved in organelle transport operate by altering their three-dimensional conformation using adenosine triphosphate (ATP) as fuel to move back and forth along a microtubule. With each step, the motor molecule releases one portion of the microtubule and grips a second site farther long the filament. Motor proteins, which are grouped into several distinct classes, attach to organelles through specialized receptors.

Since eukaryotic cells greatly depend upon the integrity of microtubules and other cytoskeletal filaments to maintain their structure and essentially to survive, many plants produce natural toxins aimed at disrupting the microtubule network

## NOTES

as a means of self-defense. Taxol, for example, is a toxic substance produced by a species of yew trees that increases microtubule polymerization (building a macromolecule) by binding to the filament and stabilizing it. Other natural toxins, such as the colchicine produced by the meadow saffron, destabilize microtubules and hinder their polymerization. Both kinds of events can be fatal to the affected cell, though in some circumstances, this can be beneficial to animals, as demonstrated by taxol, which is commonly used as a cancer medication.

### **Functions of Cytoskeleton**

Just as our skeletons give our bodies' structure and shape, the cytoskeleton gives cells structure and shape. The cytoskeleton is responsible for lots of important cellular functions:

- It allows cells to move
- Engulf particles
- Brace themselves against pulling forces
- Transport vesicles through the cytosol
- Separate chromosomes during cell division
- Allows our muscles to contract

Clearly, things just wouldn't be the same without the cytoskeleton.

In eukaryotic cells, the cytoskeleton is made up of three major kinds of filaments: actin filaments, intermediate filaments, and microtubules. Each of these filaments is a polymer, meaning that it is made up of many single sub-units, like a child's building blocks snapped together to form a long chain. The sub-units are called monomers, and each type of cytoskeletal filament is built out of a different kind of monomer.

The polymeric structure of cytoskeletal filaments means that they can be disassembled and rearranged at any time. This means that the cell can respond to signals in its environment and rapidly change its shape, motion, or attachment accordingly.

### **Microtubule Structure**

Microtubules are the largest cytoskeletal filaments in cells, with a diameter of 25 nanometers. They are made out of sub-units called tubulin. Each tubulin subunit is made up of one alpha and one beta tubulin that are attached to each other, so technically tubulin is a heterodimer, not a monomer. As you can see, it really does look like a tube, hence the name micro'tubule.'

In a microtubule structure, tubulin monomers are linked both at their ends and along their sides (laterally). This means that microtubules are quite stable along their lengths. Imagine that you have some plastic building blocks that are all identical and can attach to each other both at their ends and laterally. If you arranged them into a microtubule structure, and then wanted to take the structure apart, you

can imagine that it would be really hard to take it apart somewhere in the middle, because how would you get the first block out? If you wanted to take it apart, you'd have to start at the ends. And indeed, this is how microtubules are assembled and disassembled, only from their ends.

### Plus and Minus Ends

Since the tubulin subunits are always linked in the same direction, microtubules have two distinct ends, called the plus (+) and minus (-) ends. On the minus end, alpha tubulin is exposed, and on the plus end, beta tubulin is exposed.

Microtubules preferentially assemble and disassemble at their plus ends. An important consequence of this fact is that microtubule minus ends can be clustered together in a so-called microtubule-organizing center, or centrosome. The centrosome stays stable as the plus ends of the microtubules grow and shrink.

Microtubules are used in many important cellular functions.

### Difference between Microfilaments and Microtubules

Microfilaments	Microtubules
They are solid structures	They are hollow tubules
They bring about cytoplasmic streaming	They cause microcirculation by directing vesicles in a particular direction
They take part in endocytosis	They have no role in endocytosis
They require ATP for their assembly	They require GTP for their assembly
They are mainly made up of protein actin	Microtubules are formed of $\alpha$ and $\beta$ tubulin proteins
They are contractile	They are non contractile
They do not possess longitudinal subunits	They contain 13 protofilaments

#### Check Your Progress

3. What is the size of microfilaments?
4. What change has actin developed over the course of the cell's evolution?

## 4.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Microfilaments are solid thinner structures composed of protein actin.
2. Microfilaments are involved in intracellular motile processes such as movement of vesicles, phagocytosis and cytokinesis. Plant cells are thought to rely on microfilaments rather than microtubules for long distance transport of vesicles and organelles.

## NOTES

3. Microfilaments are usually about 7 nm in diameter.
4. Over the course of evolutionary history of the cell, actin has remained relatively unchanged.

## NOTES

### 4.5 SUMMARY

- The cytoskeleton is composed of three types of membranous structures: microfilaments, intermediate filaments and microtubules which participate in a number of cellular activities.
- Microfilaments are made up of actin, present in muscle myofibrils which constitute 10-15% of cell protein.
- They are 6-8 nm in thickness and shows beaded appearance due to helical arrangement of globular actin molecules.
- Actin filament is made of two coiled strands. Microfilaments are attached to plasmamembrane by variety of linked proteins.
- With the help of cross linking proteins, spectrin and filamin, microfilaments get arranged into a stable network having gel like properties.
- Microfilaments help in maintaining supporting framework for villi and pseudopodia.
- In pollen tubes, microfilaments guide the vesicles containing wall precursors towards the tip where growth is occurring.
- An intermediate filament has a rope like character with 8 protofilaments joined together. Intermediate filaments are rope like structures composed of variety of different proteins capable of assembling into similar type of filaments.
- Actin filament or microfilament composed of double helical polymer of actin, play key role in all types of contractibility and motility within the cells.
- It can bend but is difficult to break, they occur in cells and cell areas subject to stress.
- They do not occur in unicellular eukaryotes.
- They provide support to biomembranes, cytoplasm, sarcomeres, maintain integrity of epithelial tissue, provide protection against abrasions, provide strength to axons and dendrons.
- Microtubules are unbranched hollow, tubular structures 25 nm in diameter that are assembled from protein tubulin and in addition to cytoskeleton, form part of the mitotic spindle, centrioles, and core of cilia and flagella, tubules formed of protofilaments of tubulin.
- Colchicine prevents assembly of microtubules. In most cases, kinesins and dynein move materials along microtubules in opposite directions



- The nucleation of microtubules in vivo occurs in association with variety of MTOCs
- The microtubules of the cytoskeleton are dynamic polymers that are subject to shortening, lengthening, assembly and disassembly.
- It occurs widely in eukaryotic cell.
- They are present in specialized structures like centrioles, basal bodies, cilia or flagella, spindle apparatus, thrombocytes, sperm tails.
- Microtubules are of indefinite length. The diameter is 25 nm. The wall is formed of 13 laterally associated and helically arranged longitudinal strands called protofilaments.

*Architecture and Functions of Cytoskeleton Networks*

## NOTES

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### 4.6 KEY WORDS

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- **Nuclear matrix:** Network of fibres found throughout the inside of cell nucleus and is analogous to cell cytoskeleton
- **Actin:** It exist either as free monomer called G actin ( globular) or part of linear polymer microfilament called F actin ( filamentous), both are essential for important function as mobility and contraction of cell
- **Myosin:** They are motor proteins known for their role in muscle contraction and wide range of motility processes in eukaryotes
- **Microtubular assembly:** Polymers of tubulin that forms cytoskeleton. They are assembly of  $\alpha$  and  $\beta$  tubulin heterodimers arranged in form of protofilaments
- **Cell differentiation:** When a cell gets specialized in order to perform a specific function as liver cell, blood cell or neuron.

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### 4.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

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#### Short Answer Questions

1. Write a short note on architecture and function of microfilaments.
2. Describe the terms
  - (i) Unconventional myosins
  - (ii) Conventional myosins
  - (iii) Muscle contractibility
  - (iv) Intermediate filaments
  - (v) Neurofilaments

## NOTES

3. Write a note on the architecture of microtubules.
4. What additional proteins do microtubules contain? Discuss.
5. Write a note on microtubules, explaining them as agents of intracellular motility.
6. List a few dynamic properties of microtubules.
7. What are the various types of intermediate filaments?

### Long Answer Questions

1. “Microfilaments are involved in intracellular motile processes such as movement of vesicles, phagocytosis and cytokinesis.” Discuss in detail.
2. “The unit of IF assembly is tetramer formed by two dimmers that are aligned side by side in fashion with their N and C termini pointing in opposite direction.” Elaborate with the help of your learning from the text.
3. “Microtubules are stiff enough to resist forces that compress and bend the fibre. This property enables them to provide mechanical support.” Discuss microtubules as structural support and organizers.
4. It is said that microtubule behavior can be explained by phenomenon called dynamic instability. Discuss this phenomenon in detail.
5. What is cytoskeleton? Describe the structure made of actin.
6. Write a detailed note discussing the Dynamic properties of microtubules.

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## 4.8 FURTHER READINGS

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**BLOCK - II**  
**ASSEMBLY AND FUNCTIONS OF**  
**CELLULAR MEMBRANES**

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**NOTES**

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**UNIT 5 BIOLOGICAL MEMBRANE**

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**Structure**

- 5.0 Introduction
- 5.1 Objectives
- 5.2 Biological Membrane and Its Composition
- 5.3 Transport of Ions Across Membrane
- 5.4 Answers to Check Your Progress Questions
- 5.5 Summary
- 5.6 Key Words
- 5.7 Self Assessment Questions and Exercises
- 5.8 Further Readings

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**5.0 INTRODUCTION**

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A biological membrane or biomembrane is an enclosing or separating membrane that acts as a selectively permeable barrier within living things. Biological membranes, in the form of eukaryotic cell membranes, consist of a phospholipid bilayer with embedded, integral and peripheral proteins used in communication and transportation of chemicals and ions. The bulk of lipid in a cell membrane provides a fluid matrix for proteins to rotate and laterally diffuse for physiological functioning. Proteins are adapted to high membrane fluidity environment of lipid bilayer with the presence of an annular lipid shell, consisting of lipid molecules bound tightly to surface of integral membrane proteins. The cell membranes are different from the isolating tissues formed by layers of cells, such as mucous membranes, basement membranes, and serous membranes.

Biological molecules are amphiphilic or amphipathic, i.e., are simultaneously hydrophobic and hydrophilic. The phospholipid bilayer contains charged hydrophilic head groups, which interact with polar water. The lipids also contain hydrophobic tails, which meet with the hydrophobic tails of the complementary layer. The hydrophobic tails are usually fatty acids that differ in lengths. The interactions of lipids, especially the hydrophobic tails, determine the lipid bilayer physical properties, such as fluidity.

Membranes in cells typically define enclosed spaces or compartments in which cells may maintain a chemical or biochemical environment that differs from the outside. For example, the membrane around peroxisomes shields the rest of the cell from peroxides, chemicals that can be toxic to the cell, and the cell membrane

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separates a cell from its surrounding medium. Peroxisomes are one form of vacuole found in the cell that contain by-products of chemical reactions within the cell. Most organelles are defined by such membranes, and are called 'membrane-bound, organelles.

In this unit, you will learn about the biological membrane, i.e., lipid bilayer, membrane proteins and transport of ions and molecules across the membrane in detail.

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### 5.1 OBJECTIVES

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After going through this unit, you will be able:

- Understand biological membrane, i.e., lipid bilayer, membrane proteins
- Explain the transport of ions and molecules across the membrane

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### 5.2 BIOLOGICAL MEMBRANE AND ITS COMPOSITION

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Plasma membrane is the outer membrane covering of cell protoplasts discovered by Schwann (1838). It was called cell membrane by Nageli and Cramer (1855). The name plasmalemma of plasma membrane was given to it by Plowe (1931). Biomembranes are not visible under the light microscope because their thickness is below the resolving power of the microscope. Under electron microscope biomembranes appear to be trilaminar or tripartite. Freeze etching technique has shown that a membrane possesses particles of different sizes. Membrane occur inside the cytoplasm. Internal membranes are rare in prokaryotes. They occur in eukaryotic cells as covering of several cell organelles like nucleus, mitochondria, plastids, lysosomes, Golgi bodies, peroxisomes, etc. Vacuoles are separated from cytoplasm by a membrane called tonoplast. All membranes whether external or internal are called cell membranes or biomembranes. Average thickness is 75 Å. Biomembranes are selectively permeable for solutes but semipermeable for water.

The asymmetric functioning of such system is of little value to cells. It was unclear for many years as to how phospholipid bilayer only 5 nm thick could be strong enough to withstand stress on the plasma membrane of most cells. The size of most animal cells are small enough for adhesive forces between water molecules to maintain spherical cell shape inside a lipid bilayer surface. Plasma membrane is able to lie passively as unreinforced thin lipid barriers at the surface of cell. Monocytes surrounded by thin membranes are deformed as they migrate between vascular endothelial cells in response to injury. Cell membrane face continuous challenge to their integrity from within. An area of membrane about equal to area of entire cell surface turns over every 30 min in many cells due to endocytosis and exocytosis. Endocytosis and exocytosis depend on the cytoskeleton for movement

of vesicles to and from plasma membrane and cytoskeletal infrastructure influences the motion of membrane. The first proposal that cellular membranes might contain a lipid bilayer was made in 1925 by two Dutch scientists E. Gorter and F. Grendel. They extracted the lipid from human blood cells and measured the amount of surface area the lipid would cover. Mammalian red blood cell lack both nuclei and cytoplasmic organelles, plasma membrane is the only lipid containing structure and all of the lipids extracted from the cells can be assumed to have resided in the cells plasma membranes. Experiments conducted in 1960 led to the concept of membrane structure known as fluid mosaic model proposed in 1972 by S. Jonathan Singer and Garth Nicolson of University of California, San Diego.

### Composition of Biological Membrane

The lipid bilayer is fundamental structure of most biomembranes. Non lamellar lipid structure also form important components of biomembranes. The phospholipids that are present in biomembranes are highly amphipathic, phosphate containing groups esterified that have long chain fatty acyl groups. The phosphate containing groups are electrically charged and are hydrophilic whereas the hydrocarbon side chain of fatty acyl groups are hydrophobic. In the phospholipid bilayer, these hydrophobic side chains extend within each leaflet toward each other to form hydrophobic interior of biomembranes. In contrast, hydrophilic phosphate containing group seek position in bilayer between hydrophobic interior and either of two external aqueous phases. Animal biomembrane also contain other lipids, such as cholesterol in addition to phospholipids. Cholesterol is less amphipathic than are phospholipids because hydrophilic portion of cholesterol is due to its uncharged and small hydroxyl group rather than to electrically charged and larger phosphate containing group. The protein content of biomembranes varies from 20% to more than 70% depending on the membrane. The ratio of lipid to protein in membrane varies, depending on type of cell membrane (plasma/ endoplasmic/ Golgi), type of organism (bacteria/plant/animal) and type of cell (cartilage/ muscle /liver). Inner mitochondrial membrane has a very high ration of protein / lipid in comparison to red blood cell plasma membrane, which is high in comparison to membranes of the myelin sheath that form wrapping around nerve cell. Inner mitochondrial membrane contains protein carriers of the electron transport chain and relative to other membranes, lipid is diminished.

In contrast, myelin sheath act as electrical insulation for nerve cell it encloses, a function that is carried by thick lipid layer of high resistance. Biomembranes transport is believed to be catalyzed by integral membrane proteins, though their activities are influenced by peripheral proteins. The tertiary and quaternary structure of integral membrane proteins has been studied. For example, aquaporin-1 (CHIP28) monomer, a member of protein family forms water channel in many epithelial and non-epithelial tissue is observed using cryo electron crystallography to form tetramers of four water channel pathways through the plasma membrane. Biomembranes structure is asymmetric as well as heterogeneous (Refer Figure 5.1).

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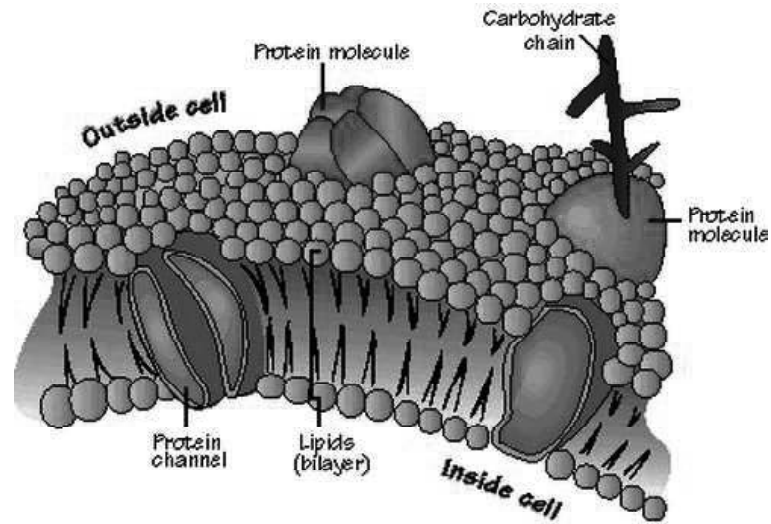


Fig. 5.1 Biomembranes Structure

**Proteins:** Proteins constitute 20 to 70 % of the membrane by mass. They occur in different ways and forms in different sites of plasma membrane. Two distinct types are common. Extrinsic proteins and intrinsic proteins. Extrinsic proteins are attached to the periphery of the lipid bilayer and can be separated from the membrane. Peripheral proteins are mostly made up of amino acids having hydrophilic side chains which act with the polar heads of lipid molecules or with surrounding water. Intrinsic proteins are partly or wholly embedded in the lipid bilayer. The constituent amino acids of these proteins form hydrophobic bonds with fatty acids of lipid molecules. These proteins are soluble in organic solvents. Due to differential distribution of peripheral proteins, two surfaces of a membrane is always asymmetric. Most integral membrane proteins are inserted into membranes in the endoplasmic reticulum by a process that involves amino acid residue signaling sequences. Integral proteins are transmembrane proteins that is they pass entirely through lipid bilayer and have domains that protrude from both extracellular and cytoplasmic side of membrane (Refer Figure 5.2).

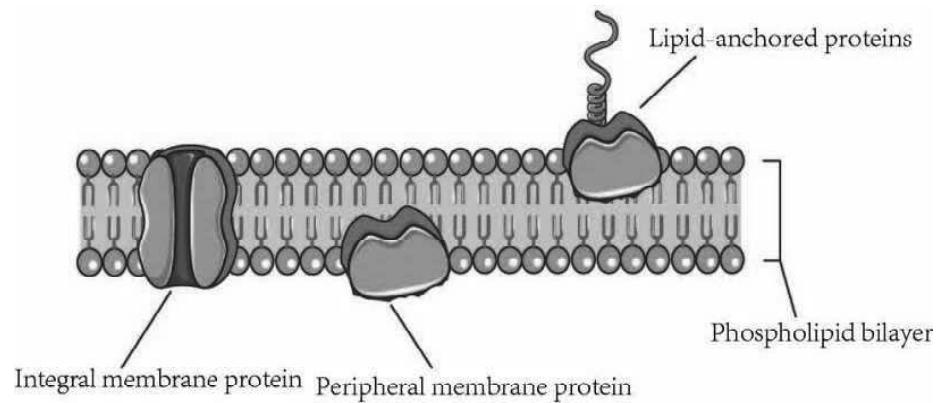


Fig. 5.2 Protein Membrane

Some membrane transport proteins undergo large conformational changes during processing and functioning. Peripheral proteins that are located entirely outside the lipid bilayer on either of cytoplasmic or extracellular side, and are associated with surface of membrane by noncovalent bonds. Lipid anchored proteins are located outside the lipid bilayer on either the extracellular surface but are covalently linked to lipid molecule situated within the bilayer. Besides structural proteins, the plasma membrane possess several enzymes which regulate cellular metabolism. More than 30 different types of enzymes are known to occur in association with plasma membrane. The carrier proteins embedded in the membrane help in transport of solutes.

**Lipids:** Lipids have asymmetric concentration across biomembranes, for example, out of four most abundant category of phospholipid in plasma membrane, the anionic one (phosphatidylserine) and a zwitterionic one (phosphatidylethanolamine) are more concentrated in the inner than the outer leaflet of bilayer. Free energy is required to move these phospholipids against their concentration gradients. The asymmetric distribution of lipids across the plasma membrane is scrambled by normal and artificial process. Cellular activation by stimuli is associated with increase in the cytosolic free  $\text{Ca}^{2+}$  concentration. Membrane lipids contain wide variety of lipids all of which are amphipathic, .i.e., they contain hydrophilic and hydrophobic regions.

There are three types of membrane lipids:

- Phosphoglycerides
- Sphingolipids
- Cholesterol

**Phosphoglycerides:** Phospholipids constitute a major portion of lipids (55-57%) in plasma membranes. They belong to mainly two categories. Neutral phospholipids and acidic phospholipids. Examples of neutral phospholipids are lecithin, cephalin, sphingomyelin, etc. Most membrane lipids contain phosphate group which make them phospholipid, most membrane phospholipids are built on glycerol backbone called phosphoglycerides.

Unlike triglycerides, which have three fatty acid and are not amphipathic, membrane glycerides are diglycerides-only two of the hydroxyl group of glycerol are esterified to fatty acid, third is esterified to hydrophilic phosphate group. These phospholipid molecules constitute the structural framework of plasma membrane.

**Sphingolipids:** A less abundant class of membrane lipids are sphingolipids, are derivatives of sphingosine, an amino alcohol that contain a long hydrocarbon chain. Sphingolipid consists of sphingosine linked to a fatty acid by its amino group. This molecule is ceramide. The various sphingosine based lipids have additional group esterified to terminal alcohol of sphingosine. If substitution is phosphorylcholine, molecule is sphingomyelin, which is only phospholipid of membrane (Refer Figure 5.3).

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**Cholesterol:** It is an amphipathic molecule like phospholipids having both hydrophilic and a hydrophobic portion. Cell membrane would be too fluid and too permeable to some molecules without cholesterol. Cholesterol is part of steroid ring that is closely attracted to part of fatty acid chain on the nearest phospholipid.

Cholesterol is sterol lipid derived from squalene, forming a major component of animal cell membrane. It is absent in higher plant membranes and bacteria. In animal cells, it affect membrane fluidity.

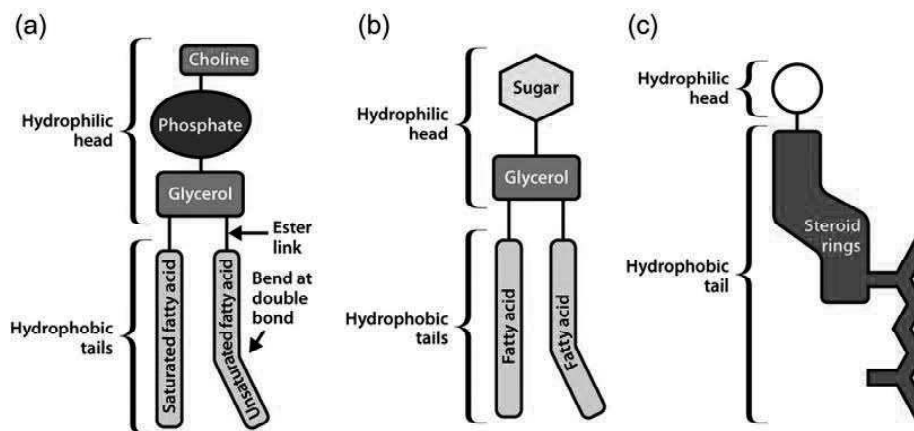


Fig. 5.3 Structure of Membrane Lipid

**Phospholipid Bilayer**

Membrane formation is due to a self-assembly process which a consequence of amphipathic nature of phospholipid molecules is. The polar hydrophilic heads of phospholipids come in contact with water while their hydrophobic tails cannot do so. There are two methods of arrangement of phospholipids in contact with water: micelle (globule) and bilayer (bimolecular sheet) in which hydrophobic tails are inside and the hydrophilic heads are towards outside. The micelle or globular form would be mechanically unsound as the bulky double tails would not be able to get adjusted in the center of globule. The driving force for the formation of lipid bilayer is hydrophobic interaction amongst the hydrocarbon tails of lipid molecules. Vander Waals forces help in close packing of tails. Polar heads of phospholipids are held to water molecules by electrostatic attractions and hydrogen bonds. Lipid bilayers are also called cooperative structures because their molecules are held together by many reinforcing non-covalent interactions which are predominantly hydrophobic. Because of these interactions lipid bilayers have a tendency to become extensive, close on themselves and the ability of self-sealing (Refer Figure 5.4).



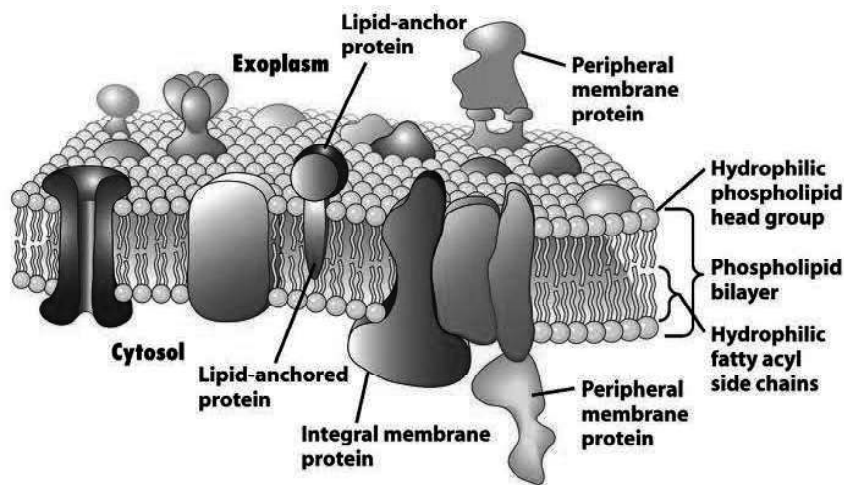


Fig. 5.4 Lipid Bilayer showing Inner and Outer Leaflets

**Carbohydrates:** More than 90% of the membrane carbohydrate is covalently linked to proteins to form glycoproteins. Their peripheral association is highly asymmetric. The remaining carbohydrates are covalently linked to lipids to form glycolipids. Unlike proteins and lipids, carbohydrates are not integral components of membranes. Their peripheral association is highly asymmetric. Oligosaccharides are covalently bound to membrane lipids and integral proteins on their non-cytosolic sides. Oligosaccharides are assembled and transferred to membrane proteins and lipids and modified in the lumen of endoplasmic reticulum and Golgi apparatus. No mechanism is known for assembly and attachment of molecules to segments of proteins and lipids at the cytosolic surface of membranes. The carbohydrate-rich zone on the surface of the cell is known as the glycocalyx. Specific glycosylation of some integral membrane proteins appears to be essential for their transport activities.

Proteins penetrate through membranes rather than remaining external to the lipid bilayer. This was derived from results of a technique called freeze fracture replication. Here tissue is frozen solid and struck with a blade, which fractures it into two pieces. Once the membrane is split, metals are deposited on their exposed surface to form a replica which is viewed in the electron microscope (Refer Figure 5.5).

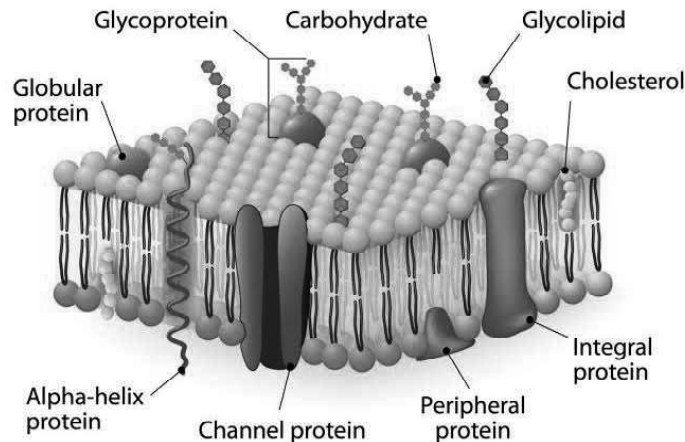
### Functions of Biological Membrane

- Membranes are continuous and enclose compartments. The plasma membrane encloses the contents of the cell, whereas the nuclear and cytoplasmic membranes enclose diverse intracellular spaces. The various membrane-bound compartments possess different contents. It allows specialized activities to proceed without external interference and enable cell activity to be regulated independently.
- Membranes prevent the unrestricted exchange of molecules from one side to another. Membranes provide the means of communication between compartments.

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## NOTES

- They form outer boundaries around the cells to maintain environment inside the cell different from those of outside environment.
- Membrane prevent the unrestricted exchange of molecules from one side to the other. At the same time, membrane provide the communication between the compartments they separate
- The plasma membrane contain machinery for physically transporting substance from one side of membrane to another from region where solute is at low concentration to a region where solute is at higher concentration. The plasma membrane is able to transport ions thereby establishing ionic gradients across itself.
- The plasma membrane of organism mediates the interaction between a cell and its neighbours. The plasma membrane allow cells to recognize and signal one another, to adhere when appropriate and to exchange material and information
- Membranes are involved in the process by which one type of energy is converted to another type (energy transduction). Membranes are involved in transfer of chemical energy from carbohydrates and fats to ATP.
- Plasma membrane plays a role in response of cell to external stimuli, a process known as signal transduction. Membranes possess receptors that combine with specific molecules. The interaction of a plasma membrane receptor with external ligand cause the membrane to generate signal that stimulate or inhibit internal activities.
- Boundaries round distinct sub-cellular compartments (Nucleus, Mitochondria, Lysosomes, Golgi bodies, etc.), compartmentalize and segregate intracellular events, and separate cells from one another,
- Membranes mediate regulation of cellular functions by: acting as selective barriers, allowing inside environment of cells or organelles to differ from outside.
- Plasma membrane is selectively permeable outer boundary of cell.
- Plasma membrane contains: Specific systems; Pumps, Channels, Transporters used for exchange of nutrients and other materials with the environment.
- Normal cellular function starts with normal cell membrane, i.e., damage to membrane can affect water balance and ion influx and therefore grossly alter most processes within the cell.
- In nerve cells, the cell membrane takes part in transmission of impulses.
- They provide sheaths for cilia and flagella.
- Secretory, excretory and waste products are thrown out by plasma membrane through exocytosis.



**Fig. 5.5** Biological Membrane with Different Protein Membranes

## NOTES

### Check Your Progress

1. What is plasma membrane?
2. Why are bio-membranes not visible under the light microscope?
3. What are internal membranes rare in?
4. What do mammalian red blood cells lack?

## 5.3 TRANSPORT OF IONS ACROSS MEMBRANE

### Fluidity of Plasma Membrane

The physical state of the lipid of a membrane is described by fluidity. Simple artificial bilayer composed of phosphatidylcholine and phosphatidylethanolamine, whose fatty acids are unsaturated. If temperature is kept warm, lipid exist in fluid state. At this temperature, lipid bilayer is described as two dimensional liquid crystal. If the temperature is lowered, point is reached where the bilayer changes. The lipid is converted from a liquid crystalline phase to a frozen gel in which movement of phospholipid fatty acid chains is restricted. The temperature at which this change occurs is called transition temperature.

Saturated fatty acids have a shape of straight, flexible rod. Cis unsaturated fatty acids have crooks in the chain at sites of double bond. Phospholipids with saturated chains pack together more tightly than those containing unsaturated chains. The greater the degree of unsaturation of fatty acids of bilayer, lower the temperature before bilayer gels.

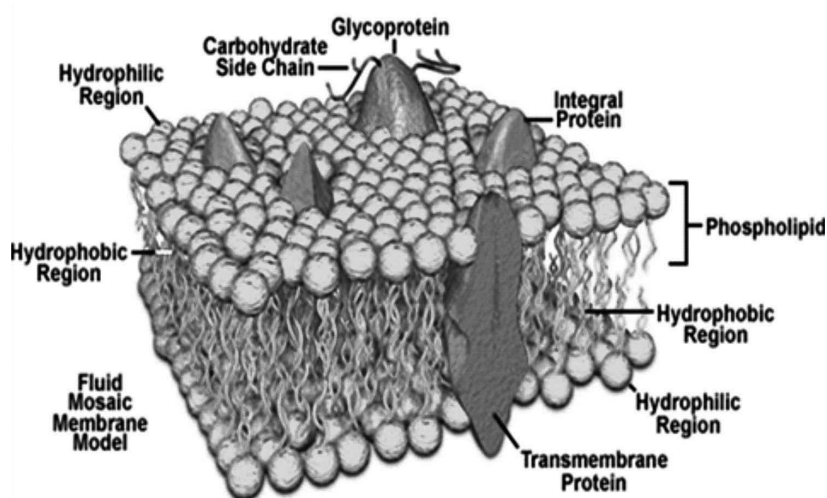
Another factor that influences bilayer fluidity is fatty acid chain length. The shorter the fatty acyl chains of phospholipid, lower is its melting temperature. The physical state of membrane is affected by cholesterol. Because of their orientation

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within bilayer, cholesterol disrupt the close packing of fatty acyl chains and interfere with mobility.

Cholesterol tends to increase durability while decreasing the permeability of a membrane. Many basic cellular processes including cell movement, cell growth, and cell division, formation of intercellular junction, endocytosis, and secretion depend on the movement of membrane components.

Cell membrane do not exist in rigid state nor any disordered state. They are partly fluid and partly rigid or stabilized. Membrane fluidity is mainly controlled by fatty acid moieties of phospholipids. Fluidity increases with the decrease in fatty acid chain and increase in the number of double bonds. Usually one fatty acid is saturated. Its straight chain helps in packing. The second fatty acid is unsaturated with one or more double bonds. Bends appear in the region of double bonds. Such bends interfere in close packing of phospholipids and increase fluidity. Bacteria are known to regulate fluidity by changing the number of double bonds and length of saturated acyl chains. Ratio of saturated to unsaturated fatty acid is 1.6 at 42 degree Celsius. Cholesterol and other lipids provide stability to membranes by preventing displacement of phospholipid due to their large size, formation of membrane complexes and interaction with adjacent phospholipids.



*Fig.5.6 Fluid Mosaic Model*

- It is the most acceptable model of a biomembranes proposed by Singer and Nicolson in 1972.
- According to this model, the membrane does not have a uniform disposition of lipids and proteins but is mosaic of two.
- The membrane is not solid but is quasi-fluid. The quasi-fluid nature of the biomembranes is shown by their properties of quick repair, dynamic nature, ability to fuse, expand and contract.
- Fluid mosaic model postulates that the lipid molecules are present in a viscous bilayer as in lamellar model.

- Protein molecules occur at places both inside and on the outer side of lipid bilayer.
- The integral proteins are called intrinsic or integral proteins while the external ones are known as extrinsic or peripheral proteins. Integral proteins account for 70% of the total membrane proteins. They cannot be extracted from the membrane without disrupting the latter.
- The plasma membrane that surrounds these cells has two layers (a *bilayer*) of *phospholipids* (fats with phosphorous attached), which at body temperature are like vegetable oil (fluid).
- Both layers of the plasma membrane have the hydrophilic heads pointing toward the outside; the hydrophobic tails form the inside of the bilayer
- Each phospholipid molecule has a head that is attracted to water (*hydrophilic*: *hydro* = water; *philic* = loving) and a tail that repels water (*hydrophobic*: *hydro* = water; *phobic* = fearing).
- The extrinsic proteins are attached covalently to phospholipid head or non-covalently to transmembrane proteins. They can be separated with mild treatment.
- Proteins provide the structural and functional specificity to the membranes. Membrane proteins provide structural and functional specificity to the membranes.
- Many membrane proteins function as enzymes. Some of them behave as permeases for allowing facilitated diffusion. A few proteins act as carriers because they actively transport different substances across the membrane.
- Some lipids and extrinsic proteins present on the outer side possess small carbohydrate molecules to form glycolipids and glycoproteins. They constitute glycocalyx.
- Conjugated oligosaccharides function as recognition centers, site of attachment, antigens, etc. Oligosaccharides provide negative charge to the outer surface.
- Because cells reside in a watery solution (extracellular fluid), and they contain a watery solution inside of them (cytoplasm), the plasma membrane forms a circle around each cell so that the water-loving heads are in contact with the fluid, and the water-fearing tails are protected on the inside.
- The lipid bilayer can exist in fluid state. For flip flop to occur, hydrophilic head group of lipid must pass through internal hydrophobic sheet of the membrane. Thus cells contain enzymes that move certain phospholipids from one leaflet to other. These enzymes play role in establishing lipid asymmetry and reverse the slow rate of passive transmembrane movement. Because lipids provide the matrix in which integral proteins of membrane are embedded, the physical state of lipid is important determinant of mobility of integral proteins.

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- Plasma membrane proteins are not totally free to drift around randomly but instead they are subjected to various influences that affect their mobility. Some membranes are crowded with proteins, so random movements of one molecule can be impeded by its neighbors. The plasma membrane possess fibrillary network or membrane skeleton consisting of peripheral proteins situated on the cytoplasmic surface of membrane. Studying the factors that affect membrane protein mobility is to genetically modify cells to produce altered membrane proteins.
- Integral proteins whose cytoplasmic portion have been genetically deleted move much greater distance than their counterparts indicating that barriers reside on cytoplasmic side of the membrane. It suggest that membrane underlying skeleton form network of fences creating compartments that restrict the distance an integral protein can travel. Proteins move across the boundaries from one compartment to another through break in fences.

### Evidences for Fluid Mosaic Model

- Fluid mosaic model can explain the presence of different types of permeability and retentively of various cell membranes.
- It accounts for dynamic nature of biomembranes with their quick repair.
- The change in permeability in different parts of the same membrane can be explained
- The model explains the passage of both electrolytes and non-electrolytes through the biomembranes
- It provides for quick growth, expansion and contraction of the membrane
- The model provides for the occurrence of protein particles both on the surface and interior of cell membranes.

### Asymmetry of Biomembranes

- The two surfaces of the biomembranes are not similar, .i.e. , the membranes are asymmetric
- Lipids present in the outer and inner side of the bilayer are commonly different, for example, lecithin on the outer side and cephalin on the inner side of erythrocyte membrane
- The amount and types of extrinsic proteins are different on the two sides. They are more abundant on the inner surface than on the outer surface
- Oligosaccharides are attached to external surface of lipids and proteins of biomembranes. They are absent on the inner side.

Proteins are huge molecules, so their movement within lipid bilayer might be restricted. Phospholipids are small molecules that make up the very fabric of lipid bilayer. When individual phospholipid molecules of plasma membrane are tagged

and followed under microscope using ultra high speed cameras, they are confined for brief period and hop from one area to another.

Analysis indicates that phospholipid diffuses freely within one compartment before jumps into neighbouring compartment and then over another fence into an adjacent compartment. Treatment of the membrane with agents that disrupts the underlying membrane skeleton removes some of the fences that restrict phospholipid diffusion. As the contents of a cell are surrounded by its plasma membrane, all communication between the cell and the extracellular medium must be mediated by this structure. In this way, plasma membrane has a dual function. On one hand, it must retain the dissolved material of the cell so that they do not leak out into the environment while on other hand, it must allow the necessary exchange of materials into and out of the cell. The lipid bilayer of membrane is suited to prevent the loss of charged and polar solutes from a cell. Several different processes are known by which substances move across membrane: simple diffusion through lipid bilayer, simple diffusion through an aqueous protein lined channel, diffusion that is facilitated by a protein transporter and active transport which requires an energy driven protein pump capable of moving substance against concentration gradient.

There are three types of membrane transport, i.e.,

- Diffusion
- Active Transport
- Bulk Transport

**Diffusion:** It is a spontaneous process in which a substance moves from a region of high concentration to a region of low concentration, eliminating the concentration difference between the two regions. It is of two types- passive and facilitated.

**Passive Diffusion:** The membrane plays a passive role in the transport of substances across it. The unassisted movement is from higher concentration side to the side where concentration is lower. Since the membranes have hydrophobic interior, simple or passive diffusion occurs only for small, non-polar molecules. For example, oxygen, carbon dioxide, small uncharged polar molecules like ethanol and glycerol

**Facilitated Diffusion:** The transport across the membrane is along the concentration gradient but is facilitated by the presence of membrane proteins without involving expenditure of energy. Membrane proteins taking part in facilitated diffusion are of two types, carrier proteins and channel proteins. Both of them are integral membrane proteins with several transmembrane segments.

Water molecules move much more rapidly through cell membrane than do dissolved ions or small polar organic solutes which are non-penetrating. Because of this difference in the penetrability of water vs solutes, membranes are semipermeable. Water moves readily through a semipermeable membrane from a region of lower solute concentration to region of higher solute concentration. The process is osmosis, and is demonstrated by placing cell in solution containing non-

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penetrating solute at a concentration different that present within cell itself. When two compartments of different solute concentration are separated by a semipermeable membrane, the compartment of higher solute concentration is said to be hypertonic relative to the compartment of lower solute concentration, known as hypotonic. When cell is placed into hypotonic solution, cell gains water by osmosis and swells. Conversely, cell placed into hypertonic solution loses water by osmosis and shrinks. It suggests that a cells volume is controlled by the difference between the solute concentration inside the cell and that in the extracellular medium. Once the internal solute concentration equals the external solute concentration, the internal and external fluids are isotonic and no net movement of water into or out of cell occurs.

### Channel Protein Mediated Facilitated Diffusion

**Ion Channels:** Cell membrane contain ion channels that is openings in membrane that are permeable to specific ions. Bert Sakmann and Erwin Neher at the Max Planck Institute in Germany in 1970s and early 1980s developed technique to monitor the ionic current passing through single ion channel. Most ion channels are highly selective in allowing only one particular type of ion to pass through the pore. Most of the ion channels that are identified can exist in either an open or closed conformation such channels are said to be gated. The opening and closing of the gates are subject to complex physiologic regulation and induced by variety of factors depending on particular channel.

Three major categories are:

- Voltage Gated Channels
- Ligand Gated Channels
- Mechano Gated Channels

**Voltage Gated Channels:** The channels open and close in response to changes in membrane potential

**Ligand Gated Channels:** Its conformational state depends on the binding of a specific molecule which is not the solute that passes through channel.

Some ligand gated channels are opened or closed following the binding of a molecule to the outer surface of the channel, others are opened or closed following the binding of ligand to the inner surface of channel. For example, neurotransmitter such as acetylcholine, act on outer surface of cation channels, while cyclic nucleotides such as cyclic AMP act on the inner surface of calcium ion channels.

**Mechano Gated Channels:** Its conformational stated depends on mechanical forces that are applied to the membrane. Members of one family of cation channels, for example, are opened by the movements of stereocilia on the hair cells of the inner ear in response to sound.

**Porins:** They are present in the outer membrane of mitochondria, chloroplasts and some bacteria. The transmembrane segment of the protein has a closed



cylindrical sheet called a barrel. It is water filled pore that is bigger than the ion channels. It allows passage of small sized hydrophilic molecules and solutes.

**Aquaporins:** They are membrane channels for the passage of water molecules. Each aquaporin has a central channel lined by 24 transmembrane segments of four proteins. The diameter of the channel is about 0.3 nm, wide enough to allow passage of water molecules. The rate of water movement is several billion molecules per second.

**Active Transport:** Like facilitated diffusion, active transport depends on integral membrane proteins that selectively bind a particular solute and move it across the membrane in a process driven by changes in protein conformation. Unlike facilitated diffusion, movement of solute against gradient requires the coupled input of energy. The endergonic movement of ions or solutes across the membrane against a concentration gradient is coupled to an exergonic process such as hydrolysis of ATP.

- **Direct or Primary Active Transport:** The active transport is directly coupled to exergonic reaction, most commonly hydrolysis of ATP. Four types of ATPases are P type, V type, F type and ABC type.
- **Indirect or Secondary Active Transport:** It is linked to direct active transport which produces high concentration of sodium ions in animal cells or hydrogen ions in bacterial cells ions on the outside. The ions have tendency to enter the cells as per their electrochemical gradient. They do so by forming symport and antiport systems. In symport, inward passage of hydrogen ions or sodium ions is coupled to passage of organic solutes. An antiport system involves exchange of potassium ions for hydrogen ions and calcium ions for sodium ions.

**Bulk Transport:** It involves the enclosure of the materials under transport in the vesicles of membrane called carrier vesicles. The vesicles are formed in response to chemical stimuli received in specific areas having receptors. It is of two types- Pinocytosis and Phagocytosis

**Pinocytosis:** It is also known as cell drinking. Invagination of the vesicles requires attachment of three types of proteins on the cytosolic side- adapter protein, clathrin and dynamin. The pinched off vesicle is initially coated and pinosome either burst in cytoplasm, fuse with lysosome or release its content into the vacuole. They are either fluid phase pinocytosis or adsorptive pinocytosis.

**Phagocytosis:** It is the bulk transport of solid matter like food, foreign particles, and pathogen across the membrane by means of carrier vesicles. Also known as cell eating. The carrier vesicles are known as phagosome. Fusion of lysosome with phagosome produces a food vacuole. The digested substances diffuse out. The vacuole with undigested material is called residual vacuole. The indigestible matter is thrown out of cell through exocytosis or cell vomiting or ephagy.

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**Other Ion Transport System:** Biologists are eagerly awaiting a high resolution structure of  $\text{Na}^+/\text{K}^+$  ATPase. A great deal can be learnt about P type pump by studying related protein, the  $\text{Ca}^{2+}$  ATPase whose three dimensional structure has been determined. The calcium pump is present in the membrane of endoplasmic reticulum where it transport  $\text{Ca}^{2+}$  ions out of cytosol into the lumen of organelle. The transport of  $\text{Ca}^{2+}$  ions by  $\text{Ca}^{2+}$  ATPase is accompanied by large conformational changes. The sodium potassium pump is found only in animal cells. Unlike P type pumps, V type pumps utilize the energy of ATP without forming a phosphorylated protein intermediate. It actively transport hydrogen ions across the walls of cytoplasmic organelles and vacuoles. Another diverse group of proteins that transport ions is ATP binding cassette transporter because all the members of this family share homologous ATP binding domain.

### Check Your Progress

5. Which membrane encloses the contents of an entire cell?
6. What is phospholipid is made up of?
7. What are the methods of arranging pf phospholipids in contact with water?
8. Briefly describe the two types of passive transport across membranes.

## 5.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Plasma membrane is the outer membrane covering of cell protoplasts.
2. Biomembranes are not visible under the light microscope because their thickness is below the resolving power of the microscope.
3. Internal membranes are rare in prokaryotes.
4. Mammalian red blood cell lack both nuclei and cytoplasmic organelles
5. Plasma membrane encloses the contents of an entire cell.
6. Phospholipid is made up of two non-polar fatty acid chains called tails and one polar group called head.
7. There are two methods of arranging pf phospholipids in contact with water- micelle and bilayer.
8. Simple diffusion and facilitated diffusion are two methods of passive transport across molecules.

## 5.5 SUMMARY

- Plasma membrane is the outer membrane covering of cell protoplasts discovered by Schwann (1838). It was called cell membrane by Nageli and Cramer (1855).

- The name plasmalemma of plasma membrane was given to it by Plowe (1931). Biomembranes are not visible under the light microscope because their thickness is below the resolving power of the microscope.
- Vacuoles are separated from cytoplasm by a membrane called tonoplast. All membranes whether external or internal are called cell membranes or biomembranes.
- Monocytes surrounded by thin membranes are deformed as they migrate between vascular endothelial cells in response to injury. Cell membrane face continuous challenge to their integrity from within.
- Endocytosis and exocytosis depend on the cytoskeleton for movement of vesicles to and from plasma membrane and cytoskeletal infrastructure influences the motion of membrane.
- Mammalian red blood cell lack both nuclei and cytoplasmic organelles, plasma membrane is the only lipid containing structure and all of the lipids extracted from the cells can be assumed to have resided in the cells plasma membranes.
- The phosphate containing groups are electrically charged and are hydrophilic whereas the hydrocarbon side chain of fatty acyl groups are hydrophobic. In the phospholipid bilayer, these hydrophobic side chains extend within each leaflet toward each other to form hydrophobic interior of biomembranes.
- Cholesterol is less amphipathic than are phospholipids because hydrophilic portion of cholesterol is due to its uncharged and small hydroxyl group rather than to electrically charged and larger phosphate containing group. The protein content of biomembranes varies from 20% to more than 70% depending on the membrane.
- Inner mitochondrial membrane contains protein carriers of the electron transport chain and relative to other membranes, lipid is diminished.
- Proteins constitute 20 to 70 % of the membrane by mass. They occur in different ways and forms in different sites of plasma membrane. Two distinct types are common. Extrinsic proteins and intrinsic proteins.
- Extrinsic proteins are attached to the periphery of the lipid bilayer and can be separated from the membrane. Peripheral proteins are mostly made up of amino acids having hydrophilic side chains which act with the polar heads of lipid molecules or with surrounding water.
- Intrinsic proteins are partly or wholly embedded in the lipid bilayer. The constituent amino acids of these proteins form hydrophobic bonds with fatty acids of lipid molecules.
- Most integral membrane proteins are inserted into membranes in the endoplasmic reticulum by a process that involves amino acid residue signaling sequences.

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- Integral proteins are transmembrane proteins that is they pass entirely through lipid bilayer and have domains that protrude from both extracellular and cytoplasmic side of membrane.
- Lipids have asymmetric concentration across biomembranes, for example, out of four most abundant category of phospholipid in plasma membrane, the anionic one (phosphatidylserine) and a zwitterionic one (phosphatidylethanolamine) are more concentrated in the inner than the outer leaflet of bilayer.
- Phospholipids constitute a major portion of lipids (55-57%) in plasma membranes. They belong to mainly two categories. Neutral phospholipids and acidic phospholipids. Examples of neutral phospholipids are lecithin, cephalin, sphingomyelin, etc.
- Most membrane lipids contain phosphate group which make them phospholipid, most membrane phospholipids are built on glycerol backbone called phosphoglycerides.
- A less abundant class of membrane lipids are sphingolipids, are derivatives of sphingosine, an amino alcohol that contain a long hydrocarbon chain. Sphingolipid consists of sphingosine linked to a fatty acid by its amino group. This molecule is ceramide.
- Membrane formation is due to a self-assembly process which a consequence of amphipathic nature of phospholipid molecules is. The polar hydrophilic heads of phospholipids come in contact with water while their hydrophobic tails cannot do so.
- The driving force for the formation of lipid bilayer is hydrophobic interaction amongst the hydrocarbon tails of lipid molecules. Vander walls forces help in close packing of tails.
- Polar heads of phospholipids are held to water molecules by electrostatic attractions and hydrogen bonds.
- Lipid bilayers are also called cooperative structures because their molecules are held together by many reinforcing non-covalent interactions which are predominantly hydrophobic.
- More than 90% of the membrane carbohydrate is covalently linked to proteins to form glycoproteins. The remaining carbohydrates are covalently linked to lipids to form glycolipids.
- Unlike proteins and lipids, carbohydrates are not integral component of membranes. Their peripheral association is highly asymmetric.
- Oligosaccharides are covalently bounded to membrane lipids and integral proteins on their non-cytosolic sides. Oligosaccharides are assembled and transferred to membrane proteins and lipids and modified in the lumen of endoplasmic reticulum and Golgi apparatus.

- Proteins penetrate through membranes rather than remaining external to lipid bilayer was derived from results of a technique called freeze fracture replication. Here tissue is frozen solid and struck with blade, which fractures block into two pieces.
- The physical state of the lipid of a membrane is described by fluidity. Simple artificial bilayer composed of phosphatidylcholine and phosphotidy-lethanolamine, whose fatty acids are unsaturated. If temperature is kept warm, lipid exist in fluid state.
- The lipid is converted from a liquid crystalline phase to a frozen gel in which movement of phospholipid fatty acid chains is restricted. The temperature at which this change occurs is called transition temperature.
- The physical state of membrane is affected by cholesterol. Because of their orientation within bilayer, cholesterol disrupt the close packing of fatty acyl chains and interfere with mobility.
- Cholesterol tends to increase durability while decreasing the permeability of a membrane. Many basic cellular processes including cell movement, cell growth, and cell division, formation of intercellular junction, endocytosis, and secretion depend on the movement of membrane components.
- Cell membrane do not exist in rigid state nor any disordered state. They are partly fluid and partly rigid or stabilized. Membrane fluidity is mainly controlled by fatty acid moieties of phospholipids.
- Fluidity increases with the decrease in fatty acid chain and increase in the number of double bonds. Usually one fatty acid is saturated. Its straight chain helps in packing.
- Bacteria are known to regulate fluidity by changing the number of double bonds and length of saturated acyl chains. Ratio of saturated to unsaturated fatty acid is 1.6 at 42 degree Celsius. Cholesterol and other lipids provide stability to membranes by preventing displacement of phospholipid due to their large size, formation of membrane complexes and interaction with adjacent phospholipids.

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### 5.6 KEY WORDS

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- **Channels:** are protein molecules that span across the cell membrane allowing the passage of ions from one side of the membrane to the other
- **Active transport:** The movement of molecules across a cell membrane from a region of their lower concentration to a region of their higher concentration in the direction against some gradient
- **Diffusion:** It is the net movement of molecules from a region of higher concentration to a region of lower concentration.

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- **Membrane lipids:** Membrane lipids are a group of compounds (structurally similar to fats and oils) which form the double-layered surface of all cells (lipid bilayer). The three major classes of membrane lipids are phospholipids, glycolipids, and cholesterol
- **Protein:** A protein molecule is very large compared with molecules of sugar or salt and consists of many amino acids joined together to form long chains, much as beads are arranged on a string
- **Glycolipid:** Glycolipids are lipids with a carbohydrate attached by a glycosidic bond. Their role is to maintain the stability of the cell membrane
- **Scaffold:** They are regulators of key signaling pathways, they interact and bind with multiple members of a signaling pathway which organized into complex.
- **Cell fusion:** It is important cellular process in which several uninuclear cells combine to form a multinuclear cell. It occurs during differentiation, embryogenesis and morphogenesis
- **Ligands:** ion or molecule that binds to a central metal atom to form coordination complex.
- **Extracellular matrix:** Three dimensional network of extracellular macromolecules such as collagen, enzymes and glycoproteins that provide structural and biochemical support surrounding cells.

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## 5.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

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### Short Answer Questions

1. What are the functions of the biological membrane?
2. Use a simple diagram to show the components parts of the lipid bilayer.
3. With the help of a simple labeled diagram describe the fluid mosaic model of membrane structure.
4. What do you mean by passive transport across membranes?
6. Briefly describe active transport across the membranes.
7. Draw the general structure of Phospholipid.
8. List the common features of the models of membrane transport systems.

### Long Answer Questions

1. “Biological molecules are amphiphilic or amphipathic, i.e., are simultaneously hydrophobic and hydrophilic.” Discuss the nature of biological molecules in detail.

2. “Membranes in cells typically define enclosed spaces or compartments in which cells may maintain a chemical or biochemical environment that differs from the outside.” From your learning of the text, explain.
3. List a few evidences for Fluid Mosaic model.
4. “Proteins are huge molecules, so their movement within lipid bilayer might be restricted. Phospholipids are small molecules that make up the very fabric of lipid bilayer.” Based on your learning of the text talk about proteins and phospholipids.
5. “Cell membrane contain ion channels that is openings in membrane that are permeable to specific ions.” Explain.

## NOTES

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### 5.8 FURTHER READINGS

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**NOTES**

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## **UNIT 6 PROTEIN SORTING IN MITOCHONDRIA, CHLOROPLAST, ER AND NUCLEUS**

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### **Structure**

- 6.0 Introduction
- 6.1 Objectives
- 6.2 Protein Sorting: General Introduction
  - 6.2.1 Protein Sorting in Mitochondria
  - 6.2.2 Protein Sorting in Chloroplast
  - 6.2.3 Protein Sorting in Endoplasmic Reticulum (ER)
  - 6.2.4 Protein Sorting in Nucleus
- 6.3 Answers to Check Your Progress Questions
- 6.4 Summary
- 6.5 Key Words
- 6.6 Self Assessment Questions and Exercises
- 6.7 Further Readings

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### **6.0 INTRODUCTION**

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Protein targeting or protein sorting is the biological mechanism by which proteins are transported to their appropriate destinations in the cell or outside it. Proteins can be targeted to the inner space of an organelle, different intracellular membranes, plasma membrane, or to exterior of the cell via secretion. This delivery process is carried out based on information contained in the protein itself. Correct sorting is crucial for the cell; errors can lead to diseases.

Targeting signals are the pieces of information that enable the cellular transport machinery to correctly position a protein inside or outside the cell. This information is contained in the polypeptide chain or in the folded protein. The continuous stretch of amino acid residues in the chain that enables targeting are called signal peptides or targeting peptides. There are two types of targeting peptides, the pre sequences and the internal targeting peptides. The pre sequences of the targeting peptide are often found at the N-terminal extension and is composed of between 6-136 basic and hydrophobic amino acids. In case of peroxisomes the targeting sequence is on the C-terminal extension mostly. Other signals, known as signal patches, are composed of parts which are separate in the primary sequence. They become functional when folding brings them together on the protein surface. In addition, protein modifications like glycosylations can induce targeting.



In this unit, you will learn about protein sorting and protein sorting in mitochondria, chloroplast, Endoplasmic Reticulum (ER) and nucleus in detail.

*Protein Sorting in  
Mitochondria, Chloroplast,  
ER and Nucleus*

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## 6.1 OBJECTIVES

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After going through this unit, you will be able to:

- Understand protein sorting
- Explain the protein sorting in mitochondria, chloroplast, Endoplasmic Reticulum and nucleus

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## 6.2 PROTEIN SORTING: GENERAL INTRODUCTION

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Knowledge of cell, different types of cells and amino acids are indispensable in understanding protein sorting and translocation. Proteins localize to various organelles or locations in the cell or moves out of the cell as secretory proteins. The organelles include nucleus, chloroplast, mitochondrion, Endoplasmic Reticulum (ER), Golgi apparatus, peroxisome, etc.

There is intermediate between a gene and its polypeptide, the intermediate is messenger RNA (mRNA). A messenger RNA is assembled as a complimentary copy of one of the two DNA strands that make up gene. The synthesis of RNA from DNA template is called transcription. Because its nucleotide sequence is complementary to that of gene from which it is transcribed, m RNA retains the same information as gene itself. The synthesis of RNA from DNA is called transcription. Because its nucleotide sequence is complementary to that of gene from which it is transcribed, m RNA retains the same information as the gene itself. The use of messenger RNA allows the cell to separate information storage from information utilization. Once in cytoplasm, the m RNA serve as a template to direct the incorporation of amino acids in an order encoded by the nucleotide sequence of DNA and mRNA. One DNA molecule serve as a template in formation of many m RNA molecules, each of which is used in formation of many polypeptide chains. Proteins are synthesized in cytoplasm by process called translation. It requires dozens of different components, including ribosomes. Ribosomes are nonspecific components of translation. Ribosomes contain both protein and RNA. The RNAs of a ribosome is called ribosomal RNAs and like mRNA each is transcribed from one of the DNA strand of a gene. tRNA constitute third major class of RNA that is required during protein synthesis.

Life on earth can be classified into prokaryotes and eukaryotes according to the difference in their cell structure. Prokaryotes are unicellular organisms like bacteria whereas eukaryotes are often multicellular organisms like plants and animals. A

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prokaryotic cell is simpler than a eukaryotic and the main difference is the lack of a well-defined nucleus in the prokaryotes. Eukaryotic cells are called so because of the presence of a true nucleus. The nucleus has a well-defined boundary defined by the nuclear membrane. In prokaryotes, the genetic material DNA is concentrated in a region called nucleoid, which do not have a membrane bound structure.

The eukaryotic DNA is linear and complexes with proteins called histones. The DNA of prokaryotes is always circular. The DNA content of prokaryotes is only around  $1 \times 10^2$  to  $5 \times 10^6$  base pairs. Eukaryotes have much more DNA content and the number of base pairs ranges from  $1.5 \times 10^7$  to  $5 \times 10^9$ . The cytoplasm of eukaryotic cells contains many large and compound collections of organelles. An organelle has its own boundary of lipid membrane which separates it from the rest of the cell and thereby allowing to perform a special function. The prokaryotes lack these membrane bound organelles like Golgi, lysosome, peroxisome, mitochondria and chloroplast. The presence of the membrane bound organelles makes eukaryotic cell more complex. The membrane bound structure of the organelles enhances the efficiency of functions by restricting them to occur within well-defined boundary, thus limiting the span of communication and movement within the organelle itself. The eukaryote cell is much bigger, typically 10-100 micrometers in diameter, compared to the prokaryotic cell which is typically 1 micrometer in diameter.

The size of the ribosomes present in the prokaryotic cell is smaller than that of eukaryotic cell. Cytoskeleton, the organelle responsible for giving structure to the cell, is not found in the prokaryotes. In prokaryotes, the cell division happens in simple steps by binary fission or simple fission. In eukaryotes the cell division is of two types called mitosis and meiosis, which are complex multi-stage process. Within the eukaryotes, there is difference in cell structure between plant and animal cell. Plant cell has a cell wall which is made of cellulose and is intricately cross-linked with fibers of other carbohydrate molecules.

This structural pattern allows each cell to withstand the increased internal pressure from osmosis, when the plant absorbs water. Animal cells do not have rigid cell walls like plant cells and this allows them to take up a variety of shapes. The chloroplasts in the plant cell are the site of photosynthesis. This is absent in the animal cells. In chloroplast, carbon dioxide is turned into sugar as part of photosynthesis. This is in opposite to energy production in animal through mitochondria where sugar is broken down to carbon dioxide to make energy. The vacuole present in plant cells are large compared to animal cells. The plant cell communicates by linking pores in their cell wall to connect to each other and pass information.

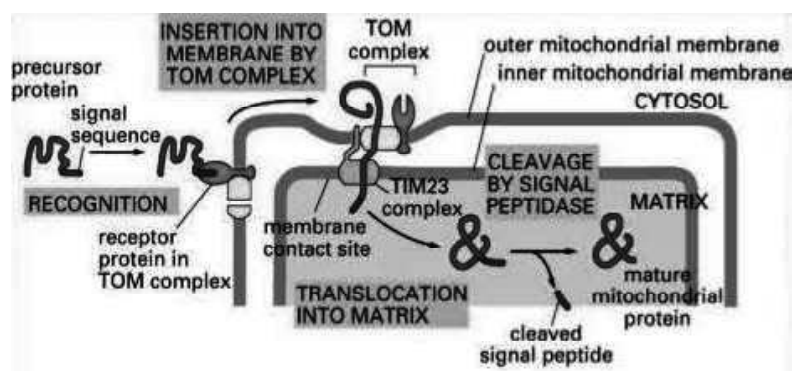
### 6.2.1 Protein Sorting in Mitochondria

Mitochondria are multifunctional double-membrane-bound organelles that arose from a bacterial endosymbiont during the evolution of eukaryotic cells. Known as

the powerhouses of the cell, mitochondria harbor the oxidative phosphorylation machinery for ATP synthesis, but also a large number of biosynthetic pathways. Moreover, they are intimately involved in complex cellular processes, like calcium homeostasis and programmed cell death. As a relic of their evolutionary origin, mitochondria contain their own genetic material and machineries to manufacture their own RNAs and proteins. However, the small circular mitochondrial genome encodes only a few proteins (8 and 13 polypeptides in yeast and humans, respectively). All remaining mitochondrial proteins (approximately 99%) are encoded by the nuclear genome and synthesized on cytosolic ribosomes in their precursor forms. To acquire their mature, functional state these precursor proteins need to be efficiently targeted and imported into mitochondria and sorted to the correct sub mitochondrial compartment: outer membrane, Inter Membrane Space (IMS), inner membrane, and matrix.

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The inner mitochondrial membrane is further subdivided into the inner boundary membrane, which is closely opposed to the outer membrane, and large tubular invaginations, termed cristae membranes. Within the four mitochondrial compartments, sophisticated translocation, sorting, and assembly machineries serve to establish incoming precursors in a functional state within the context of their new environment. Advances in the last decade, particularly because of the application of proteomic approaches, have significantly extended the number of components and machineries known to be involved in mitochondrial protein import.



*Fig. 6.1 Protein Transport in Mitochondria*

Mitochondria contain components required for protein synthesis, including DNA, DNA dependent RNA polymerase, RNA, ribosomes and amino acid activating enzymes. Isolated mitochondria contain multi enzyme system and co factors required to catalyze coupled oxidation by molecular oxygen of acetyl CoA or intermediates of citric acid cycle. These simple compounds arise from preliminary steps in metabolism of carbohydrates, proteins and fats of diet. Citric acid cycle enzymes have dual localization, appearing in both mitochondria and soluble fraction of the cell. They contain enzyme systems that catalyze the coupled oxidation of fatty acid and ketone bodies. Short chain fatty acid and ketone bodies provide important part of fuel used by tissue of ruminants. Citric acid cycle intermediates

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lie on pathways for interconversion of carbohydrates, fats and proteins and activity of mitochondrial enzymes contribute to biosynthetic processes. Many of the carbon skeleton can be produced from carbohydrate via citric acid cycle and transamination reaction. In liver and kidney mitochondria, amino acid are converted to cycle intermediates which are converted to glucose and glycogen via enzymatic pathways outside mitochondria. The key enzymes in amino acid metabolism are glutamate dehydrogenase, localized in mitochondria and glutamate linked aminotransferase. Together catalyze the deamination of amino acids. The regulation of mitochondrial metabolism is very complex. There are many other factors to be considered in addition to regulation imposed by the permeability properties of mitochondrial membrane and regulation of membrane by availability of ADP and phosphate E.g. citric acid cycle activity of mitochondria is influenced by feedback inhibition, enzyme activation by precursors, removal of intermediates and competition for cofactors and hydrogen acceptors. Superimposed on these regulatory mechanism are effect of hormones which regulate basal oxygen consumption of tissue. Physiological amount of hormones influence the rate of tissue respiration by promoting the synthesis of respiratory enzymes. Respiratory chain phosphorylation is partially uncoupled and may occur in tissue when excessive amount of hormone is secreted by thyroid.

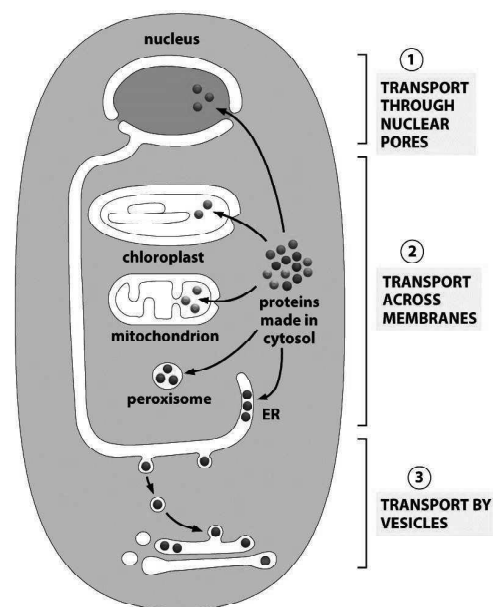


Fig. 6.2 Transport Mechanism Process

### 6.2.2 Protein Sorting in Chloroplast

The newly synthesized proteins by free ribosomes are imported into chloroplasts as in mitochondria. Calvin cycle enzymes fix atmospheric CO<sub>2</sub> into carbohydrates during photosynthesis. Accurate trafficking of nuclear encoded proteins to the stroma of primary plastids is controlled by a characteristic N-terminal signal sequence named the Transit Peptide (TP). Many of the proteins of the outer

compartments insert into the membrane without such a targeting peptide. The transit peptide shows no strict consensus sequence, but is characterized by common features such as a positive net charge and a high frequency of hydroxylated amino acids.

Similarly, most nuclear encoded proteins destined to complex plastids studied to date possess an N-terminal signal, named the bipartite targeting signal. In accordance with the need to traverse one or two additional membranes, these peptides contain an additional element, a sequence equivalent to the Signal Peptide (SP) of secretory proteins. The journey thus commences with entry to the secretory system, likely by the mechanism described for a large variety of secretory proteins: courtesy of the Sec61 transposon. The SP element in the bipartite leader peptide has been studied in detail for proteins that target to the apicoplast (the non-photosynthetic plastid of apicomplexan parasites). The signal was shown to be sufficient to guide reporter proteins to the secretory system. The sequence can be replaced with a canonical SP from a secretory protein, this chimera still confers plastid targeting of a reporter. The SP is likely removed cotranslationally. These first steps occur rapidly and may conclude before the synthesis of a full-length protein is concluded. As a result of SP cleavage the TP is exposed and available to lead the rest of the way. Once the protein has reached the stroma the TP is proteolytically cleaved like the SP before. Pulse-chase measurements in Apicomplexa showed that it takes 45–60 minutes from the moment of translation to TP removal. A homolog of the plant chloroplast transit peptide peptidase was identified in the nuclear genome of the apicomplexans *T. gondii* and *P. falciparum* and proposed to execute this cleavage. Interestingly, the TP elements of complex plastid proteins from apicomplexans and cryptophytes were shown to be sufficient for targeting into isolated pea chloroplasts and cryptomonads peptide was substrate for the pre stromal processing peptidase.

## NOTES

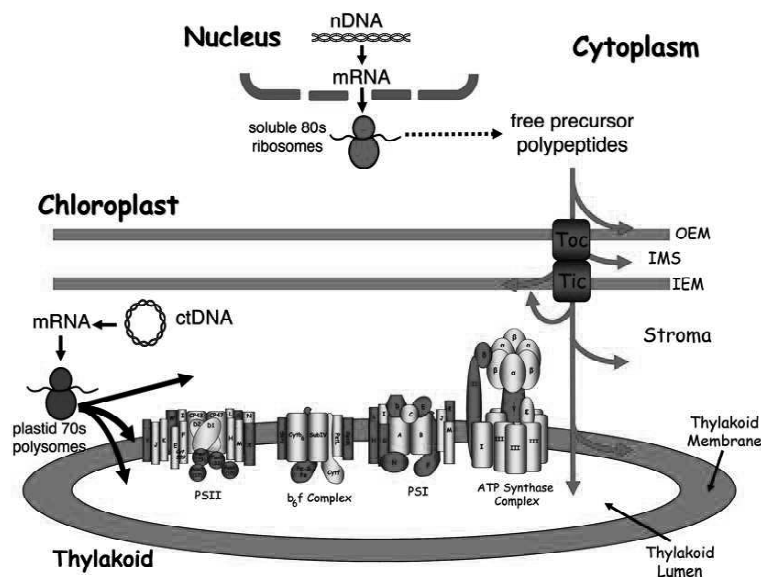


Fig. 6.3 Protein Sorting in Chloroplast

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The primary sequence of the apicoplast TP is not conserved, yet several features reminiscent of the plant chloroplast TP have been noted. In apicomplexans it was demonstrated that an overall positive charge is essential for targeting to the apicoplast lumen, while the position of the positively charged amino acids in the TP can vary. In addition, the compartmentalization of complex plastids imposes the need for specific signals to discriminate between proteins that continue all the way to the stroma from those that home to the outer compartments. The minute size of the apicoplast and the proximity of its four membranes have been a challenge to the study of these signals in Apicomplexa. However, in diatoms intermediates that are transported across only one or two of the four plastid membranes can be discriminated from those imported across all four, and studies in the model system *Phaeodactylum tricornerutum* revealed a complex set of signals. It was shown that the positive net charge of the TP is important to cross the two innermost membranes, and required to cross the second outermost membrane into the PPC. Negative charges in the TP were shown to inhibit entry into the PPC of diatoms, but in contrast are required for PPC targeting in chlorarachniophytes.

This may represent a fundamental difference in these pathways originating from different algal lineages. In diatoms and in cryptophytes the presence of a specific residue, a highly conserved aromatic amino acid at position + 1 of the TP, is crucial for import into the lumen, and the lack of this residue results in PPC residence. Finally, it was shown that the sequences outside of the TP, for example, within the N-terminal part of the mature protein can contribute to the correct targeting of certain proteins. Plastocyanin is a nuclear-encoded chloroplast thylakoid lumen protein that is synthesized in the cytoplasm with a large N-terminal extension (66 amino acids). Transport of plastocyanin involves two steps: import across the chloroplast envelope into the stroma, followed by transfer across the thylakoid membrane into the lumen. During transport the N-terminal extension is removed in two parts by two different processing proteases. In this study we examined the functions of the two cleaved parts, C1 and C2, in the transport pathway of plastocyanin. The results show that C1 mediates import into the chloroplast. C1 is sufficient to direct chloroplast import of mutant proteins that lack C2. It is also sufficient to direct import of a non-plastid protein and can be replaced functionally by the transit peptide of an imported stromal protein. C2 is a prerequisite for intra organellar routing but is not required for chloroplast import. Deletions in C2 result in accumulation of intermediates in the stroma or on the outside of the thylakoids.

The fact that C1 is functionally equivalent to a stromal-targeting transit peptide shows that plastocyanin is imported into the chloroplast by way of the same mechanism as stromal proteins, and that import into and routing inside the chloroplasts are independent processes. N-terminal leader sequences have also been characterized for the proteins targeting to the three membrane bounded plastids of euglenoids and dinoflagellates. Bioinformatic analyses in euglenoids revealed two classes of plastid targeted proteins. In addition to those with bipartite signals there are those with tripartite leaders. In the latter, a third, usually

hydrophobic, domain is present that acts as a stop transfer signal and allows the protein to be transported as an integral membrane protein.

This model is in agreement with earlier experimental evidence from *Euglena*. A recent study identified short introns within regions encoding the complex presequences of *Euglena* plastid proteins. This likely indicates the involvement of introns and exon-shuffling in the acquisition of these targeting signals. Interestingly, dinoflagellate plastid proteins feature leader sequences similar to those of *Euglena* indicating a potential link to the three-membrane topology of these plastids. While most proteins destined to complex plastids are led by their N-terminal sequence, some proteins seem to possess a non-canonical targeting signal.

## NOTES

### 6.2.3 Protein Sorting in Endoplasmic Reticulum (ER)

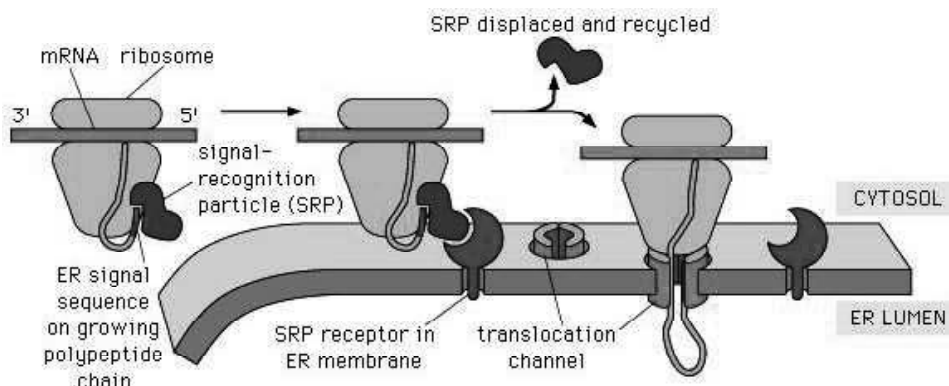
The amount of endoplasmic reticulum varies with type of cell, stage of development, level of activity in terms of proportion between rough and smooth. There is huge data that indicates that protein storage and modification, lipid synthesis and some carbohydrate synthesis take place in endoplasmic reticulum. Many membrane components are of endoplasmic reticulum origin. Both normal changes in the amount of endoplasmic reticulum accompanying development and induced changes in the amount resulting from introduction of toxic agents. Most of the induction experiments and comparisons of them with normal development changes have been carried out on liver cells.

In rapidly differentiating early stages cell formation of rough endoplasmic reticulum precedes that of smooth. So it was postulated that membrane phospholipids and proteins were assembled in rough endoplasmic reticulum and in some manner transferred to smooth endoplasmic reticulum. Tzur and Shapiro (1964) showed that the amount of phospholipid synthesis was regulated by the amount of protein available for binding. Amount of new membrane formed was determined by amount of membrane protein available in the rough endoplasmic reticulum where linkage of phospholipids and proteins take place. They observed that in instance of induction of membrane extension, rough endoplasmic reticulum and smooth endoplasmic reticulum increase simultaneously. They suggest that in normal development smooth endoplasmic reticulum results from detachment of ribosomes from rough endoplasmic reticulum. It was deduced from studies of heterogenous population of microsomes that sectors of endoplasmic reticulum vary with respect to enzymatic activities. This suggest functional differentiation in what appear to be uniform membranes.

This is consistent with some suggestions from morphological and cytochemical studies and with the general postulate that different activities characterize different regions of endoplasmic reticulum. Some aspects of this differentiation may relate to modifying the membrane for transfer. There is convincing electron micrographic evidence that such transfer takes place. Blebbing from one membrane of the endoplasmic reticulum is observed. Transport of proteins between organelles within

## NOTES

the secretory pathway occurs via spherical membrane-bounded vesicles that bud from a donor organelle and fuse with an acceptor in another part of the cell. This fission and fusion transport strategy allows secretory proteins to cross membrane barriers without perturbing the functional segregation conferred by organelles (Refer Figure 6.4).



**Fig. 6.4** Protein Sorting in ER

Conserved sets of cytoplasmic proteins generate distinct classes of transport vesicles, which are largely classified by the protein coats that drive their formation. The three main vesicular frameworks found across eukaryotic life (clathrin, COPI, and COPII) come from evolutionarily related coat proteins. COPII-coated vesicles transport cargo proteins from the ER to the Golgi; COPI-coated vesicles transport cargo in the retrograde direction (from the cis-Golgi back to the ER) and between Golgi cisternae; and clathrin-coated vesicles form from the plasma membrane and the TGN to fuse with endosomes or lysosomes. Vesicle coats perform two central functions: deforming the membrane into a spherical vesicle and populating the vesicle with specific cargo. By coupling cargo selection to vesicle formation. Studies on the internalization of cell surface receptors via clathrin-mediated endocytosis first established the principle that specific protein-based signals mediate capture of cargo into vesicles. Subsequent biochemical, structural, and genetic dissection of clathrin and other vesicle systems has defined how these different coat assemblies couple cargo sorting with the general formation of vesicles. Central to the appropriate sorting of cargo, specific coat subunits (known as cargo adaptors) contain binding surfaces that recognize sorting signals present in the cytoplasmic domains of cargo proteins.

Interaction between coat and signal is responsible for capture of cargo into the forming vesicles. Most binary cargo-coat interactions measured *in vitro* are relatively low affinity, which may be important in the context of coat dynamics during traffic. During the lifetime of the vesicle, coat proteins are shed from the vesicle surface to expose fusion machinery; therefore, interactions between coat and vesicle components must be reversible. However, cargo adaptors also often have affinity for lipids, which can contribute both to the specificity of recruitment and to the global affinity of the adaptor for a donor organelle. One limitation of the signal-mediated sorting of coat-bound cargo adaptors is access to a signal; the



many soluble secreted and lysosomal proteins are precluded from interacting directly with the vesicle coat subunits by the barrier formed by the lipid bilayer. Sorting of these cargo proteins occurs by receptor-mediated transport. Cargo receptors are thus defined as proteins that span the membrane, binding simultaneously to cargo proteins and coat adaptors, to efficiently recruit soluble proteins to nascent vesicles. Some plasma membrane proteins require a cargo receptor despite containing their own ER export motifs. In this case, efficient ER export requires multiple signals: one signal is present in the cargo protein itself, and a second one comes from a cargo receptor (Refer Figure 6.5).

## NOTES

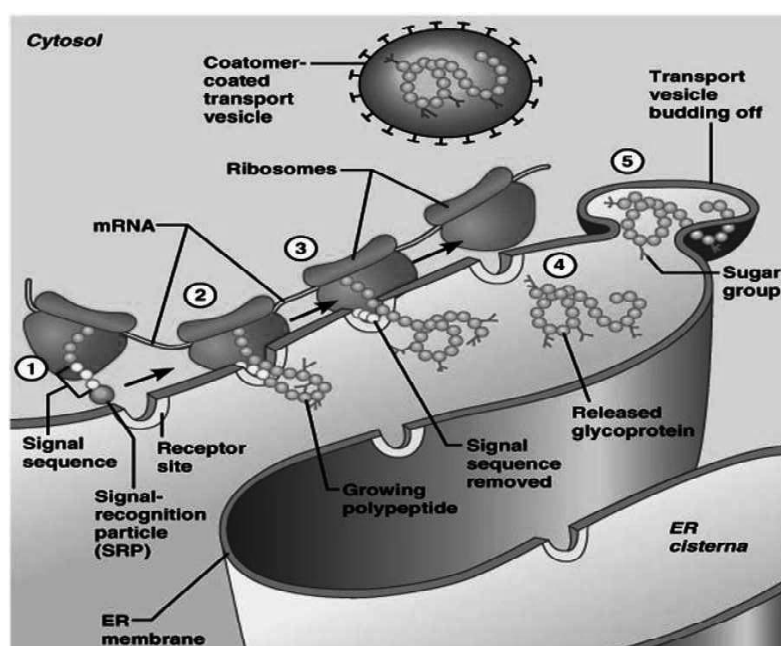


Fig. 6.5 ER Protein Synthesis

### 6.2.4 Protein Sorting in Nucleus

The nuclear envelope consists of outer and inner membranes and has inter-membranous space between them. The outer membrane is continuous with ER and has ribosomes on it. Proteins for the nucleus are synthesized on free ribosomes in the cytosol and imported into nucleus through 3000-4000 nuclear pores known as nuclear pore complexes which are special gates. The proteins that are imported into nucleus are in fully folded state and do not require any chaperones. Proteins imported into nucleus have targeting signal sequences on them which are called Nuclear Localization Signals (NLS). Each one has 4-8 amino acids and they are internal sequences and not terminal. NLS is not cleaved from the protein. Due to this feature proteins can re-enter the nucleus whenever the nuclear envelope is lost during cell division. Proteins are the most abundant macromolecules in the cell. They are the workhorses, carrying out vital biological functions. They perform critical roles in growth, giving structure to cell, maintenance in tissues etc.

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Proteins have a wide range of functions as enzymes, hormones, antibodies, structural protein, storage protein and transport protein to name a few. Enzymes facilitate biochemical reactions and are vital to metabolism. Hormones like insulin, oxytocin and somatotropin are messenger proteins, giving signals to coordinate various activities. Antibodies are proteins that defend the body from antigens. Structural proteins like keratin, collagen, actin and elastin are fibrous and stringy which help in providing structure, stiffness and rigidity to otherwise-fluid biological components. Storage proteins like ovalbumin and casein store amino acids. Transport proteins like hemoglobin and cytochromes are carrier proteins which move molecules from one place to another around the body. Proteins are made of amino acids, connected together by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. One end of this amino acid chain has a free amino group and is called amino terminal or N-terminal. The other end, with a free carboxyl group, is called the carboxyl terminal or C-terminal.

The amino acid sequence is the order in which amino acid residues appear in the protein. The amino acid sequence is written in the order, starting from N-terminal and ending in C-terminal. The linear order of the amino acids in a protein or peptide constitutes the primary structure of the protein. Proteins cannot perform its intended function in the primary structure level. They fold to form secondary, tertiary and quaternary structure. Secondary structure is formed by the hydrogen bonds between the amino acids in the polypeptide. Secondary structures are regularly repeating local structures. Multiple secondary structures can be present in a single protein. Alpha helix and beta sheets are examples of secondary structure. Tertiary structure is the three-dimensional structure of the polypeptide chain into which it folds naturally or with the assistance of chaperones. The function of a protein depends on its tertiary structure (Refer Figure 6.6).

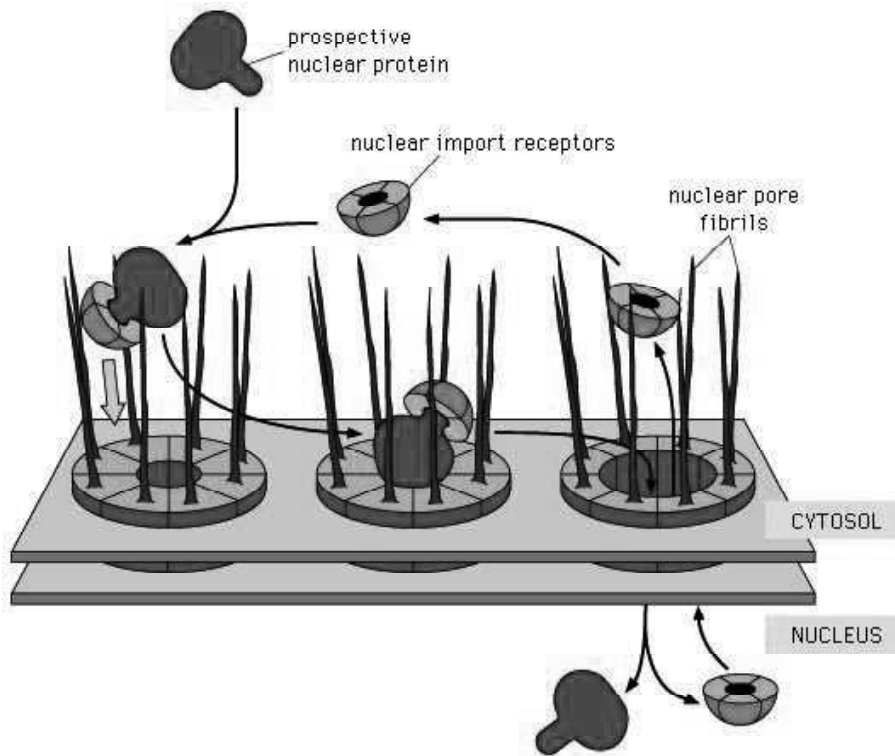
When denatured, the protein tertiary structure is disrupted and the protein loses its activity. The tertiary structure is the spatial arrangement of secondary structures interacting through hydrophobicity, salt bridges, hydrogen bonds, disulfide bonds, and post-translational modifications. In quaternary structure, separate peptide chain, known as subunits join together to form a complex. The sequence of amino acids in a protein is decided by the three letter codons in the messenger RNA (mRNA) from which the protein was translated. The sequence of codons in the mRNA is, in turn, decided by the sequence of codons in the DNA from which the mRNA was transcribed. The coding portion of DNA is known as genes. Thus, the instructions to define a protein are written in the genes which reside in the nucleus. Except for a small number of proteins, coded in the genomes of mitochondria and chloroplasts, most of the proteins in a cell are encoded by nuclear DNA and are synthesized on ribosomes in the cytosol. For proper functioning, these proteins are to be distributed to their correct destinations in the cell.

In 1999, Gunter Blobel was awarded Nobel Prize in Physiology and Medicine for the discovery that 'proteins have intrinsic signals that govern their transport and localization in the cell'. The sorting signals are present in the primary

amino acid sequence levels mostly at its N terminal. For further sorting within the organelle, additional targeting information may be located in a secondary targeting sequence, either placed adjacent to the original targeting sequence or in other regions of the protein. Proteins are translocated to their targeted location either cotranslationally or posttranslationally. In cotranslational translocation, the translocation starts while the protein is still being synthesized on the ribosome. Proteins targeted for ER, Golgi apparatus, plasma membrane, lysosome, vacuole and extracellular space uses the SRP-dependent pathway and are translocated cotranslationally.

The N-terminal signal sequence of these proteins, is recognized by a Signal Recognition Particle (SRP), while the proteins being translated in the free ribosome. The ribosome-protein complex is transferred to a SRP receptor on the ER and the synthesis pauses. There, the nascent protein is inserted into the translocon that passes through the ER membrane. Transfer of the ribosome-mRNA complex from the SRP to the translocon opens the gate on the translocon and allows the translation to resume. The signal sequence is immediately cleaved from the polypeptide once it has been translocated into the ER by signal peptidase in secretory proteins. Within the ER, chaperone helps protein to fold correctly. From ER, proteins are transported in vesicles to the Golgi apparatus where they are further processed and sorted for transport to endosomes, lysosomes, plasma membrane or secretion from the cell. The proteins for ER will have various ER retention signals to keep them in the ER itself.

## NOTES



**Fig. 6.6** Protein Sorting in Nucleus

## NOTES

Most of the proteins targeted for mitochondria, chloroplast, nucleus and peroxisome are translocated post translationally. In contrast to the cotranslationally translocated proteins, these proteins are translated in the free ribosomes in the cytosol. Once the translation is complete, they are released into the cytosol. These proteins which enter the non-secretory pathway are sorted to their destination site based on the presence of the targeting signal. Once the protein has reached its destination, the targeting signals are cleaved off. The targeting sequence for mitochondrial proteins, mitochondrial Transfer Peptide (mTP), will have 3 - 5 non-consecutive Arg or Lys residues, often with Ser and Thr, at the N-terminal of the polypeptide chain. No Glu or Asp residues are generally found here. In the case of chloroplast, Chloroplast Transit Peptide (CTP), no common sequence motifs are found but the N-terminal is generally rich in Ser, Thr, and small hydrophobic amino acid residues and the region is poor in Glu and Asp residues. For peroxisome proteins, the sorting signal is generally found at extreme C-terminal usually as Ser-Lys-Leu and these signals are not cleaved off after reaching the destination. Proteins destined for nucleus have a distributed sorting signal which is not cleaved off after sorting. One cluster of 5 basic amino acids or two smaller clusters of basic residues, separated by around 10 amino acids are usually found as nuclear localization signal.

### Check Your Progress

1. Define Phosphorylation.
2. What leads to protein sorting in nucleus?
3. What is cytoskeleton?
4. Define proteins and give its function.
5. What are cargo receptors?
6. Define mitochondria.

## 6.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Phosphorylation is an important mechanism by which activity of proteins are altered by the addition of phosphate group.
2. Transport through nuclear pores, transport across membrane, and vesicular transport leads to protein sorting in nucleus.
3. Cytoskeleton is network of filaments and tubules that extends throughout a cell, through cytoplasm, which is all of material within a cell except nucleus

4. Proteins are the most abundant macromolecules in the cell. They are the workhorses, carrying out vital biological functions. They perform critical roles in growth, giving structure to cell, maintenance in tissues etc.
5. Cargo receptors are defined as proteins that span the membrane, binding simultaneously to cargo proteins and coat adaptors, to efficiently recruit soluble proteins to nascent vesicles. Some plasma membrane proteins require a cargo receptor despite containing their own ER export motifs.
6. Mitochondria are multifunctional double-membrane-bound organelles that arose from a bacterial endosymbiont during the evolution of eukaryotic cells.

## NOTES

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### 6.4 SUMMARY

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- The synthesis of RNA from DNA template is called transcription. Because its nucleotide sequence is complementary to that of gene from which it is transcribed, mRNA retains the same information as gene itself. The synthesis of RNA from DNA is called transcription.
- One DNA molecule serve as a template in formation of many mRNA molecules, each of which is used in formation of many polypeptide chains. Proteins are synthesized in cytoplasm by process called translation.
- A prokaryotic cell is simpler than a eukaryotic and the main difference is the lack of a well-defined nucleus in the prokaryotes. Eukaryotic cells are called so because of the presence of a true nucleus.
- Eukaryotes have much more DNA content and the number of base pairs ranges from  $1.5 \times 10^7$  to  $5 \times 10^9$ . The cytoplasm of eukaryotic cells contains many large and compound collections of organelles.
- The vacuole present in plant cells are large compared to animal cells. The plant cell communicates by linking pores in their cell wall to connect to each other and pass information.
- Mitochondria are multifunctional double-membrane-bound organelles that arose from a bacterial endosymbiont during the evolution of eukaryotic cells.
- The key enzymes in amino acid metabolism are glutamate dehydrogenase, localized in mitochondria and glutamate linked aminotransferase. Together catalyze the deamination of amino acids. The regulation of mitochondrial metabolism is very complex.
- A homolog of the plant chloroplast transit peptide peptidase was identified in the nuclear genome of the apicomplexans *T. gondii* and *P. falciparum* and proposed to execute this cleavage.

## NOTES

- In addition, the compartmentalization of complex plastids imposes the need for specific signals to discriminate between proteins that continue all the way to the stroma from those that home to the outer compartments. The minute size of the apicoplast and the proximity of its four membranes have been a challenge to the study of these signals in Apicomplexa.
- Bioinformatic analyses in euglenoids revealed two classes of plastid targeted proteins. In addition to those with bipartite signals there are those with tripartite leaders.
- Interaction between coat and signal is responsible for capture of cargo into the forming vesicles. Most binary cargo-coat interactions measured in vitro are relatively low affinity, which may be important in the context of coat dynamics during traffic.
- Cargo receptors are defined as proteins that span the membrane, binding simultaneously to cargo proteins and coat adaptors, to efficiently recruit soluble proteins to nascent vesicles. Some plasma membrane proteins require a cargo receptor despite containing their own ER export motifs.
- Proteins for the nucleus are synthesized on free ribosomes in the cytosol and imported into nucleus through 3000-4000 nuclear pores known as nuclear pore complexes which are special gates. The proteins that are imported into nucleus are in fully folded state and do not require any chaperones.
- Proteins are the most abundant macromolecules in the cell. They are the workhorses, carrying out vital biological functions. They perform critical roles in growth, giving structure to cell, maintenance in tissues etc.
- The linear order of the amino acids in a protein or peptide constitutes the primary structure of the protein. Proteins cannot perform its intended function in the primary structure level. They fold to form secondary, tertiary and quaternary structure. Secondary structure is formed by the hydrogen bonds between the amino acids in the polypeptide.
- Secondary structures are regularly repeating local structures. Multiple secondary structures can be present in a single protein. Alpha helix and beta sheets are examples of secondary structure.
- Tertiary structure is the three-dimensional structure of the polypeptide chain into which it folds naturally or with the assistance of chaperones. The function of a protein depends on its tertiary structure.
- In quaternary structure, separate peptide chain, known as subunits join together to form a complex. The sequence of amino acids in a protein is decided by the three letter codons in the messenger RNA (mRNA) from which the protein was translated.

- Except for a small number of proteins, coded in the genomes of mitochondria and chloroplasts, most of the proteins in a cell are encoded by nuclear DNA and are synthesized on ribosomes in the cytosol. For proper functioning, these proteins are to be distributed to their correct destinations in the cell.
- In 1999, Gunter Blobel was awarded Nobel Prize in Physiology or Medicine for the discovery that ‘proteins have intrinsic signals that govern their transport and localization in the cell’. The sorting signals are present in the primary amino acid sequence levels mostly at its N terminal.
- The N-terminal signal sequence of these proteins, is recognized by a Signal Recognition Particle (SRP), while the proteins being translated in the free ribosome. The ribosome-protein complex is transferred to a SRP receptor on the ER and the synthesis pauses.
- Most of the proteins targeted for mitochondria, chloroplast, nucleus and peroxisome are translocated posttranslationally. In contrast to the cotranslationally translocated proteins, these proteins are translated in the free ribosomes in the cytosol.
- Once the translation is complete, they are released into the cytosol. These proteins which enter the non-secretory pathway are sorted to their destination site based on the presence of the targeting signal. Once the protein has reached its destination, the targeting signals are cleaved off.
- For peroxisome proteins, the sorting signal is generally found at extreme C-terminal usually as Ser-Lys-Leu and these signals are not cleaved off after reaching the destination. Proteins destined for nucleus have a distributed sorting signal which is not cleaved off after sorting.

## NOTES

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### 6.5 KEY WORDS

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- **Phosphorylation:** It is an important mechanism by which activity of proteins are altered by the addition of phosphate group.
- **Cytoskeleton:** Network of filaments and tubules that extends throughout a cell, through cytoplasm, which is all of material within a cell except nucleus.
- **Transamination:** A chemical reaction that transfers an amino group to a ketoacid to form new amino acids. It is responsible for the deamination of most amino acids.
- **Feedback inhibition:** The process of the end product of particular metabolic reaction inhibiting an allosteric enzyme involved in that reaction.
- **Endosymbiont:** Any organism that lives within the body or cells of another organism in a mutualistic relationship.

## NOTES

- **Translocon:** Complex of proteins associated with the translocation of polypeptides across membranes or complex that transports nascent polypeptides with targeting signal sequence.
- **SRP receptor:** It is a dimer composed of two different subunits that are associated with rough ER in mammalian cells.

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## 6.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

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### Short Answer Questions

1. Brief a note on protein sorting.
2. Describe briefly about protein sorting in mitochondria.
3. Write a short note on in protein sorting in nucleus.
4. Define the following terms:
  - Phosphorylation
  - Cytoskeleton
  - Endosymbiont
  - Translocon
5. Brief a note on protein sorting in chloroplast.

### Long Answer Questions

1. How is protein sorting carried out? Explain in detail.
2. Explore the latest trends in protein sorting in nucleus?
3. Elaborate a note on protein sorting in mitochondria.
4. Explain with the help of well labelled diagram the protein sorting in chloroplast.
5. Write in detail about protein sorting in endoplasmic reticulum, also give its well labeled diagram.

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## 6.7 FURTHER READINGS

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## NOTES

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## **UNIT 7 PROTEIN PROCESSING AND TRAFFICKING FROM ER TO GOLGI; CELL DIVISION AND CELL CYCLE**

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### **Structure**

- 7.0 Introduction
- 7.1 Objectives
- 7.2 Protein Processing and Trafficking: General Introduction
- 7.3 Cell Division and Cell Cycle
- 7.4 Answers to Check Your Progress Questions
- 7.5 Summary
- 7.6 Key Words
- 7.7 Self Assessment Questions and Exercises
- 7.8 Further Readings

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### **7.0 INTRODUCTION**

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Translation completes the flow of genetic information within the cell. The sequence of nucleotides in DNA has now been converted to the sequence of amino acids in a polypeptide chain. The synthesis of a polypeptide, however, is not equivalent to the production of a functional protein. To be useful, polypeptides must fold into distinct three-dimensional conformations, and in many cases multiple polypeptide chains must assemble into a functional complex. In addition, many proteins undergo further modifications, including cleavage and the covalent attachment of carbohydrates and lipids that are critical for the function and correct localization of proteins within the cell. Many of the proteins now known to function as molecular chaperones were initially identified as heat-shock proteins, a group of proteins expressed in cells that have been subjected to elevated temperatures or other forms of environmental stress.

Membrane protein trafficking first requires a newly synthesized protein to travel from the endoplasmic reticulum through the multiple layers of the Golgi apparatus. These organelles not only serve to modify membrane proteins with appropriate carbohydrate moieties but also serve as ‘quality control’ pathways to ensure that immature or misfolded proteins are removed from the biosynthetic pathway and ultimately targeted for degradation. The compartmentalization of eukaryotic cells has considerable functional advantages for the cell, but requires elaborate mechanisms to ensure that nascent proteins are correctly targeted to the appropriate compartment.

In this unit, you will learn about protein processing and trafficking from Endoplasmic Reticulum to Golgi- cell division and cell cycle in detail.

*Protein Processing and  
Trafficking from ER to Golgi;  
Cell Division and Cell Cycle*

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## 7.1 OBJECTIVES

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After going through this unit, you will be able to:

- Understand protein processing
- Explain protein processing and trafficking from Endoplasmic Reticulum to Golgi- cell division and cell cycle

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## 7.2 PROTEIN PROCESSING AND TRAFFICKING: GENERAL INTRODUCTION

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Membrane protein trafficking first requires a newly synthesized protein to travel from the endoplasmic reticulum through the multiple layers of the Golgi apparatus. These organelles not only serve to modify membrane proteins with appropriate carbohydrate moieties but also serve as ‘quality control’ pathways to ensure that immature or misfolded proteins are removed from the biosynthetic pathway and ultimately targeted for degradation. The compartmentalization of eukaryotic cells has considerable functional advantages for the cell, but requires elaborate mechanisms to ensure that nascent proteins are correctly targeted to the appropriate compartment. This targeting occurs as the result of a series of binary (yes/no) decisions that commence at the time of synthesis of the nascent polypeptide. Proteins carry codes in their sequences that are read by targeting machinery at every stage of their voyage to their ultimate location. Proteins may be targeted to the cytosol, mitochondria, peroxisomes or chloroplasts. These proteins (if encoded in the nucleus) are synthesized on free ribosomes.

However, proteins destined for secretion, for the lumen of the ER, Golgi or lysosomes, or for the membrane of any of these organelles or the plasma membrane are synthesized on the membrane bound ribosomes of the rough ER. They are then targeted to the appropriate cellular compartment. Protein import, oligomerization and folding are aided by chaperones (BiP) and protein disulphide isomerase within the ER lumen. Misfolded proteins are not permitted to pass further down the secretory pathway, but instead are degraded. Their continued association with chaperones prevents them from being packaged into transport vesicles destined for the Golgi. It has become clear recently that these proteins are degraded in the cytosol using the same ubiquitin-dependent machinery as cytosolic proteins. This means that polypeptides that are to be degraded have to be re-directed back through the translocation machinery. The Golgi apparatus consists of an ordered series of compartments in which N-linked oligosaccharide chains are processed, O-linked oligosaccharides are added, and the proteoglycans of the extracellular matrix are assembled. Distinct steps in the processing of N linked

## NOTES

## NOTES

oligosaccharides occur in the cis, medial and trans compartments of the Golgi. The Golgi also has important sorting functions. O-linked glycosylation Sugar moieties are added sequentially (in contrast to N-linked glycosylation in which residues are added en bloc) to the hydroxyl groups of serine or threonine residues. An extreme example is the attachment of glycosaminoglycans via a link tetra saccharide to serine residues of the core protein of proteoglycans. Tagging Lysosomal Hydrolases GlcNAc-phosphotransferases phosphorylate sugar residues on lysosomal hydrolases. These mannose-6-phosphate residues are recognized by a receptor that targets these proteins to the lysosome. All known Golgi residents are membrane proteins. The signal for Golgi retention seems to be in the trans membrane domain of these proteins. These sequences may cause oligomerization of the proteins, thus preventing their incorporation in transport vesicles. The trans Golgi network is an important sorting compartment within the secretory pathway.

Here, lysosomal hydrolases bind to the mannose-6-phosphate receptor and are targeted to the lysosome. Proteins destined for regulated secretion (examples include digestive enzymes secreted in response to hormonal stimulation, or the hormones themselves) are packaged into vesicles that await a signal to discharge their contents. It is also a site for sorting proteins to the appropriate plasma membrane.

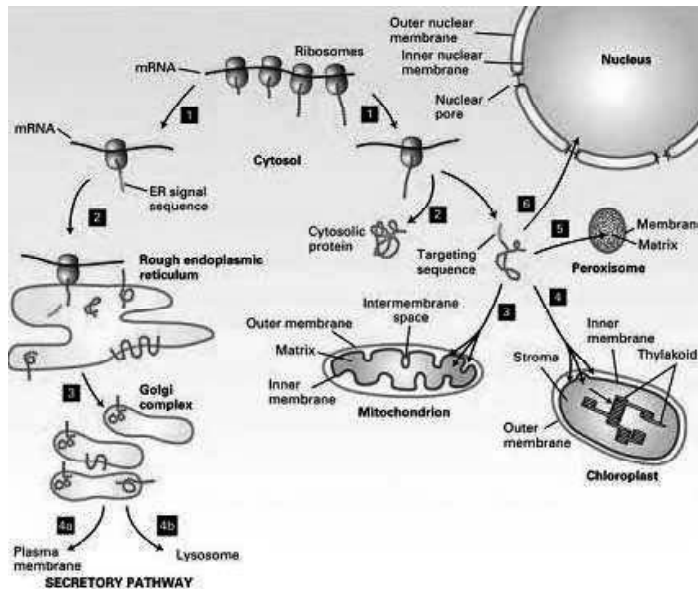
### **Protein Processing and Trafficking from ER to Golgi**

A typical mammalian cell may contain numerous kinds of proteins and numerous individual protein molecules. The eukaryotic cell is a multi-compartmental structure. Its many organelles each requires different proteins. Except a few of them which are synthesized in mitochondria and chloroplasts all other proteins necessary for the cell and the ones to be secreted by the cell are synthesized in the cytosol on free ribosomes and on ribosomes bound to the endoplasmic reticulum. Most proteins are coded by the nuclear genome and synthesized in the cytoplasm. The proteins are present in the ER, mitochondria, chloroplasts, Golgi, peroxisomes, nucleus, in the cytosol and in the membranes of all these organelles. They are selectively transported into their appropriate organelles inside the cell and across the plasma membrane to be secreted outside the cell. Some of them are carried into membrane bound vesicles which bud off from one organelle and transported in definite pathways. Different destinations of different proteins require sophisticated system for labelling and sorting newly synthesized proteins and ensuring that they reach their proper places. This transportation of proteins to their final destinations is called protein targeting.

Proteins destined for cytoplasm and those to be incorporated into mitochondria, chloroplasts and nuclei are synthesized on free ribosomes in the cytoplasm. Proteins destined for cellular membranes, lysosomes and extracellular

transport, use a special distribution system. The main structures in this system are the Rough Endoplasmic Reticulum (RER) and Golgi complex. The RER is a network of interconnected membrane enclosed vesicles or vacuoles. The Endoplasmic Reticulum is coated with polyribosomes to give it a rough appearance. The Golgi complex is also a stack of membrane bound sacs but they are not interconnected. The Golgi complex acts as a switching center for proteins to various destinations (Refer Figure 7.1).

## NOTES



*Fig. 7.1 Major Protein Sorting Pathway in Eukaryotes*

Proteins to be directed to their destinations via Golgi complex are synthesized by ribosomes associated with endoplasmic reticulum. Some of them are carried into membrane bound vesicles which bud off from one organelle and transported in definite pathways. Different destinations of different proteins require sophisticated system for labelling and sorting newly synthesized proteins and ensuring that they reach their proper places. This transportation of proteins to their final destinations is called protein targeting. Proteins destined for cytoplasm and those to be incorporated into mitochondria, chloroplasts and nuclei are synthesized on free ribosomes in the cytoplasm. Proteins destined for cellular membranes, lysosomes and extracellular transport, use a special distribution system. The main structures in this system are the Rough Endoplasmic Reticulum (RER) and Golgi complex. The RER is a network of interconnected membrane enclosed vesicles or vacuoles. The endoplasmic reticulum is coated with polyribosomes to give it a rough appearance. The Golgi complex is also a stack of membrane bound sacs but they are not interconnected. The Golgi complex acts as a switching center for proteins to various destinations (Refer Figure 7.2 and 7.3).

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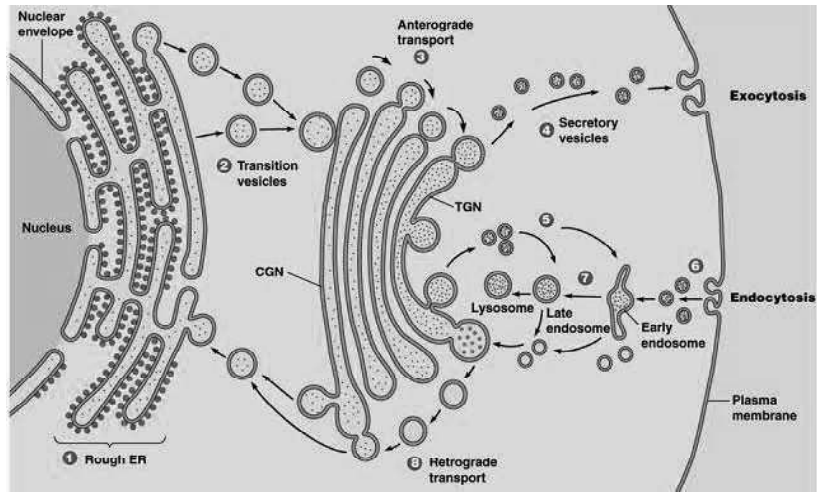


Fig. 7.2 Rough ER and Golgi

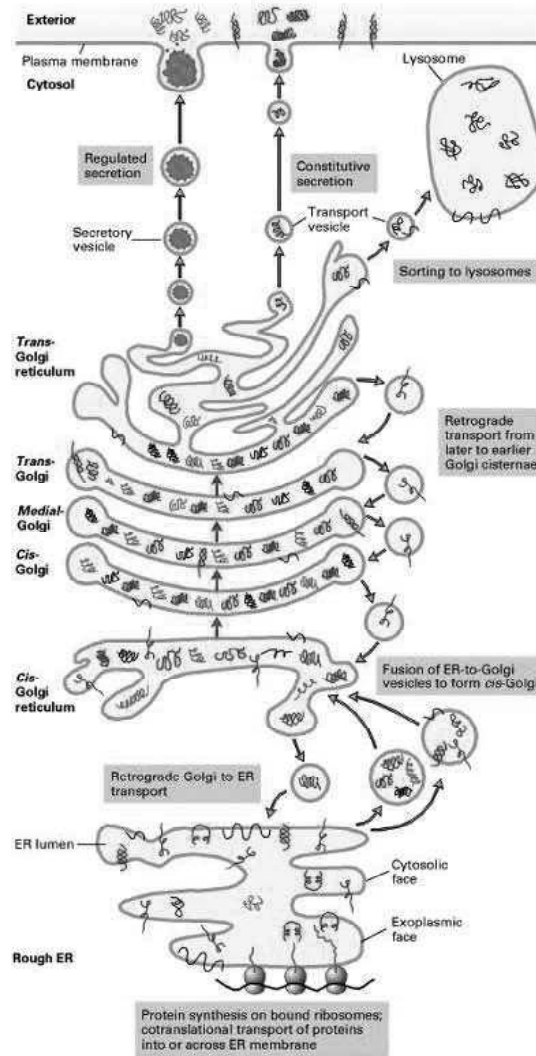


Fig. 7.3 Sort of Proteins

Proteins to be directed to their destinations via Golgi complex are synthesized by ribosomes associated with endoplasmic reticulum. Some of them are carried into membrane bound vesicles which bud off from one organelle and transported in definite pathways. Different destinations of different proteins require sophisticated system for labelling and sorting newly synthesized proteins and ensuring that they reach their proper places. This transportation of proteins to their final destinations is called protein targeting. Proteins destined for cytoplasm and those to be incorporated into mitochondria, chloroplasts and nuclei are synthesized on free ribosomes in the cytoplasm. Proteins destined for cellular membranes, lysosomes and extracellular transport, use a special distribution system. The main structures in this system are the Rough Endoplasmic Reticulum (RER) and Golgi complex. The RER is a network of interconnected membrane enclosed vesicles or vacuoles. The endoplasmic reticulum is coated with polyribosomes to give it a rough appearance. The Golgi complex is also a stack of membrane bound sacs but they are not interconnected. The Golgi complex acts as a switching center for proteins to various destinations. Proteins to be directed to their destinations via Golgi complex are synthesized by ribosomes associated with endoplasmic reticulum

## NOTES

It consists of a Signal Recognition Particle (SRP) present in the cytosol. SRP binds to the signal sequence of the nascent protein as soon as it emerges out of ribosome and directs it towards the ER membrane. The binding of SRP stops further synthesis of protein chain when it is about 70 amino acids long. This prevents it from folding. The SRP-ribosome complex binds to the SAP receptor, which is an integral membrane protein in the wall of ER and is a docking protein of the ER. At this point GTP hydrolysis hydrolyses frees SRP which is ready for the next round of directing next nascent protein of ER. It consists of a Signal Recognition Particle (SRP) present in the cytosol. SRP binds to the signal sequence of the nascent protein as soon as it emerges out of ribosome and directs it towards the ER membrane. The binding of SRP stops further synthesis of protein chain when it is about 70 amino acids long. This prevents it from folding. The SRP-ribosome complex binds to the SAP receptor, which is an integral membrane protein in the wall of ER and is a docking protein of the ER.

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The lysosomal enzymes and lysosomal membrane proteins are synthesized in rough ER and transported to Golgi cisternae and ultimately to lysosomes. The sorting signal that directs the lysosomal enzymes from the Trans-Golgi Network (TGN) to lysosomes is Mannose 6-Phosphate (M6P). The attachment of M6P to lysosomal enzymes prevents their further modification. Separation of M6P bearing lysosomal enzymes from other proteins takes place in TGN. The wall of TGN contains M6P receptors. These M6P receptors bind to lysosomal proteins. The vesicles containing these receptor bearing proteins bud off from TGN. These vesicles are called lysosomes. Later these vesicles fuse with vesicles which have arisen by pinocytosis and phagocytosis to form secondary lysosomes. Low pH of Lysosomes triggers the dissociation of enzymes from the receptors. The M6P receptors are recycled back to trans-Golgi network in vesicles. Lysosomes contain hydrolyzing proteolytic enzyme, which digests proteins meant for degradation. A protein named ubiquitin marks the proteins meant for destruction. Ubiquitin is present in all eukaryotic cells. This mechanism degrades only those proteins which are meant for destruction and not the proteins which are to be left alone.

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## 7.3 CELL DIVISION AND CELL CYCLE

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New cells originate only from other living cells and the process is called cell division. For a multicellular organism, human or oak tree, countless division of single celled zygote produce an organism of cellular complexity and organization. Cell division does not stop with formation of mature organism but continues in tissue throughout. Millions of cells residing within marrow of bones or lining of tract are undergoing division. For multicellular organism such as human or oak tree, countless division of single celled zygote produce organism of cellular complexity and organization. Cell division occurs in all organism, it takes place differently in prokaryotes and eukaryotes. Mitosis leads to production of cells that are genetically identical to their parent, whereas meiosis leads to production of cells with half the genetic content of parent. Mitosis serve as a basis for producing new cells, meiosis as the basis for producing new sexually reproducing organism.

### The Cell Cycle

In a population of dividing cell whether inside the body or in culture dish, each cell passes through a series of defined stages that constitutes cell cycle. The cell cycle is divided into two major phases based on activities visible with light microscope: M phase and interphase. M phase includes the process of mitosis during which duplicated chromosomes are separated into two nuclei and cytokinesis, during which the entire cell divides into two daughter cells.



Interphase, the period between cell divisions is a time when the cell grows and engages in diverse metabolic activities. Whereas M phase last only an hour or in mammalian cells, extend for days, weeks or longer depending the cell type. Although M phase is the period when the contents of a cell are actually divided, numerous preparation for mitosis occur during interphase including replication of DNA. Though cell engages in replication throughout interphase. DNA replication can be monitored by the incorporation of thymidine into newly synthesized DNA. If thymidine is given to culture of cells for a short period and a sample of cell population is fixed, dried into slide and examined by autoradiography, only fraction of cells are found to have radioactive nuclei.

Among cells that were engaged in mitosis at the time of fixation none is found to have radioactively labeled nucleus. These mitotic cells have unlabeled chromosomes because they were not engaged in DNA replication during the labeling period. It can be concluded that there is definite period of time between the end of DNA synthesis and beginning of M phase. This period is termed G<sub>2</sub> (second gap).

The duration of G<sub>2</sub> is revealed as one continues to take samples of cells from culture until labeled mitotic chromosomes are observed. The first cells whose mitotic chromosomes are labeled must have been at last stages of DNA synthesis at the start of incubation with thymidine. The length of time between the start of labeling period and appearance of cells with labeled mitotic figures corresponds to duration of G<sub>2</sub>.

DNA replication occurs during a period of cell cycle termed S phase. S phase is the period when cell synthesizes the additional histones that will be needed as cell doubles the number of nucleosomes in its chromosomes. The length of S phase can be determined directly. The percentage of cells engaged in a particular activity is a measure of percentage of time that is this activity occupies in the lives of cell.

If we know the length of entire cycle, length of S phase can be calculated from the percentage of cells whose nuclei are radioactively labeled during brief pulse with thymidine.

### **Cell Cycles In-Vivo**

One of the properties that distinguish various type of cell within multicellular plant or animal is their capacity to grow and divide, i.e., cells such as nerve cell, muscle cell or red blood cells that are highly specialized and lack the ability to divide, cells that don't divide but can be induced to begin DNA synthesis and divide when given an appropriate stimulus, cells that normally possess a relatively high level of mitotic activity. Cell cycles can range in length from as short as 30 minutes in a cleaving frog embryo, whose cell cycle lack both G<sub>1</sub> and G<sub>2</sub> phases to several months in slowly growing tissue. Cells that are arrested in this state which includes the majority of cells in the body are said to be in G<sub>0</sub> state to distinguish them from G<sub>1</sub> phase cells that soon enter S phase.

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### Control of Cell Cycle

The study of cell cycle is not important in cell biology, but has enormous practical implication in combating cancer, a disease that results from breakdown in cells ability to regulate its division. Rao and Johnson wanted to know whether the cytoplasm of cells contain regulatory factors that affect cell cycle activities. In one experiment, they fused cells in G1 stage with cells in S stage and tried understanding the following questions: does the cytoplasm donated by the non-replicating G1 cell contain factors that block DNA replication in the S phase, does the cytoplasm of replicating S phase cell contain factors that stimulate DNA replication in G1 phase nucleus.

Nucleus in hybrid cell had been donated by G1 phase cell and was activated by S phase cytoplasm to begin DNA replication. The cytoplasm of a replicating cell contain diffusible factors that stimulate initiation of DNA synthesis in G1 phase nuclei. In contrast when G2 phase and S phase cells were fused, G2 phase nuclei didn't initiate another round of DNA synthesis. This suggests that G2 phase nuclei, can no longer respond to initiation factors present in S phase cell cytoplasm.

### M Phase Mitosis and Cytokinesis

Our knowledge of M phase is based on more than century of microscopic and biochemical research on animals and plants. The term mitosis comes from Greek word mitosis meaning 'thread'. The name was coined in 1882 by biologist Walther Flemming to describe threadlike chromosomes that appeared in animal cells before they divided into two. Mitosis is a process of nuclear division in which replicated DNA molecules of each chromosome are segregated into two nuclei.

Mitosis is accompanied by cytokinesis: a process by which dividing cell splits into two. The two daughter cells resulting from mitosis and cytokinesis possess a genetic content identical to each other and to the mother cell from which they arose.

Mitosis maintains the chromosome number and generates new cells for growth and maintenance of an organism. Mitosis can take place in either haploid or diploid cells. Haploid mitotic cell are found in fungi, gametophytes and few animals. Mitosis is a stage of cell cycle where cell devotes all its energy to single activity- chromosome segregation. Most metabolic activities of the cell, including transcription and translation are curtailed during mitosis.

### Prophase

There is division of the centriole. Such granule is found in the cells of primitive plants and all animals that contains RNA protein and is situated outside the nucleus. Distinct chromosomes become visible. Each chromosome is a double filament, such chromosomes lie parallel and joined to each other at a single point. This attachment point is called centromere. During the first stage of mitosis, that of

prophase, duplicated chromosomes are prepared for segregation and the mitotic machinery is assembled.

*Protein Processing and  
Trafficking from ER to Golgi;  
Cell Division and Cell Cycle*

### **Formation of Mitotic Chromosomes**

The nucleus of an interphase cell contains tremendous lengths of chromatin fibres. The extended state of interphase chromatin is ideally suited for processes of transcription and replication but not for segregation into two daughter cells. Before segregating its chromosomes, cell converts them into shorter, thicker structures by process of chromosome compaction which occurs during early prophase. Prior to replication, DNA of each interphase chromosomes become associated at sites along its length with multiprotein complex called cohesion. Following replication, cohesion function as a physical bridge that holds two sister chromatids together through G2 and into mitosis when they are separated.

The most notable landmark on a mitotic chromosome is primary constriction which marks the position of centromere. The centromere is highly repeated DNA sequences that serve as the binding sites for specific proteins. Examination of section through mitotic chromosomes reveals the presence of proteinaceous button like structure called kinetochore at outer surface of centromere of each chromatid. As a cell progresses past G2 into mitosis, the microtubules, microtubules of the cytoskeleton undergoes disassembly in preparation for their reassembly as components of a complex, micro sized machine called mitotic spindle. The first stage in the formation of mitotic spindle is the appearance of microtubules in arrangement called aster. Microtubules grow by addition of subunits to their plus ends, while their minus ends associates with centrosome. The process of aster formation is followed by separation of centrosomes from one another and their movement around nucleus towards opposite ends of cell.

### **Metaphase and Anaphase**

Early during metaphase, the spindle poles, marked by centrioles in animal cells, reach their position at opposite sides of the cell. Spindle and asters attain their maximal growth at this stage. The chromosome pairs, scattered randomly through central portion of the cell, begin to migrate. It is the centromere of each chromosome pair which occupy station within this plane. Each centromere divides and entire independent chromosomes are produced. One set of chromosomes migrate away from metaphase plate towards spindle pole, and an identical twin set migrates in the opposite direction towards other spindle pole. This poleward migration of chromosomes represents anaphase of mitotic divisions.

### **Telophase**

The beginning of telophase is marked by the appearance of a cleavage furrow in animal cells and of division plate in plant cells. The cleavage furrow is a shallow groove circling the surface of cell. This groove deepens, cuts through the spindle

## **NOTES**

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fibrils and constricts the cell into daughter cells. The chromosome within each daughter cell aggregate near the spindle pole. A new nuclear membrane forms which envelops the chromosome, but centriole remains outside in the cytoplasm. The chromosomes in each newly forming nucleus manufacture new nucleoli.

### **Meiosis**

Reproductive cells of all kinds are manufactured in specialized reproductive tissue. New cells are produced by mitotic divisions just as in formation of new cells in other tissue. New cells then undergo a process of maturation which transform them into cells with unique developmental patterns. Chromosomes do stay constant from one generation to the next, and constancy is brought by meiosis. It reduces the chromosome number of reproductive cells by half. The unreduced chromosome number before meiosis is called diploid number  $2n$ , after meiosis is the haploid number,  $n$ . The meiotic divisions occur one after the other and have many features in common with mitotic divisions. Each meiotic division passes through prophase, metaphase, anaphase and telophase as in mitosis.

As in mitotic divisions also, the centriole divides, spindle fibres and asters form, nuclear membrane dissolves during prophase and reforms each telophase. In the first meiotic division,  $2n$  chromosomes duplicate before or during prophase. These  $2n$  pairs, the members of each pair again joined at the centromere, migrate into metaphasic plate. Every pair in one plane comes to lie next to corresponding type of chromosome pair in other plane. The metaphasic plate is made up of paired chromosome pairs. During anaphase, two chromosomes of each foursome migrate to one spindle pole, two to the other.

At the end of the first of meiotic division, there are two cells each with  $n$  pairs of chromosomes. In the metaphase of second meiotic division, the  $n$  pairs of chromosomes line up in same plane and  $n$  chromosome migrate to each of the poles during anaphase. At the termination of meiosis, four cells are present, each with  $n$  single chromosome, a complete haploid set. In males, all four haploid cells produced by meiosis become functional sperms. In females, only one cell becomes functional egg.

Of the two cells produced by the first meiotic divisions, one is small and it degenerates. Its remnant, the first polar body, remain attached to other cell.

This cell passes through the second meiotic division. Of the two cells produced, one becomes egg, other is small and degenerates. Its remnants form the second polar body which remains attached to the egg. It outlines the nuclear maturation of gametes. In parallel with it, cytoplasmic maturation occurs. The form of cytoplasmic maturation varies for different animal species. In the sperms of vertebrates, much of cytoplasm degenerates altogether. The nucleus enlarges into an oval sperm head. Mature eggs are the largest cells, their cytoplasm become specialized for accumulation and storage of yolk, food reserve for future embryo.

### **Check Your Progress**

1. What is interphase?
2. Where are lysosomal enzymes synthesized?
3. When does DNA replication occur?
4. Where are lysosomal enzymes synthesized?
5. What is the function of Golgi complex?
6. What does mitosis leads to?
7. What is the function of mitosis?

### **NOTES**

## **7.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS**

1. Phase of cell cycle in which a typical cell spends most of its life is called interphase. No division occurs here.
2. Lysosomal enzymes are synthesized in the Golgi bodies.
3. DNA replication occurs during a period of cell cycle termed S phase. S phase is the period when cell synthesizes the additional histones that will be needed as cell doubles the number of nucleosomes in its chromosomes.
4. The lysosomal enzymes and lysosomal membrane proteins are synthesized in rough ER and transported to Golgi cisternae and ultimately to lysosomes. The sorting signal that directs the lysosomal enzymes from the Trans-Golgi Network (TGN) to lysosomes is Mannose 6-Phosphate (M6P).
5. The Golgi complex is also a stack of membrane bound sacs but they are not interconnected. The Golgi complex acts as a switching centre for proteins to various destinations.
6. Mitosis leads to production of cells that are genetically identical to their parent, whereas meiosis leads to production of cells with half the genetic content of parent.
7. Mitosis serve as a basis for producing new cells, meiosis as the basis for producing new sexually reproducing organism.

## **7.5 SUMMARY**

- Membrane protein trafficking first requires a newly synthesized protein to travel from the endoplasmic reticulum through the multiple layers of the Golgi apparatus.

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- Proteins carry codes in their sequences that are read by targeting machinery at every stage of their voyage to their ultimate location. Proteins may be targeted to the cytosol, mitochondria, peroxisomes or chloroplasts. These proteins (if encoded in the nucleus) are synthesized on free ribosomes.
- Protein import, oligomerization and folding are aided by chaperones (BiP) and protein disulphide isomerase within the ER lumen. Misfolded proteins are not permitted to pass further down the secretory pathway, but instead are degraded.
- A typical mammalian cell may contain numerous kinds of proteins and numerous individual protein molecules. The eukaryotic cell is a multi-compartmental structure.
- Proteins destined for cytoplasm and those to be incorporated into mitochondria, chloroplasts and nuclei are synthesized on free ribosomes in the cytoplasm. Proteins destined for cellular membranes, lysosomes and extracellular transport, use a special distribution system.
- The Golgi complex is also a stack of membrane bound sacs but they are not interconnected. The Golgi complex acts as a switching center for proteins to various destinations.
- Proteins to be directed to their destinations via Golgi complex are synthesized by ribosomes associated with endoplasmic reticulum. Some of them are carried into membrane bound vesicles which bud off from one organelle and transported in definite pathways.
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- Mitosis leads to production of cells that are genetically identical to their parent, whereas meiosis leads to production of cells with half the genetic content of parent. Mitosis serve as a basis for producing new cells, meiosis as the basis for producing new sexually reproducing organism.

- Interphase, the period between cell divisions is a time when the cell grows and engages in diverse metabolic activities. Whereas M phase last only an hour or in mammalian cells, extend for days, weeks or longer depending the cell type.
- Although M phase is the period when the contents of a cell are actually divided, numerous preparation for mitosis occur during interphase including replication of DNA.
- DNA replication occurs during a period of cell cycle termed S phase. S phase is the period when cell synthesizes the additional histones that will be needed as cell doubles the number of nucleosomes in its chromosomes. The length of S phase can be determined directly.
- Cell cycles can range in length from as short as 30 minutes in a cleaving frog embryo, whose cell cycle lack both G1 and G2 phases to several months in slowly growing tissue.
- Cells that are arrested in this state which includes the majority of cells in the body are said to be in G0 state to distinguish them from G1 phase cells that soon enter S phase.
- Nucleus in hybrid cell had been donated by G1 phase cell and was activated by S phase cytoplasm to begin DNA replication. The cytoplasm of a replicating cell contain diffusible factors that stimulate initiation of DNA synthesis in G1 phase nuclei.
- Mitosis is accompanied by cytokinesis: a process by which dividing cell splits into two. The two daughter cells resulting from mitosis and cytokinesis possess a genetic content identical to each other and to the mother cell from which they arose.
- Prior to replication, DNA of each interphase chromosomes become associated at sites along its length with multiprotein complex called cohesion. Following replication, cohesion function as a physical bridge that holds two sister chromatids together through G2 and into mitosis when they are separated.
- Microtubules grow by addition of subunits to their plus ends, while their minus ends associates with centrosome. The process of aster formation is followed by separation of centrosomes from one another and their movement around nucleus towards opposite ends of cell.
- Spindle and asters attain their maximal growth at this stage. The chromosome pairs, scattered randomly through central portion of the cell, begin to migrate. It is the centromere of each chromosome pair which occupy station within this plane.
- The chromosome within each daughter cell aggregate near the spindle pole. A new nuclear membrane forms which envelops the chromosome, but

## NOTES

## NOTES

- centriole remains outside in the cytoplasm. The chromosomes in each newly forming nucleus manufacture new nucleoli.
- Reproductive cells of all kinds are manufactured in specialized reproductive tissue. New cells are produced by mitotic divisions just as in formation of new cells in other tissue. New cells then undergo a process of maturation which transform them into cells with unique developmental patterns.
  - Chromosomes do stay constant from one generation to the next, and constancy is brought by meiosis. It reduces the chromosome number of reproductive cells by half. The unreduced chromosome number before meiosis is called diploid number  $2n$ , after meiosis is the haploid number,  $n$ .
  - The meiotic divisions occur one after the other and have many features in common with mitotic divisions. Each meiotic division passes through prophase, metaphase, anaphase and telophase as in mitosis.
  - As in mitotic divisions also, the centriole divides, spindle fibres and asters form, nuclear membrane dissolves during prophase and reforms each telophase. In the first meiotic division,  $2n$  chromosomes duplicate before or during prophase.
  - At the termination of meiosis, four cells are present, each with  $n$  single chromosome, a complete haploid set. In males, all four haploid cells produced by meiosis become functional sperms. In females, only one cell becomes functional egg.
  - The nucleus enlarges into an oval sperm head. Mature eggs are the largest cells, their cytoplasm become specialized for accumulation and storage of yolk, food reserve for future embryo.

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## 7.6 KEY WORDS

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- **Mitosis:** Type of cell division that results in two daughter cells each having same number and kind of chromosomes as the parent nucleus
- **Meiosis:** Type of cell division that results in four daughter cells each with half the number of chromosomes of the parent cell, as in production of gametes.
- **Golgi body:** Complex of vesicles and folded membranes within the cytoplasm of most eukaryotic cells involved in secretion and intracellular transport.
- **Endoplasmic reticulum:** A network of membranous tubules within the cytoplasm of a eukaryotic cell, continuous with the nuclear membrane.
- **Protein trafficking:** Proteins translated within the rough endoplasmic reticulum are transferred to the Golgi.



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## 7.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

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### Short Answer Questions

1. What is mitosis and meiosis?
2. Brief a note on SRP.
3. Give a brief note lysosomal enzymes.
4. Write a short note on cell cycle.
5. Write about metaphase and anaphase.
6. Write a brief note on telophase.

### Long Answer Questions

1. What is protein synthesis?
2. Explain with the help of labelled diagram major protein sorting pathway in eukaryotes.
3. Give a detailed note on cell division and cell cycle.
4. Explain in detail about the formation of mitotic chromosomes.
5. Elaborate a note on meiosis.
6. What is mitosis and meiosis? How would you differentiate it?
7. How cell cycle regulates?

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## 7.8 FURTHER READINGS

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**UNIT 8 BASIC ACCOUNT ON  
MENDELIAN GENETICS  
AND GENE INTERACTION**

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**Structure**

- 8.0 Introduction
- 8.1 Objectives
- 8.2 Mendelian Genetics
- 8.3 Gene Interaction
- 8.4 Linkage
- 8.5 Crossing Over
- 8.6 Gene Mapping: Genetic or Chromosomal or Sturtevant Maps
- 8.7 Answers to Check Your Progress Questions
- 8.8 Summary
- 8.9 Key Words
- 8.10 Self Assessment Questions and Exercises
- 8.11 Further Readings

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**8.0 INTRODUCTION**

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Genetics deal with the transfer of biological information from cell to cell, from parents to offspring and generation to generation. They are concerned with ‘whys’ and ‘hows’ of transfer which is the basis for certain differences and similarities recognized in group of living organisms. It deals with physical and chemical nature of information itself. Then what is the source of genetic variation? How are differences distributed in populations? Not all variation among living things is inherited. Change that become established through these mechanisms over long period of time in living things is called evolution.

In the 1860’s, an Austrian monk named Gregor Mendel introduced a new theory of inheritance based on his experimental work with pea plants. Prior to Mendel, most people believed inheritance was due to a blending of parental ‘essences’, much like how mixing blue and yellow paint will produce a green color. Mendel instead believed that heredity is the result of discrete units of inheritance, and every single unit (or gene) was independent in its actions in an individual’s genome. According to this Mendelian concept, inheritance of a trait depends on the passing-on of these units. Based on his pea plant studies, Mendel proposed that traits are always controlled by single genes. However, modern studies have revealed that most traits in humans are controlled by multiple genes

as well as environmental influences and do not necessarily exhibit a simple Mendelian pattern of inheritance(see “Mendel’s Experimental Results”).

The ABO blood group system is used to denote the presence of one, both, or neither of the A and B antigens on erythrocytes. In human blood transfusions it is the most important of the 36 different blood type (or group) classification systems currently recognized. The ABO blood types were discovered by Karl Landsteiner in 1901, for which he received the Nobel Prize in Physiology or Medicine in 1930.

In this unit, you will learn in detail about the works and results of Mendelian, his genetic experiments. You will also learn about crossing over and the ABO blood type.

## NOTES

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### 8.1 OBJECTIVES

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After going through this unit, you will be able to:

- Discuss Mendelian genetics and his experiments
- Explain the chemical nature of gene
- Elaborate the reasons for Mendel’s success
- Analyze the law of independent assortment
- ABO blood type alleles in humans
- Discuss the mechanism of crossing over

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### 8.2 MENDELIAN GENETICS

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Gregor Mendel is called the father of genetics. His experiments with garden peas were conducted in a space of garden, while he was employed as teacher. The conclusions that he drew from his investigations constitute the foundation of today’s science of genetics. Mendel was not the first to perform hybridization experiments but he was the one of the first to consider the result in term of single traits. His predecessors could only observe that similarities and differences occurred among parents and progeny. Employing the scientific method, Mendel designed the necessary experiments, counted and classified the peas resulting from his cross. Although Mendel devised a precise mathematical pattern for transmission of hereditary traits, he had no concept of biological mechanism involved. On the basis of his experiments, he predicted and verified his predictions with result of later crosses. William Bateson, an Englishman gave this science the name ‘Genetics’ in 1905. Gregor Mendel was born in 1822 to a family of poor farmers in Silisian, a village in Heizendorf which is now a part of Czechoslovakia. He completed his high school at the age of 18. He got his early education in a monastery in Bruno. At very early age, he was ordinate a priest of Augustinian monastery of St Thomas at Bruno. A few years after he was sent to the University of Vienna for

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training in Physics, Mathematics and Natural Sciences. It was at Vienna that Mendel was influenced by two scientists, Franz Unger, a plant physiologist and Christian Doppler, discoverer of well-known Doppler effect in Physics. Mendel picked up knowledge here about Kolreuter's and Gaertner's hybridization experiments. After completing his studies he returned to Bruno in 1854, where he continued to work as priest and as teacher in high school. In 1857, he began his famous experiments on peas in monastery garden. Mendel presented data and conclusion derived from his experiments in paper entitled 'Experiments in plant hybridization' which was read before Brunn Natural history society in 1865 and was published in Annual proceedings of Natural history society in 1866. Mendel's observations went unnoticed on account of:

- He published his work in an obscure journal
- Failure of scientists to notice his work because scientific world was at that time busy in the controversy arisen by the Darwin theory of origin of species
- His ideas was ahead of his time as the ignorance was prevalent in that period about cytological basis of heredity

In 1900, three eminent biologists, Hugo de vries of Holland, Karl correns of Germany and Eric Von Tachermak of Austria working independently on heredity discovered the same phenomenon originally undiscovered by Mendel. These scientists referred to the importance of Mendel's forgotten paper and extended the work by experiments on various plants and animals. Due to his great contribution in field of genetics, he is referred as the 'Father of genetics.'

### Mendel's Experiment

Mendel chose garden pea (*Pisum sativum*) as plant material for his experiments, since it had following advantages:

- Pea plant was self-fertilizing, because petals enclose the reproductive organs till fertilization. The self-fertilization through many generations helps in obtaining the pure lines with constant trait in pea plants.
- The pea plant was easy to cultivate and from one generation to next took only a single growing season (annual).
- Peas had many sharply defined inherited characters. Thus they possess many desirable features.
- The cross pollination and fertilization can be achieved easily.
- The flowers are bisexual and hermaphrodite.

Mendel made sure that his plants were bred pure for the single trait which he wanted to study. He did this by letting the plant self-pollinate for many generations. Then Mendel performed hundreds of crosses. Seven pairs of contrasting characters were chosen for the study. For cross pollination, garden pea, being self-fertilizing plant, anthers have to be removed before maturity.

This operation of removal of anthers is called emasculation. The stigma is protected against any undesired foreign pollen with the help of a bag. Pollen grains are collected from male parent and dusted on stigma of female flower. At the time of cross pollination, the pollen should be mature and stigma should be receptive.

### **Mendel Contribution**

Mendel performed his experiments in three stages. First he made sure that his plants were pure bred (14 varieties for 7 pairs of contrasting characters). He did this by letting the plants, fertilize themselves to eliminate any offspring that was not true to the form of the trait. It was thus made sure that offspring of each generation were all like parent plant. These true breeding plants constitute the Parent (P). Second stage was to hybridise the plants. He made several crosses by dusting the pollen of one kind on stigma of plants of another kind. He pollinated plants from a strain whose seeds were always round, with pollen from a strain whose seeds were always wrinkled. Such offspring of different parents with contrasting characters form the first filial generation or  $F_1$ . Mendel made reciprocal crosses. Third stage was to let the  $F_1$  plants pollinate themselves. Plants thus produced were called second filial generation. Similarly  $F_3$  and  $F_4$  were also obtained.

### **Concept of Gene**

Bateson promoted Mendel's view of paired genes. He used the word 'allelomorph' later known as allele to identify members of pairs that control different alternative traits. During 1900s, a Frenchman showed that genes controlled fur color in mouse. W.L. Johannsen studied the influence of heredity and environment in plants. Johannsen began using the word gene. They were able to build on the basic principles of cytology established between 1865. Why were Mendel's important discoveries not recognized for long time (35 years) after studies were completed.

### **Chromosome Theory**

William Roux postulated as early as 1883 that chromosomes within the nucleus of the cell were the bearers of hereditary factors. To explain the mechanics of gene transmission from cell to cell, he suggested that nuclei must be invisible structures held in chains that duplicate themselves when cell is divided. Experiments of T. Boveri and W. S. Sutton in 1902 brought evidence that a gene is a part of chromosome. The theory of gene as a discrete unit of chromosome was developed by T.H. Morgan from studies of fruit fly.

### **Chemical Nature of Gene**

The gene was first characterized as an indivisible unit of structure, unit of mutation and unit of function with all three of attributes considered as equivalent.

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A physician A.E Garrod indicated in 1909 that genes in human function through enzymes. Prokaryotes were chosen for experimental material even though eukaryotes had more practical significance for geneticists. The Avery et al experiments showed that a chemical deoxyribonucleic acid could bring about genetic change in a pneumococcus bacterium. Hershey and Chase demonstrated that nucleic acid component and not the protein is the genetic material carried by bacteriophage. J.D Watson and F.H.C Crick worked out the double helix structure of chemical DNA. The central problem of genetics was resolved with discovery that DNA is genetic material. Genes accomplish their function through replication that results in more units like themselves and through transcription and translation, whereby proteins that function as determiners in metabolism of cell is synthesized. Although genes are stable, they are susceptible to occasional change, which provide altered form of gene. Genes are defined chemically and are known for what they do in directing the formation of traits through specificity of protein enzymes. In animal body, the same set of genes is present in all nucleated cell. Different genes become active at different times during development. Information contained in a gene is to be decoded by processes of transcription and translation to produce proteins. Proteins are enzymes that catalyze cellular biochemical reactions. Each principle of genetics contain that gene is a unit of inheritance, they are arranged in linear order of chromosomes, genes are unit of DNA and capable of replication, they carry coded message that can be transcribed and translated into polypeptides which are either enzymes or structural proteins.

Mendel chose Garden Pea as his experimental organism because it is an annual plant with well-defined characteristic and can be crossed easily. Garden pea have perfect flowers containing both female and male (pollen producing parts) and are self-fertilized. Mendel was fortunate in choosing a diploid plant with two sets of chromosomes. If he had chosen a polyploidy organism with more than two sets, he would have obtained simple results. Through many generation of self-fertilization, pea had developed into pure lines. A single alternation in a trait was demonstrated by visible difference between varieties. In the seven pair of contrasting traits, Mendel chose to study, one form was dominant over well-defined contrasting alternative. Vines were tall or dwarf, unripe pods were green or yellow and inflated or constricted between seeds, nutritive parts of ripe seed were green or yellow. Seeds with white seed coats were produced by plants that had white flowers and those with grey seed coat come from plants that had violet flowers.

### **Mendel's Experiments**

To prevent self-fertilization in test flowers, anthers were removed from those chosen to be parents before their pollen receiving parts are mature. Pollen from parent was transferred at appropriate time to stigma of parent flower. Hybridisation

experiments were carried through several generations and backcrosses were made between hybrids and pure parent varieties. He observed that weather, soil and moisture conditions affected the growth characteristic of peas but heredity was the main factor under condition of his experiment. In experiment, Mendel crossed tall and dwarf varieties of garden pea. All offspring in first  $F_1$  generation were tall. The dwarf trait had disappeared in  $F_1$  progeny. When tall hybrid plants were self-fertilized and progeny was analyzed, some were tall, some were dwarf. Mendel predicted what would occur in  $F_3$  generation and planted  $F_2$  seeds to test this prediction.  $F_2$  short plants were expected to produce all short  $F_3$  progeny. Though when results obtained, Tall  $F_2$  plants produced both tall and short in the proportion of two tall to one short and short  $F_2$  plants produced only short progeny. The significant deduction from Mendel's result was that the separation of pairs resulted in purity of gametes. The paired genes separate from one another and distributed to different cells.

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### Results of Mendel's Experiment

The following are the results of Mendel's experiments:

- **$F_1$  Only of One Type:** Mendel tested the seven characters. Individually by crossing a variety carrying a particular trait of character (tall) with another variety carrying a different trait of same character (dwarf). Uniformly, Mendel's crosses between the two different varieties for each character always produced  $F_1$  that was only of one type. For example, when he crossed smooth seed shaped variety with a wrinkled one, he obtained seeds that were all smooth. Similarly all the crosses between plants with round seeds and plants with wrinkled seeds produced offspring whose seeds were always round. One trait seemed to dominate the alternate trait.
- **$F_2$  Generation (Selfing of  $F_1$ ):** Mendel next performed the second major step in his experiments. He let the  $F_1$  plants pollinate themselves. In the generation of plants that resulted (the  $F_2$  generation) the dominant traits produced 75% offspring, while 25% of other trait reappeared. He found in all such crosses the ratio was approximately 3:1. The regular reappearance of various hidden recessive traits was a notable contribution to concepts of heredity and apeaks against all traits as becoming blended as diluted in hybrid offspring.
- **$F_3$  Generation:** Upon self-fertilization of  $F_2$  generation, Mendel found that wrinkled plants always give rise to wrinkled in all generations (bred true). It shows that there was no smooth S factor within them. On the other hand  $F_2$  plants that appeared smooth did not always breed true. Of 565 self-fertilized smooth plant only 193 bred true to smooth while 372 each produced smooth and wrinkled plants in proportion of 3 smooth: 1 wrinkled.

## Interpretation of Mendel's Results

The following principles of inheritance were given by Mendel:

### Law of Dominance

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Out of two contrasting factors, only one expresses itself in an individual. The factor that expresses itself is called dominant while the other which has not shown its effect is called recessive. When homozygous tall pea plants are crossed with homozygous dwarf plants, the plants appeared in first filial generation are tall though they receive factor for dwarfness. This recessive hidden character reappeared, unchanged in second filial generation. The Danish botanist Johanssen introduced the term gene for Mendel's factor in 1909 (Refer Figure 8.1).

- Every gene has two alleles that can code for a trait.
- One allele is dominant, meaning it will always show.
- One allele is recessive, meaning it will be masked by the presence of the dominant allele.
- Hybrids will always show the dominant phenotype.

Ex: PP = purple

pp = white

Pp = purple

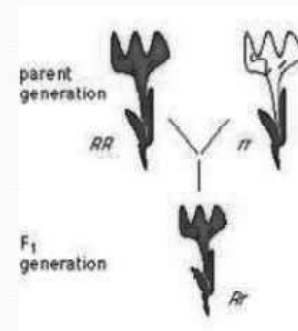


Fig. 8.1 Law of Dominance

### Law of Segregation (Purity of Gametes)

It states that when a pair of contrasting factors or genes are brought together in a hybrid, these factors don't blend but associate themselves and remain together and separate at the time of gamete formation. To understand the idea of law of segregation, monohybrid cross is taken. A cross between pea plant bearing axial flowers (AA) and a pea plant bearing terminal flowers (aa). If two pure varieties are crossed together, in first filial generation, F1 hybrid is produced with genotype Aa. In F2 generation, the two varieties appear in ratio of 3:1. In hybrid, two types of male and female gametes are formed in equal quantity. Law of segregation is defined as allele pairs separate or segregate during gamete formation and the paired condition is restored by random fusion of gametes during fertilization (Refer Figure 8.2).



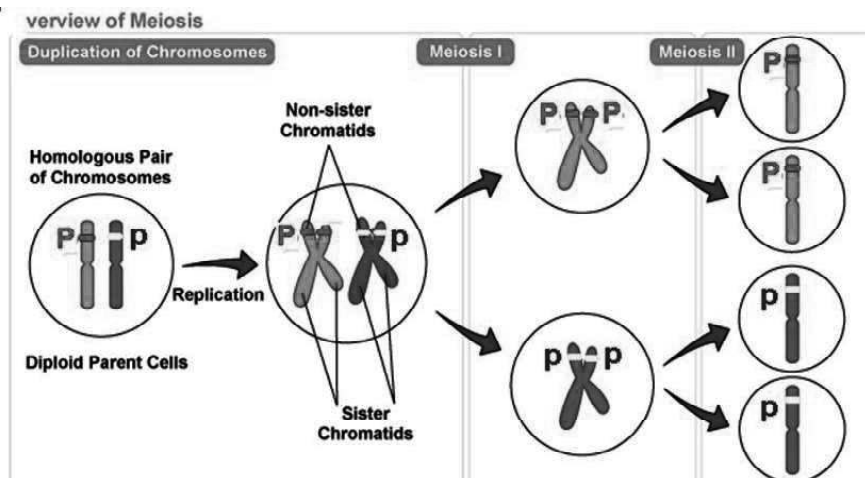


Fig. 8.2 Law of Segregation

### Law of Independent Assortment

The genes of different characters located in different pairs of chromosomes are independent of one another in their segregation during gamete formation. If we consider the inheritance of two or more genes at a time, their distribution in the gametes and in the progeny of generations is independent of each other. He crossed a variety having round and yellow seeds with one having wrinkled and green seeds. This plant will produce yellow and round seeds because R dominates r and Y dominates y. For  $F_2$  generation, four types of gametes are produced, RY, rY, Ry, ry. The four types of alleles are assorted in four types of gametes. Gametes are produced with  $F_2$  in the ratio of 9:3:3:1 (Refer Figure 8.3).

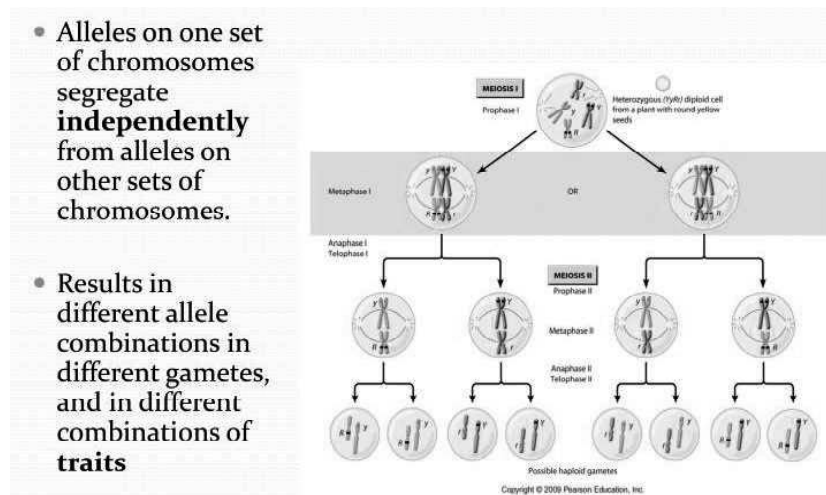


Fig. 8.3 Mendel's Second Law

## NOTES

## NOTES

### Importance of Mendelism

The importance of Mendelism is listed in the below points:

- Hybridisation is used for obtaining improved varieties of plants. This process results in combinations of desirable characters of two or more species. In other words, desirable characters of one species are transferred to the other.
- Mendelism has enabled the plant breeders to improve the races of domestic animals. Hybridisation through artificial insemination has proved highly successful in improving the quality of milk, meat, egg and other animal products.
- Laws of heredity postulated by Mendel is equally applicable to mankind.
- Study of inheritance of the blood group can solve the disputed parentage of a child.
- Genetic counselor can predict the possibility of hereditary defect in a future and even detect genetic disorders in foetus.

### Dihybrid Cross

Consider a dihybrid cross, a cross in which two pairs of alleles are segregating. Look at cross in plants, assume both genes are autosomal, that one allele of each gene is completely dominant to the other allele, that no epistasis is involved.

Parental genotypes:  $AABB \times aabb$

Parental gametes:  $AB \times ab$

Progeny or  $F_1$  genotype  $\times AaBb$

Suppose that  $F_1$  plants are test crossed, i.e., crossed to homozygous recessive plants. All the gametes produced by homozygous recessive test cross parent will be  $ab$  in genotype. Only crosses where the two genes involved were located on different chromosomes and showed independent assortment. Now suppose that gene  $C$  is located on the same chromosome as gene  $A$ . A dihybrid cross in which allele of genes  $A$  and  $C$  are segregating. And  $F_1$  plants would produce gametes of only two types  $AC, ac$ . If so genes  $A$  and  $C$  would be completely linked and all gametes would carry parental combination. No recombinant gametes would be produced in contrast to 50% recombinant gametes characteristic of independent assortment. The actual result of such test cross involve two genes located on same chromosome are somewhere between the results expected for complete linkage and for independent assortment. At some frequency less than 50% recombinant gametes are formed. Gametes that contain recombinant combination of genetic marker located on same chromosome are produced by crossing over. Genetic markers are said to be linked whenever over 50% of the gametes produced contain parental combination of the markers and less than 50% of gametes contain recombinant combinations (Refer Figure 8.4).

YyRr x YyRr		YR	Yr	yR	yr	
YR	YYRR	YYRr	YyRR	YyRr		9/16 yellow round
Yr	YYRr	YYrr	YyRr	Yyrr		3/16 green round
yR	YyRR	YyRr	yyRR	yyRr		3/16 yellow wrinkled
yr	YyRr	Yyrr	yyRr	yyrr		1/16 green wrinkled

Fig. 8.4 Dihybrid Cross of Color and Seed Shape

## NOTES

### Reasons for Mendel's Success

Herein below are the reasons for Mendel's success:

- His choice of Pea plants (*Pisum sativum*) for his breeding experiments was excellent. He later on selected pure breeding varieties for his experiments.
- Mendel kept complete record of every cross.
- Mendel was fortunate also that the characters which by chance he selected for his breeding experiments did not show linkage, incomplete dominance, gene interaction, etc.
- Mendel formulated theoretical explanations for interpreting his results. He tested his explanation for its validity.
- His predecessors studied many traits at the same time but he took one or two traits at one time for his experiments.
- He didn't attempt to solve all the variations reported in his breeding experiments, which were not clear to him such as linkage of flower and seed colour.

### 8.3 GENE INTERACTION

The emphasis has been given to the fact that genes studied by Mendel were segregating independently of each other. What is not yet been mentioned is that these genes must have been functioning independently of one another.

If each gene were expressing itself in a separate test tube, it would be reasonable to expect all genes to be functionally independent. But these genes are not in separate test tube, they are located in the same nuclei of the same cells. Thus expression of an allele of one gene will alter the expression of one or more of allele of a second gene. An example of gene interaction was reported by William Bateson and his associate R.C Punnett. Bateson began to conform and extend

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Mendel's work after its discovery in 1900 and became pioneer in transmission genetics. Domestic breeds of chickens have different comb shape. Wyandottes have a type of comb called rose whereas brahmas have a pea comb. Leghorns have single combs. The investigators crossed wyandottes and brahmas and all the  $F_1$  chickens had walnut combs, a phenotype not expressed in either of parent. When  $F_1$  chickens were mated among themselves and large  $F_2$  populations were produced. A dihybrid ratio was recognized but phenotypes were different from those of parents. These two phenotypes were expressed as a result of gene product interaction. Genes R and P were non allelic, but each was dominant over its allele. When R and P were together, as in  $F_1$ , the two different products interacted to produce a walnut comb. The two non-allelic genes R and P acted independently in different ways, similar to ways in which codominant allele act.

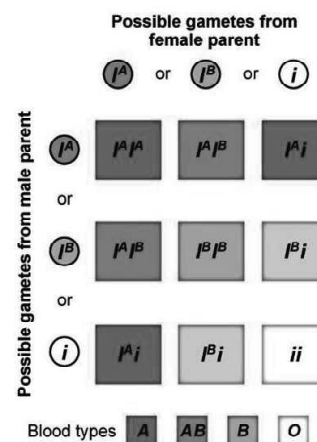
**Allelic Interaction: Multiple Alleles**

An allele is a specific form of gene. It may be mutant allele, resulting in an altered phenotype or wild type allele producing active gene product and normal phenotype. Different wild type alleles occur in population. They all produce phenotype within wild type range. Similarly mutant allele of gene exhibit wide range of gene product activity from no activity to wild type level of activity. Alleles may produce a whole series of variable phenotype ranging from an extreme mutant phenotype to wild type phenotype. When more than two different forms of gene exist in a species, they are referred as multiple alleles. E.g. Gene controlling eye color in fruit fly. *Drosophila* have red eyes, but vast array of eye colour have been studied extensively for many decades. Mutant allele of one gene result in flies with eye color ranging from pure white through series of intermediate color upto wild type red when present in homozygous condition, as shown in Figure 8.5.

**Multiple Alleles**

**Blood groups in humans**

- Some forms of inheritance are determined by more than two alleles, referred to as multiple alleles.
- The ABO blood group has three forms of alleles, sometimes called AB markers.



*Fig. 8.5 Multiple Alleles*

**ABO Blood Type Alleles in Humans**

The ABO locus has three common alleles:  $I^A$ ,  $I^B$  and  $I^O$ .  $I^A$  and  $I^B$  are codominant (both A and B antigens on their red blood cells) and  $I^O$  is recessive (no antigen) on

their red blood cells. The ABO locus controls the type of glycolipids found on the surface of erythrocytes by specifying the type of glycosyl transferase synthesized in the red blood cells. The specific type of glycolipids on the red cell surface provide the antigenic determinants that react with specific antibodies present in blood serum

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### Rh Factor Alleles in Human

The Rh factor was discovered in 1940 by K. Landsteiner and A.S Wiener from rabbits immunized with the blood of monkey *Macaca rhesus*. A test for Rh incompatibility is accomplished by placing a drop of blood on a slide and introducing anti RH serum. Agglutination of erythrocytes indicates incompatibility whereas even distribution of erythrocytes indicates no reaction. Cross matching of the Rh factor as well as of ABO types of donor and recipient blood is used to avoid incompatibility agglutination reaction following transfusion. Blood is exchanged between the mother and the foetus during childbirth. Thus Rh negative mothers are often immunized by blood from Rh positive foetus to which they give birth. Usually no ill effects are associated with exposure of mother to Rh positive antigen during first child birth. Rh positive children carried by same mother may be exposed to antibodies produced by mother against Rh antigen which are carried across placenta in blood serum. Children develop symptoms of haemolytic jaundice and anemia, condition known as erythroblastosis fetalis.

### Codominance

When the dominant character is not able to suppress, even incompletely the recessive character and both the characters appear side by side in F1 hybrids, the phenomenon is called co dominance. For example in cattles, if a cattle with black coat is crossed to a cattle with white colour, F1 hybrid possess roan coat. In roan coat, both black and white patches appear separately. So the alleles which are able to express themselves independently when present together are called co dominant alleles.

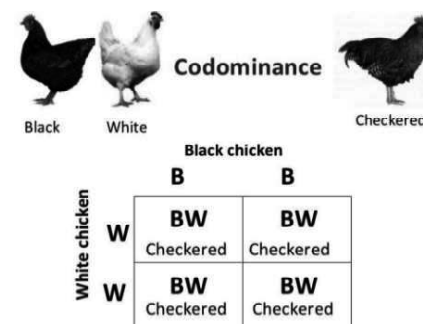


Fig. 8.6 Codominance

### Incomplete Dominance

The law of dominance is not always correct as there can be found where the complete dominance is absent. In such cases some traits of F<sub>1</sub> phenotype is intermediate between those of parental traits. In incomplete dominance the genes

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of allelomorphic pair are not expressed as dominant and recessive but express themselves partially when present together in a hybrid. As a result F<sub>1</sub> hybrid show characters intermediate to effect of two genes of the parents, for example, *Mirabilis Jalapa* (Refer Figure 8.7).

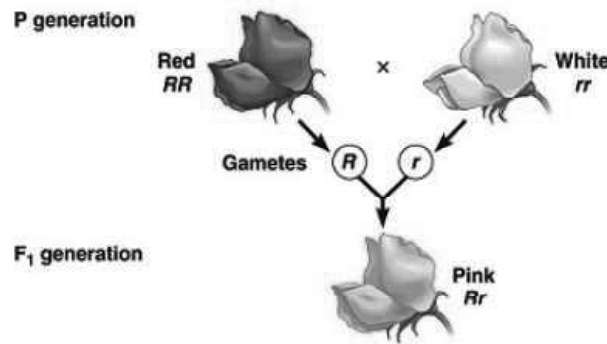


Fig. 8.7 Incomplete Dominance

**Pleiotropy:** It refers to multiple phenotypic effects of a single mutant gene. The traits that are affected have no physiological connection. The most evident expression of a pleiotropic gene is called as major or primary effect, while less evident effects are called as minor or secondary effects. Pleiotropic genes usually change the normal Mendelian ratio. A classic example of pleiotropy is sickle cell anemia. It appears due to presence of pleiotropic gene Hb<sup>s</sup>. This leads to formation of haemoglobin –S. This is one of the secondary effects. The gene Hb<sup>s</sup> is lethal in homozygous condition but has a slight detectable effect in heterozygous condition. Sickle cell anemia is common in person of African descent and is also found in other parts of the world where malaria is or has been major cause of death.

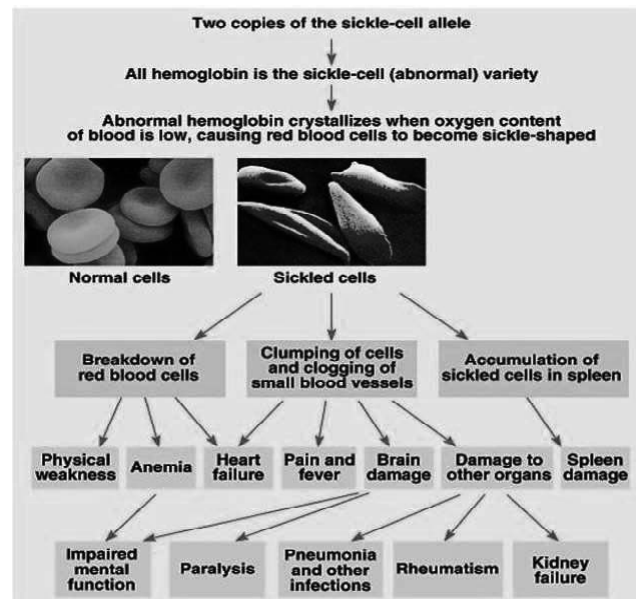


Fig. 8.8 Sickle Cell Anemia

### Non-allelic Interaction: Epistasis

The functional interaction between different genes occur when an allele or genotype at one locus inhibits the expression of a non-allele or genotype at distinct locus, such interaction is known as epistasis. Any gene that masks the expression of another non allelic gene is epistatic to that gene. Epistasis is the interaction between different genes (non-alleles). Dominance is the interaction between different alleles of the same gene. Metabolic processes in living organism take place via sequence of enzyme catalyzed reaction. Each step requires the activity of a specific enzyme. Each enzyme is the product of a specific gene. Some enzymes are coded by two or more different genes (Refer Figure 8.9).

### NOTES

#### EPISTASIS:

interaction between 2 non-allelic genes in which one modifies the phenotypic expression of the other

Example: gene for pigment deposition is epistatic to gene for melanin production in mice

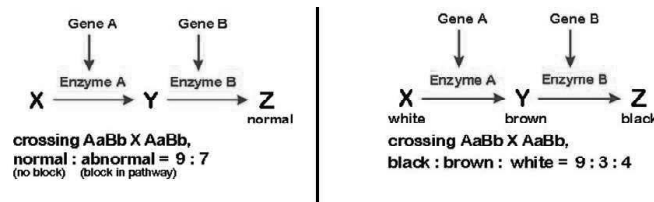


Fig. 8.9 Epistasis

**Duplicate Genes:** Two or more than two independent genes located on different chromosomes produce the same effect of trait. Such genes are called as duplicate genes (Refer Figure 8.10).

Same effect is given by either of two genes (A) or (B)  
 Example is Capsella bursa -pastoris (Shepherd's Purse ) Fruit shape of two types  
 a) Triangular (Heart shape ) capsule  
 b) Top shaped (Narrow ) capsule

Genetic Expression  
 Parents AABB X aabb  
 Triangular Top shaped  
 P gametes AB ab  
 F1 (Hybrid) AaBb triangular

F1 gametes	AB	Ab	aB	ab	
AB	AB 1	Ab 2	aB 3	ab 4	AB- 1,2,3,4,5,7,9,10,13=9
Ab	AB 5	Ab 6	aB 7	Ab 8	ab-11,12,15=3 (triangular) ab-16=1 (top shaped) So the ratio is 15:1
aB	aB 9	Ab 10	aB 11	ab 12	This is example of gene interaction,two genes involved in same pathway.It is based on the idea that some genes may be present more than once in the genome
ab	AB 13	Ab 14	aB 15	ab 16	

**F2 generation**  
 15 (Triangular): 1 (Top)

Fig. 8.10 Duplicate Genes

**Supplementary Genes:** There are two independent pairs of genes interacting in such a manner that one dominant factor produces its effect, whether the other is

present or not, while the second one produce its effect only in the presence of first (Refer Figure 8.11).

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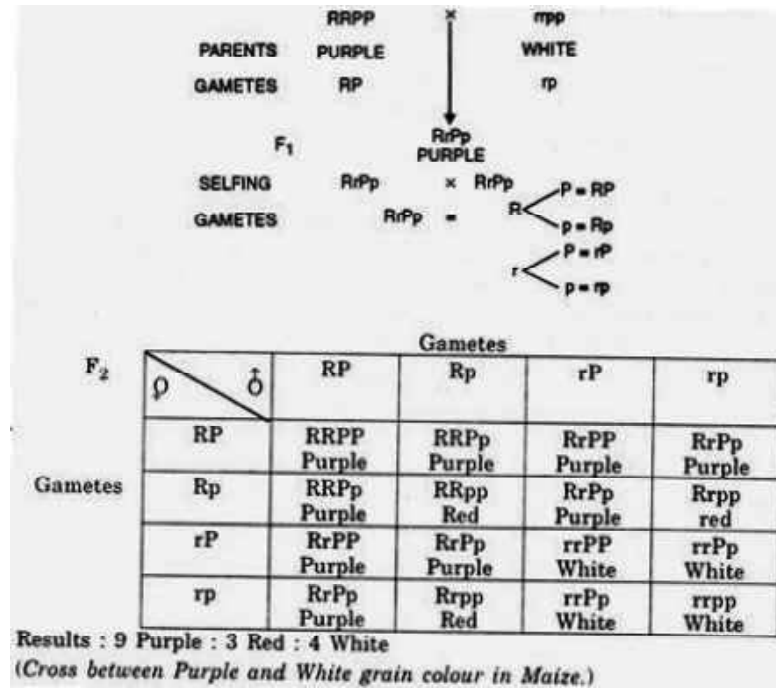


Fig. 8.11 Supplementary Genes

**Complementary Genes:** Two pairs of non allelic genes, which interact to produce only one phenotypic trait, but neither of them if present alone produces the phenotypic trait in the absence of other. As sweet Pea shown below (Refer Figure 8.12).

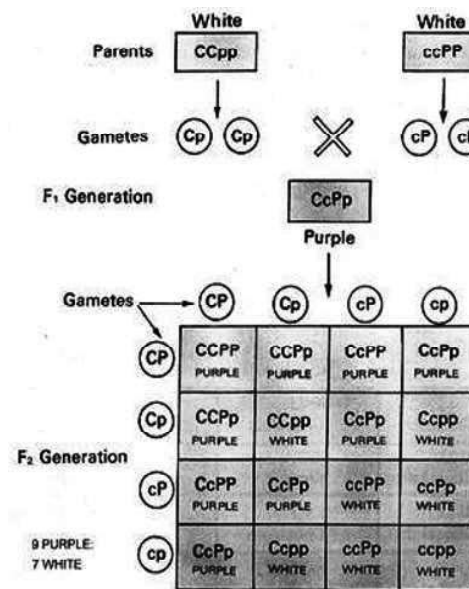


Fig. 8.12 Complementary Genes



**Polygenic Inheritance:** Also called as multiple factor inheritance. They are quantitative traits that are expressed in continuous fashion. There are a whole series of traits, many of great economic importance in which the difference are in degree only and are quantitative.

### Check Your Progress

1. Who is called the father of genetics?
2. What did Mendel choose as plant material for his experiments?
3. What was the gene first characterized as?
4. What is an allele?

## NOTES

### 8.4 LINKAGE

*Drosophila melanogaster* (Fruit Fly) was investigated intensively in the laboratories of Columbia University. Thomas Hunt Morgan in 1910, discovered a fruit fly with white eyes in a vial of flies with normal red eyes. T.H Morgan mainly studied the inheritance of mutant traits in *Drosophila*. *Drosophila* is a suitable material for the genetic experiments due to following reasons:

- Its generation time is 12-14 days, which is helpful in rapid study and analysis of results in laboratory.
- It can be multiplied in large number under laboratory conditions.
- A large number of flies are produced in each progeny. A pair of flies in a small milk is able to produce hundreds of progeny in a single mating.
- Fly breeding could be done throughout the year in a laboratory with inexpensive material.
- Each cell of *Drosophila melanogaster* has four pairs of chromosomes. Out of which three pairs of chromosomes are similar in male and female and called autosomes. The males possess one X chromosome and one Y chromosome producing two kinds of sperms; half with X chromosomes and half with Y chromosome. Y chromosome is J shaped. Being homogametic, females produce only one kind of eggs, each with one X chromosome.

#### Theory of Linkage

Morgan began his work on *Drosophila melanogaster* around 1910. Morgan and his associates A.H Strutevant, H.J Muller, C.B Bridges were engaged in studies on chromosome theory of heredity. From their studies on mutants in *Drosophila*, they could assign several genes to chromosomes. He learnt the advantages of using fruit fly under laboratory conditions. He noticed that fruit flies with 2 mm size can be raised easily in bottles, fed on simple food like banana and yeast. These flies were found hovering around ripe fruits of banana and mango. Female flies are easily distinguished from male by their larger size and by presence of ovipositor.

While raising population of *Drosophila*, he noticed a single white eyed male among normal pure red eyed flies. The white eyed character was sex linked and carried by X chromosome in *Drosophila*.

## NOTES

### Experiments by W. Bateson, Saunders and R.C Punnet

Bateson, Saunders and Punnet (1905) found different results in a dihybrid cross in *Lathyrus odoratus*. While crossing plants having purple flowers and long pollen and red flowers and round pollen, they found deviation from F<sub>2</sub> ratio (9:3:3:1 of Mendelian dihybrid cross). They obtained purple long, purple round, red long and red round in the ratio of 14:1:1:3 respectively in F<sub>2</sub> generation. Bateson and Punnett coined the term coupling for referring to the situation where two dominant alleles of a gene are both present in one parent and two recessive alleles in the other. Thus both dominant genes pass together in one gamete in one parent. Similarly, both recessive pass together in the gamete of other parent.

### Complete Linkage

If linkage is complete, there would be parental combinations and no recombination. Morgan (1919) reported a complete linkage in *Drosophila*. When ordinary male wild fly with gray body and normal wings was crossed with female having black body and vestigial wings. In F<sub>1</sub> hybrids were all gray bodied and normal winged. But when F<sub>1</sub>, male is back crossed with recessive female parent only two types of individuals were produced instead of four. Thus indicating the complete linkage (Refer Figure 8.13).

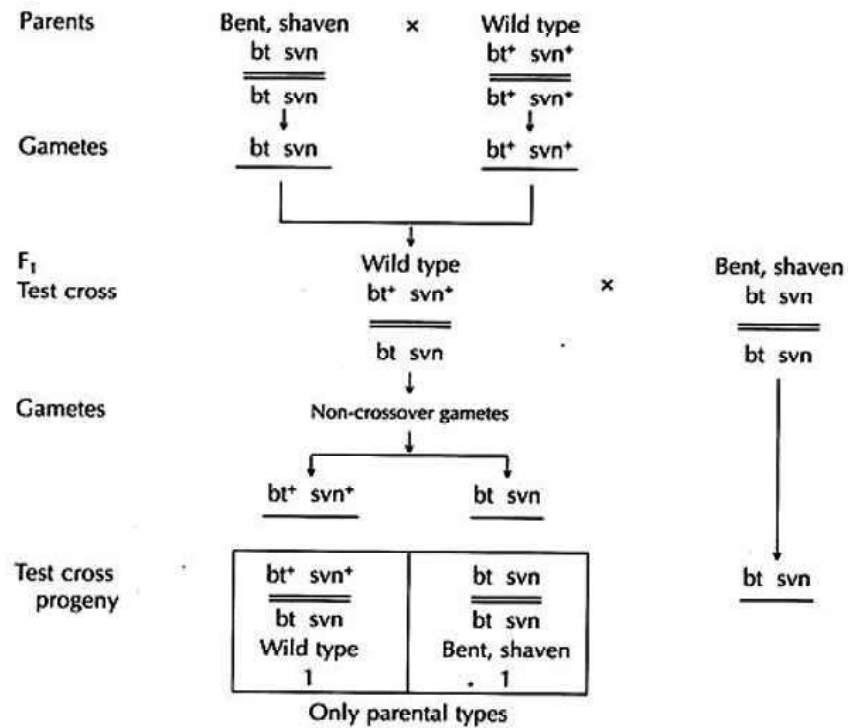


Fig. 8.13 Complete Linkage

## Incomplete Linkage

It produces new combinations of genes in the progeny due to the formation of crossing over in between linked genes present on homologous chromosomes. When in sweet pea, a cross is made between blue flower and long pollen with red flower and round pollen in F<sub>1</sub> expected blue flower and long pollen. However test cross between blue and long and double recessive give parental combinations 87.4% while new combinations are 12.6%, genes got separated due to breaking of chromosomes at the time of crossing over

## NOTES

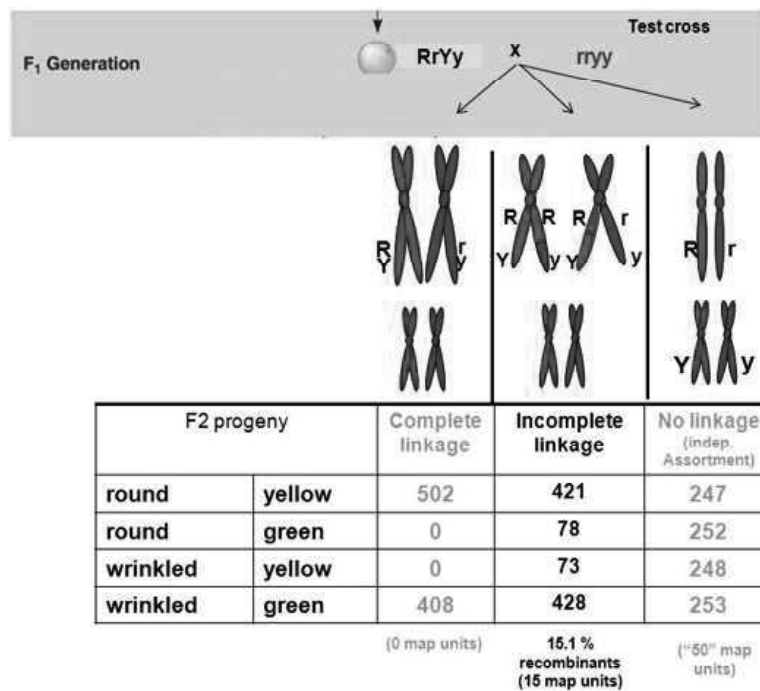


Fig. 8.14 Incomplete Linkage

## 8.5 CROSSING OVER

It is the process of exchange of genetic material or segments between non sister chromatids of two homologous chromosomes. It occurs due to interchange of sections of homologous chromosomes. Normally, if independent assortment take place, i.e., when genes are present on different chromosomes, we expect a test cross ratio of 1:1:1:1. It can be concluded that two genes are on the same chromosome and the appearance of recombinants in low number has resulted from crossing over. The chromosomes undergo breakage during gametogenesis. The percentage of crossing over which is obtained between different linked genes varies according to the distance between genes on the chromosomes. The two genes are apart on a chromosome, more likely is the occurrence of crossing over between them. The genes in such a case, should be located so much apart on the same chromosome as to allow crossing over in all the mother cells during reductional

## NOTES

division. Mendel's law of Independent assortment holds good only under following conditions:

- When the genes are located on different chromosomes
- If the genes are located on the same chromosome but the distance between them is so good as to produce 50 % recombinant gametes due to crossing over.

### Mechanism of Crossing Over

- The homologous chromosomes pair lengthwise due to force of attraction in zygotene in prophase I of meiosis. The pairing starts at few points and proceeds along the whole length. This process of pairing is called synapsis. The paired homologous chromosomes are called bivalents. During synapsis, a scaffold called synaptonemal complex aligns DNA molecule of two homologous chromosomes side by side.
- Synapsis is followed by the duplication of chromosomes which changes the bivalent nature of chromosome to four stranded stage. Four stranded stage of chromatids occurs due to splitting of homologous chromosomes into sister chromatids attached with unsplit centromeres.
- In pachytene, crossing over occurs. Non sister chromatids of homologous pair twist over each other due to enzyme endonuclease. The chromatids get connected with each other at points called chiasmata. The number of chiasmata formed is proportional to the length of chromatids. During diakinesis of prophase I, chiasmata move towards the end of bivalent by process called terminalization.
- Frequency of crossing over is directly proportional to the distance between two genes. More the distance between two genes, better are the chances of crossing over and chiasmata formation.
- The number of offspring resulting from recombination expressed as a percentage of total offspring is termed as crossover value.
- A crossover value of 50% between two genes gives an offspring phenotypic ratio of 1:1:1:1 which is indistinguishable from the independent ratio. Crossing over fails to take place in male drosophila and female silkworm
- Few factors which affect the process of crossing over are as follows:
  - o The frequency of crossing over decreases with increase in age
  - o Change in temperature affects the frequency of crossing over
  - o Inversion in chromosomes decreases the process of crossing over
  - o The chances of crossing over are much more in distantly placed genes as compared to the genes located in close proximity
  - o Genes lying close to centromere in chromatids show reduced tendency of crossing over

- o Factor for crossing over is present in cytoplasm and is transmitted to next generation

### **Significance of Crossing Over**

- They provide an inexhaustible store of genetic variability in sexually reproducing organisms
- This process produces new combination of genes. Green revolution and white revolution are due to selective picking up of useful genetic recombination developed by crossing over
- Crossing over provides a proof for linear arrangement of genes
- The process of crossing over leads to construction of linkage maps.

### **NOTES**

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## **8.6 GENE MAPPING: GENETIC OR CHROMOSOMAL OR STURTEVANT MAPS**

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With the help of processes like linkage and crossing over following facts were established:

- The genes are present in linear fashion on chromosomes
- The genes are located in definite positions on chromatids
- Linkage groups are equal to number of chromosomal pairs in any diploid organism
- The strength of linkage is inversely proportional to the distance between two linked genes
- Chances of crossing over increase in distantly placed genes

The chromosomal maps represent the condensed, graphic representations of relative distance between the genes in a linkage group, expressed in percentage of recombination, located in a single group of chromosomes. Thus, the graphic representation of genes is known as chromosome maps. Sturtevant was the first to construct a genetic map based on his idea of recombination frequencies that shows the location of various genes on a chromosome. Recombination frequency is defined as one map unit or one centimorgan. Map is one dimensional.

Morgan (1911) predicted that frequency of crossing over is governed by distance between genes. Thus probability of occurrence of crossing over between two particular genes increase as the distance between them becomes larger. The cross over frequency is directly proportional to distances between genes. A map unit is equal to 1% cross over (recombinants). It represents the linear distance along the chromosome for which a recombination frequency of 1% is observed. Genetic maps of chromosomes are also known as chromosome maps. Here chromosomes are shown by straight lines, proportional to their length with position of genes. These maps represent linkage groups.

## Cytological Basis of Crossing Over

### NOTES

Morgan first proposed crossing over to explain the formation of recombinant combination of genes that were shown to be linked by genetic data. He hypothesized that this linkage was the result of location of these genes on the same chromosome. If crossing over occurs, one may expect to observe it cytologically. Cross shaped structure in which two of the four chromatids of homologous chromosomes pair appear to exchange pairing partners are detected in cytological studies of meiosis in many organisms.

Crossing over occurs after chromosome duplication, when each homologous chromosome is represented by two chromatids. Each pair of synapsed homologs is called tetrad, because it consists of four chromatids. Crossing over refers to the exchange of segments of chromosomes whereas recombination implies the formation of new combination of genes. Thus crossing over occurs in completely homozygous organisms, but new combination can be formed only in organism that are heterozygous at two or more loci.

#### Check Your Progress

5. Why do the homologous chromosomes pair lengthwise?
6. What is synapsis followed by?
7. What do the chromosomal maps represent?
8. When does crossing over occur?

### 8.7 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Gregor Mendel is called the father of genetics.
2. Mendel chose garden Pea (*Pisum sativum*) as plant material for his experiments.
3. The gene was first characterized as an indivisible unit of structure.
4. An allele is a specific form of gene.
5. The homologous chromosomes pair lengthwise due to force of attraction in zygotene in Prophase I of meiosis.
6. Synapsis is followed by the duplication of chromosomes.
7. The chromosomal maps represent the condensed, graphic representations of relative distance between the genes in a linkage group.
8. Crossing over occurs after chromosome duplication.

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## 8.8 SUMMARY

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- Mendelian genetics is based on the transmission of genes from parents to progeny and thus from generation to generation.
- The law of heredity is based on the segregation of monohybrid cross. It states that two independent assortment, the independent segregation of members of different pairs of alleles.
- Genetics deal with the transfer of biological information from cell to cell, from parents to offspring and generation to generation.
- In the 1860's, an Austrian monk named Gregor Mendel introduced a new theory of inheritance based on his experimental work with pea plants.
- Gregor Mendel is called the father of genetics.
- Although Mendel devised a precise mathematical pattern for transmission of hereditary traits, he had no concept of biological mechanism.
- The gene was first characterized as an indivisible unit of structure, unit of mutation and unit of function with all three of attributes considered as equivalent.
- J.D Watson and F.H.C Crick worked out the double helix structure of chemical DNA.
- Genes are defined chemically and are known for what they do in directing the formation of traits through specificity of protein enzymes.
- Each principle of genetics contain that gene is a unit of inheritance, they are arranged in linear order of chromosomes, genes are unit of DNA and capable of replication, they carry coded message that can be transcribed and translated into polypeptides which are either enzymes or structural proteins.
- After completing his studies he returned to Bruno in 1854, where he continued to work as priest and as teacher in high school.
- William Roux postulated as early as 1883 that chromosomes within the nucleus of the cell were the bearers of hereditary factors.
- To prevent self-fertilization in test flowers, anthers were removed from those chosen to be parents before their pollen receiving parts are mature.
- Hybridisation is used for obtaining improved varieties of plants. This process results in combinations of desirable characters of two or more species.
- Mendelism has enabled the plant breeders to improve the races of domestic animals.
- Laws of heredity postulated by Mendel is equally applicable to mankind.
- Study of inheritance of the blood group can solve the disputed parentage of a child.

## NOTES

## NOTES

- Genetic counselor can predict the possibility of hereditary defect in a future and even detect genetic disorders in foetus.
- If each gene were expressing itself in a separate test tube, it would be reasonable to expect all genes to be functionally independent.
- When the dominant character is not able to suppress, even incompletely the recessive character and both the characters appear side by side in F1 hybrids, the phenomenon is called co dominance.
- The law of dominance is not always correct as there can be found where the complete dominance is absent.
- Sickle cell anemia is common in person of African descent and is also found in other parts of the world where malaria is or has been major cause of death.
- The functional interaction between different genes occur when an allele or genotype at one locus inhibits the expression of a non-allele or genotype at distinct locus, such interaction is known as epistasis.
- Two or more than two independent genes located on different chromosomes produce the same effect of trait.
- First law of heredity is based on the segregation of monohybrid cross. It states that two factors for each trait remain distinct in an individual and segregate during gamete formation.
- If genes are associated and always transmitted together, linkage is said to be complete. However, the chromosomes can have new combination of linked genes due to exchanges involving two loci between chromosomal region, creating recombinants.
- There are allelic and non allelic interaction between genes. Allelic interaction includes incomplete dominance, codominance, multiple alleles and non allelic includes complementary genes, supplementary genes, epistasis, polygenic inheritance.
- Complementary genes are two pairs of non allelic dominant genes located on separate loci, interacting to influence a single phenotype.
- Epistasis is a situation where allele or genotype at one locus suppresses the expression of a non allelic locus.

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## 8.9 KEY WORDS

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- **Linkage:** It is a phenomenon of genes staying together during inheritance through several generations
- **Monohybrid cross:** It is a cross between two organism of a species which is made to study inheritance of a single pair of genes



- **Dihybrid cross:** It is a cross between two organisms of a species which is made to study the inheritance of two pairs of factors
- **F<sub>1</sub> generation:** It is referred to the generation of hybrids produced from cross between the genetically different individuals called parents.
- **Homozygote:** It refers to the individual which contains identical genes of a character on its homologous chromosomes.

## NOTES

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### 8.10 SELF ASSESSMENT QUESTIONS AND EXERCISES

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#### Short Answer Questions

1. What is known as Mendel's experiment?
2. What were the results of Mendel's experiment?
3. What do you mean by the law of dominance?
4. Write a note on the law of segregation.
5. Briefly describe the law of independent assortment.
6. Write a note on dihybrid cross.
7. What were the reasons for Mendel's success?
8. What is allelic interaction?
9. What are ABO Blood type alleles in humans?
10. Discuss briefly about incomplete dominance.

#### Long Answer Questions

1. "Out of two contrasting factors, only one expresses itself in an individual. The factor that expresses itself is called dominant while the other which has not shown its effect is called recessive." Discuss.
2. What do you mean by crossing over? What is the mechanism of crossing over? Also list its significance.
3. "The chromosomal maps represent the condensed, graphic representations of relative distance between the genes in a linkage group, expressed in percentage of recombination, located in a single group of chromosomes." Explain.
4. What is the chemical nature of gene? Also discuss about gene interaction.
5. Write in detail about gene mapping. Also explain about genetic or chromosome or strutevant maps.
6. Write a detailed note on the chromosome theory.

7. “The genes of different characters located in different pairs of chromosomes are independent of one another in their segregation during gamete formation.” Explain the statement with the help of your learning of the text.

## NOTES

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### 8.11 FURTHER READINGS

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## UNIT 9 PRINCIPLES OF GENETICS

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### Structure

- 9.0 Introduction
- 9.1 Objectives
- 9.2 Sex Linked Genes in Drosophila and the Pattern of Sex Linked Gene Transmission
- 9.3 Cytoplasmic Inheritance
- 9.4 Characteristics and Detection of Cytoplasmic Inheritance
- 9.5 Extra-Nuclear Inheritance by Cellular Organelles
- 9.6 Origin, Induction and Application of Prions
- 9.7 Answers to Check Your Progress Questions
- 9.8 Summary
- 9.9 Key Words
- 9.10 Self Assessment Questions and Exercises
- 9.11 Further Readings

### NOTES

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### 9.0 INTRODUCTION

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Genes on sex chromosomes are sex linked. A gene that is on only the X chromosome is X linked, one only on Y chromosome is Y linked or holandric. Homologous genes that occur on both the X and Y chromosomes are partially X linked or psuedoautosomal. They exhibit the same pattern of inheritance as autosomal genes. Psuedoautosomal segments of X and Y chromosomes exhibit pairing at meiosis.

The inheritance of most of the characters of an individual is governed by nuclear genes. But in some cases, the inheritance is governed by cytoplasmic factors or genes. When the transmission of characters from parents to offspring is governed by cytoplasmic genes; it is known as cytoplasmic inheritance or extra nuclear inheritance or extra chromosomal inheritance or non-mendelian inheritance or organellar inheritance. The first case of cytoplasmic inheritance was reported by Conens in 1909 in four 'o' clock (*Mirabilis jalapa*) for leaf colour. Later on, cytoplasmic inheritance was reported by various workers in various organisms.

Male sterility in plants implies an inability to produce or to release functional pollen, and is the result of failure of formation or development of functional stamens, microspores or gametes. As a consequence male sterility is sometimes divided into: (a) 'Pollen sterility' in which male sterile individuals differ from normal only in the absence or extreme scarcity of functional pollen grains; (b) 'Structural or staminal male sterility' in which male flowers or stamens are malformed and non-functional or completely absent; (c) 'Functional male sterility' in which perfectly good and viable pollen is trapped in indehiscent anthers and thus prevented from functioning.

Of the three types of male sterility mentioned, pollen sterility is by far the most common and the only one that has played a major role in plant breeding and hybrid seed production.

## NOTES

In this unit, you will learn about sex linkage, cytoplasmic inheritance, male sterility, and applications of prions in detail.

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### 9.1 OBJECTIVES

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After going through this unit, you will be able to:

- Discuss sex linked genes in drosophila
- Analyse the effects of deficiency of enzyme G6PD
- Explain the characteristics and detection of cytoplasmic inheritance
- Describe the characteristics of Mendelian inheritance
- Elaborate the extra-nuclear inheritance in eukaryotes
- Describe plastid inheritance in mirabilis

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### 9.2 SEX LINKED GENES IN DROSOPHILA AND THE PATTERN OF SEX LINKED GENE TRANSMISSION

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It is believed that sex chromosomes were once ordinary homologous chromosomes and that they carried a number of ordinary genes in addition to certain sex determining genes. A physical modification of one of these one homologous chromosomes can be visualized with light microscope, for example, one of the two sex chromosomes has become much smaller than the other, as in human Y chromosome and become highly heterochromatic. In drosophila, the heterochromatic Y chromosome became larger than its X homologue and acquired the shape of hook. Normally a female fly carries two X chromosomes, whereas a male has one X chromosome and one Y chromosome. Females are typed as XX and males as XY. An occasional abnormal fertilization give rise to fly with one X but no Y, such an individual though sterile is male phenotypically. In humans, like Drosophila, mechanical errors in separation of homologues during meiosis can produce XO individuals having an X but no Y as in Turner syndrome. Likewise, XXY exception exhibits Klinefelter syndrome, where XX fails to show separation. A third kind of sex chromosome organization is found in birds and butterflies. It is necessarily the same in Drosophila and humans except that the male has two large chromosomes called Z, and female carries one Z and one smaller chromosome called W. The males are ZZ and females are ZW.

In organisms with X and Y chromosomes, the female is termed as the homogametic and male as the heterogametic, meaning that as far as sex chromosomes are concerned female produces only X carrying eggs, whereas male produces both X and Y carrying sperms. Because off spring that are XY are males, the inheritance of the X chromosome shows a specific pattern. It follows that a male will always inherit his X chromosome from his mother since his father must contribute the Y. Moreover, a male can transmit the information in his X chromosome to his grandchildren only through his daughters. Genetic traits which are transmitted by way of this specific pattern is said to be sex linked called criss cross or zig-zag pattern of inheritance.

## NOTES

### White Eye Locus

When a cross is made between wild type females (+, red eyed) and white eyed males w, all the F<sub>1</sub> flies are wild type indicating that w is recessive to normal allele. However when the F<sub>1</sub> flies are inbred, three quarter of the resulting flies possess the wild phenotype and one quarter is of white phenotype. Apparently it is a straight forward example of segregation of pair of alleles + and w. When the F<sub>2</sub> flies are classified for both eye color and sex, it is found that all of the females are wild type, whereas half of males are wild and other half have white eyes. When we examine the reciprocal cross, where white females are crossed to wild type males. In this case, the F<sub>1</sub> progeny is no longer have all wild type, instead we find wild type females and white males. Moreover when the F<sub>1</sub> progeny is inbred, half the progeny is wild type in phenotype and other half is white eyed. The transmission of w and its wild type allele +, when assumed that w is located on the X chromosome accounts for the observed phenotypic ratios.

### Sex Linkage in Humans

The human X chromosome contain many genes that are required in both sexes, whereas Y chromosome contains only few genes, primarily genes related to maleness. About 200 traits have been estimated to be X linked compared to a few, which are Y linked. The traits controlled by genes located on the X chromosome are called sex linked genes.

### X Linked Dominant Genes

Any such gene is expressed in both sexes and is not different in way from autosomal dominant inheritance pattern. One dose of X linked dominant allele produces its effect in males as well as females. The hemizygous male transmits the gene to all its daughter but not to his sons. There is no male father to male son transmission. The heterozygous female transmit the trait to half of their children irrespective of sex (Refer Figure 9.1 (a), (b)).

NOTES

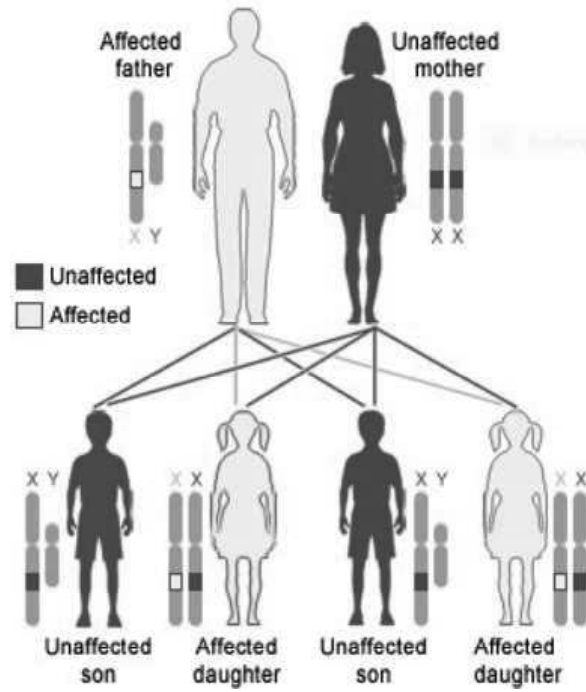


Fig. 9.1 (a) X-Linked Dominant, Affected Father

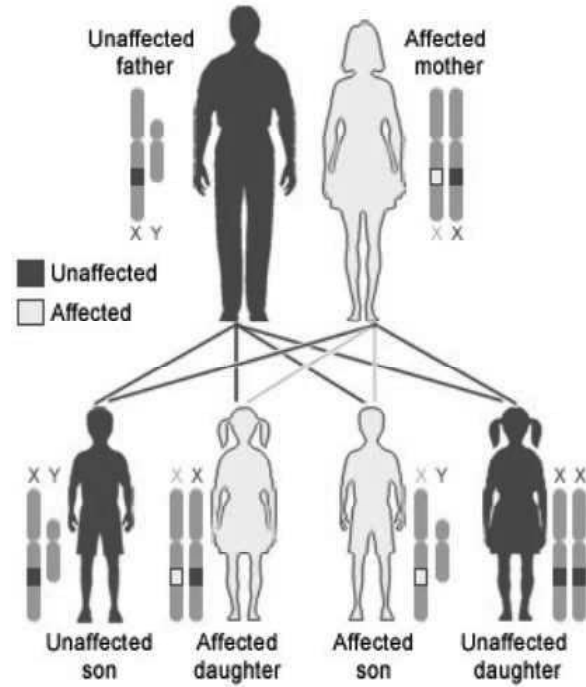
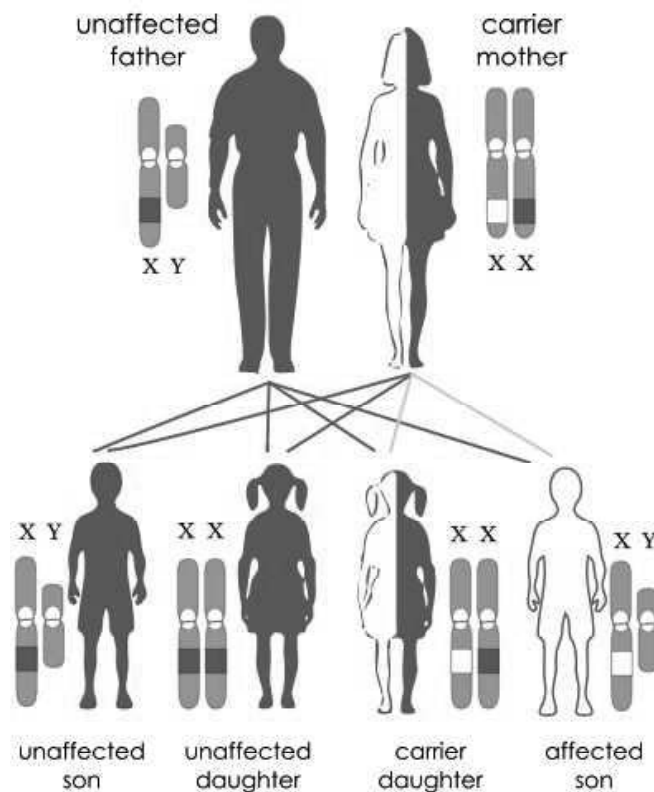


Fig. 9.1 (b) X-Linked Dominant, Affected Mother

## X Linked Recessive Genes

The recessive X linked alleles females express recessive allele only when they are homozygous, whereas males show in hemizygous condition. The frequency of X linked recessive traits is always lower in females than in males. As a rule, males transmit their X chromosome to every daughter and their Y chromosome to every son showing a pattern of inheritance in which phenotype is expressed only in males of alternate generations. A male bearer transmits the recessive allele to the daughters, wherein it is not expressed as being masked by heterozygous condition. The heterozygous female is a carrier of the allele. The X linked allele show a criss cross pattern of inheritance (Refer Figure 9.2).

## NOTES



**Fig 9.2** X Linked Recessive Genes

## Colour Blindness

The first to be described is a form of red green colorblindness in which the green sensitive cones are defective and is called deutan colour blindness. It is due to an X linked recessive gene. It affects about 8% of human males but only about 0.7% females.

A different kind of red green defect called protan colour blindness is manifested due to a defect in the red sensitive cones. It is less common than the

deutan type, occurring in about 2% of males but in women 4 out of 10,000. Other forms of colour blindness, some sex linked can be detected by visual examination through special charts.

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### Haemophilia

It is a disorder in which one of the factors required for the normal clotting of blood is deficient and the blood fails to clot. Even minor injury can cause profuse internal and external bleeding that if not treated may lead to death. Haemophilia A is the classic sex linked type of the genetic disease. It is characterized by the reduction in the amount of antihemophilic globulin known as factor VIII. About 80% of the cases account for this type of haemophilia. Another form is Haemophilia B or Christmas disease. It is the result of defective plasma thromboplastin component. The gene for haemophilic B is not allelic to that of haemophilia A. A rare autosomal gene can cause haemophilia C. This interferes with the production of plasma thromboplastin antecedent or factor XI. Less than 1% of the haemophilics are due to this type of mutation.

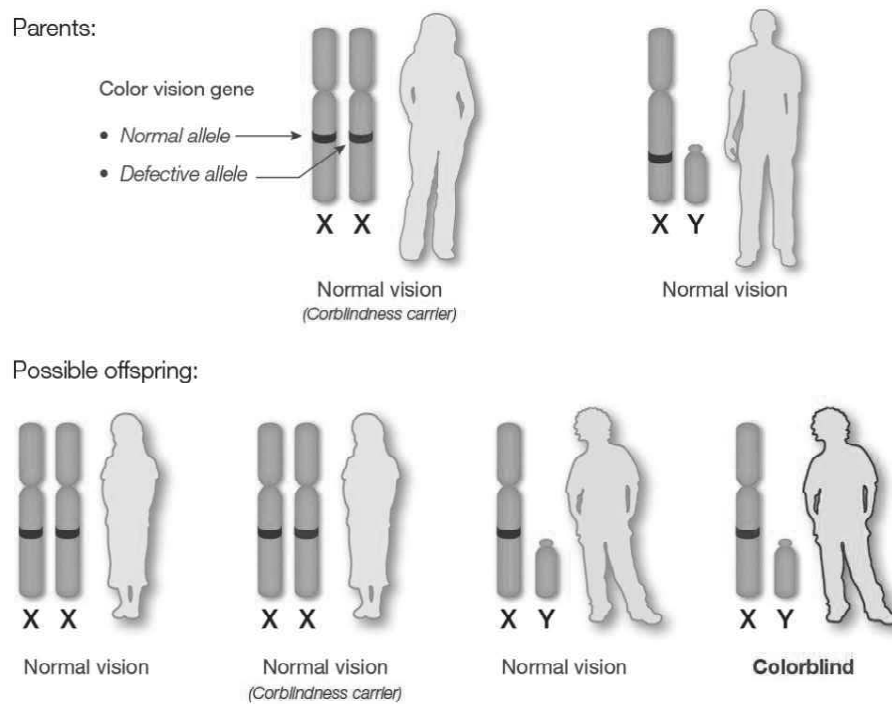
### Deficiency of Enzyme G6PD

The glucose 6 phosphate dehydrogenase is an important enzyme which results from the action of recessive X linked gene that affect 100 million person. The enzyme is involved in a minor glycolysis pathway in red blood cells. Persons from Mediterrean also show this defect. This disorder is rare in whites in areas where malaria is not indigenous. This conditions is noteworthy for large scale destruction of erythrocytes with haemolytic anemia. Inhalation of pollen or ingestion of seeds of broad bean produces the same effect.

### X Linkage

During meiosis in males, the X and Y chromosomes don't line up side by side in normal synapsis. They pair end to end. Atleast 140 genes are assigned to the X chromosome and 171 more are suspected of being X linked. Because few genes are known on Y chromosome, terms X linked and sex linked are used synonymously. X linked traits have a unique mode of inheritance because females have two doses of an X linked gene while males have one. Males are hemizygous for X linked traits. Dominant X linked genes are always expressed in both sexes just as autosomal traits. Because females have two X chromosomes, the frequency of dominant X linked traits is higher in females than in males. Such traits are expressed in both homozygous and heterozygous females. The opposite is true for recessive alleles. Because they are hemizygous, males always express recessive X linked alleles. Females express recessive alleles only when they are homozygous. Males transmit their X chromosome to every daughter, their Y chromosome to every son (Refer Figure 9.3).





**Fig. 9.3** X Linkage

### Red Colour Blindness

People cannot see certain colours. The most common such defect is inability to distinguish red from green. Two X linked loci are known to cause partial colour blindness. The recognition that genes are located on chromosomes led to association of partial colour blindness and the X chromosome both of which show the criss cross inheritance.

### Haemophilia

Also known as Bleeder's disease is condition characterized by the inability to form blood clots. An affected individual is in danger of bleeding to death from smallest cut. At least three different loci control different forms of hemophilia. The rarest is controlled by an autosomal recessive gene but the other two result from recessive alleles at two X linked loci. Haemophilia B comprises about 20% of all haemophilia and is caused by deficiency of plasma thromboplastin.

### Holandric Genes

The Y chromosome does bear gene responsible for the testis determining factor, on the short arm. Histocompatibility antigen genes have been located on the Y chromosome in human, rat, mouse and guinea pig. Genes that contribute to height are also assigned on this chromosome along with genes that determine slow maturation of the individual. It was proposed that a gene that determines excessive hair development on the ears is holandric in nature. This trait is common in East

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Indians, Australia inhabitants and in Japanese populations. Other example is that of porcupine trait. During the course of disease, skin turns yellow, gradually turns black and covered with rough scales with bristly outgrowth.

## NOTES

### Testis Determining Factor

This gene on the Y chromosome is responsible for the development of the testis and hence called the Testis Determining Factor (TDF). The gene has been isolated, cloned and structurally characterized and sequenced. It encode a protein that acts by regulating the expression of other genes. It acts as master regulator that triggers the expression of a large number of genes that produce male sex phenotype.

### Y Linkage

Y linked or holandric genes, genes should have the simplest possible pattern of inheritance. Because only males have Y chromosome, Y linked traits appear in males. Because they are hemizygous, every Y linked gene should be expressed. Each male contributes his one and only Y chromosome to every male offspring, so Y linked traits should show strict father to son inheritance.

### X Inactivation

Individuals who are AA produce twice as much of compound as individuals who are Aa. So it is reasonable to ask if homozygous normal females produce twice as much gene product for X linked genes as hemizygous normal males. X linked traits are phenotypically similar in males and females due to phenomenon known as dosage compensation. Giemsa staining of somatic cells reveals X inactivation as sexual dimorphism. In females, one darkly staining region is found at edge of nucleus called barr body and is inactivated X chromosome. One X chromosome is essential for normal development in both sexes but if individual has more than one, all but one are inactivated and visible in stained somatic cells as a Barr body

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## 9.3 CYTOPLASMIC INHERITANCE

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The existence of genes as segments of nucleic acid molecules, located in chromosome of nucleus, has been demonstrated by several experiments. The nuclear genes control the phenotypes of the organisms and are concerned with the transmission of hereditary character from one generation to next generation is known and predictable Mendelian fashion.

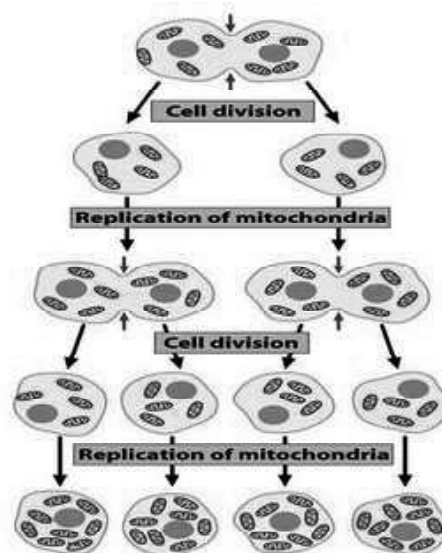
Carl Correns in 1908 observed a difference in the results of reciprocal crosses and first apparent deviation from Mendelian heredity. Different shades of colour from white to dark green in the leaves of some plants were investigated. Instead of equal inheritance from seed and pollen parent as shown by Mendel in garden peas, Correns showed in studies of *Mirabilis jalapa* plants that inheritance of certain traits came entirely from the seed parent. Colour difference were related to plastids, cytoplasmic organelles in plant cells, most important of which are

chloroplasts which carry chlorophyll. Chloroplasts arise from cytoplasmic particles called proplastids that contain DNA and duplicate themselves independently of other cell parts. They are distributed more or less equally during cell division. Although some proplastids are transmitted in the cytoplasm of the egg, if any are transmitted in the pollen of most plants. Thus some chloroplast characteristics are controlled by non-chromosomal inheritance independently of the nuclear genes and are inherited from maternal or seed parent cytoplasm.

Many variations in plant colors depend directly or indirectly on nuclear genes. Phenotypes on which leaves are mosaic of pale green or white or dark green areas are dependent on nuclear genes but their occurrence in a particular plant is controlled by the cytoplasm of the maternal line. Some variations in chloroplasts can be traced to mutational changes in the DNA of the proplastids and are entirely independent of nuclear genes. The most accepted hypothesis for persistently mottled or variegated leaves, branches or whole plants is the two kinds of chloroplasts, normal and mutant are present in the same plant.

Normal green chloroplasts are produced in the cell through independent multiplication of proplastids. Mutant forms give rise to abnormal plastids. Daughter cells resemble parent cells from which they have arisen. In rapidly dividing cells, the multiplication of proplastids does not keep pace with cell division and the reduced numbers make chance distribution effective in changing the characteristics of daughter cells (Refer Figure 9.4).

- Inheritance of DNA in cytoplasm (mitochondria or chloroplasts)
- Inherited from mother only
  - Sperm contributes nucleus, but no cytoplasm
- Characteristics exhibit extensive phenotypic variation
  - Each cell can contain hundreds of mitochondria, and may not have same genetic information
  - Homoplasmy – all the same
  - Heteroplasmy – different genetic information
    - Ratio of "normal" to "mutant"



**Fig. 9.4** Cytoplasmic Inheritance

The inheritance of genes of nuclear chromosomes is characterised by the fact that the genes from male and female parents contribute equally to the genetic constitution of the offspring. Therefore, in it the reciprocal crosses between parents of different homozygous genotype will produce offspring's of identical phenotypes except for sex-linked genes.

## NOTES

## NOTES

However, in certain cases, although male and female parents contribute equally their nuclear genes to the offspring's, the results show a non-Mendelian inheritance pattern and the result of reciprocal crosses varies.

These variations suggest that the genes for the inheritance of certain characters do not occur within the nucleus, but they are present in cytoplasm and play an important role in transmission of certain specific traits, which are not controlled by nuclear genes. Therefore, it builds up the concept of cytoplasmic inheritance. The genes for cytoplasmic inheritance are independent, self-replicating nucleic acids.

Evidence for cytoplasmic inheritance was first reported by Correns in *Mirabilis jalapa* and by Bar in *Pelargonium zonule* in 1908. Rhoades described cytoplasmic male sterility in maize in 1933. In 1943, Sonneborn discovered kappa particles in *Paramecium* and described its cytoplasmic inheritance. Presence of DNA in chloroplasts was first demonstrated by Ris in plant cell.

In 1963, Nass and his co-workers proved the existence of DNA in mitochondria. Subsequently, from time to time, observations by several scientists have been reported the important role of cytoplasm in genetics. Thus, on the basis of observations made on cytoplasmic inheritance of some specific traits, it has been suggested that cytoplasm is also genetically active.

Extra-chromosomal inheritance, extra-nuclear inheritance, somal inheritance and maternal inheritance are all synonyms. All these terms can be defined as the inheritance of characteristics of only one of the two parents, usually the female parent to the progeny. The reciprocal crosses show consistent differences as well as there is a lack of segregation in  $F_2$  and subsequent generations

The genes controlling cytoplasmic inheritance are present outside the nucleus and, in the cytoplasm, they are known as plasma genes, cytoplasmic genes, cytogeneses, extra nuclear genes or extra chromosomal genes.

The sum total of the genes present in cytoplasm of a cell is known as Plasmon. All the genes present in a plastid are known as plastoms. Similarly, all the genes present in a mitochondrion are known as chondriosoms. The genes present in plastid and in mitochondrion are located in their own DNAs and are known as cpDNA and mtDNA, respectively. These DNAs are collectively termed organelle DNA.

### Cytoplasmic Male Sterility

It is associated with pollen failure. It occurs in many flowering plants and results in male sterility. In maize, wheat, sugar beet, onions and other crop plants, fertility is controlled at least in part by cytoplasmic factors. It was discovered and analyzed by M.M Rhoades. Pollen was aborted in the anthers of corn plants causing them to be male sterile but female structure and fertility were normal. It was transmitted from generation to generation through egg cytoplasm. A particular male sterile variety produced only male sterile progeny when fertilized with pollen from normal

grains. The male sterile seed parent plants were backcrossed repeatedly with pollen fertile lines until all chromosomes from male sterile line had been exchanged for male fertile line. In the genetically restored sterile line, male sterility persisted, suggesting that inheritance was maternal and not controlled by chromosomal genes (Refer Figure 9.5).

## NOTES

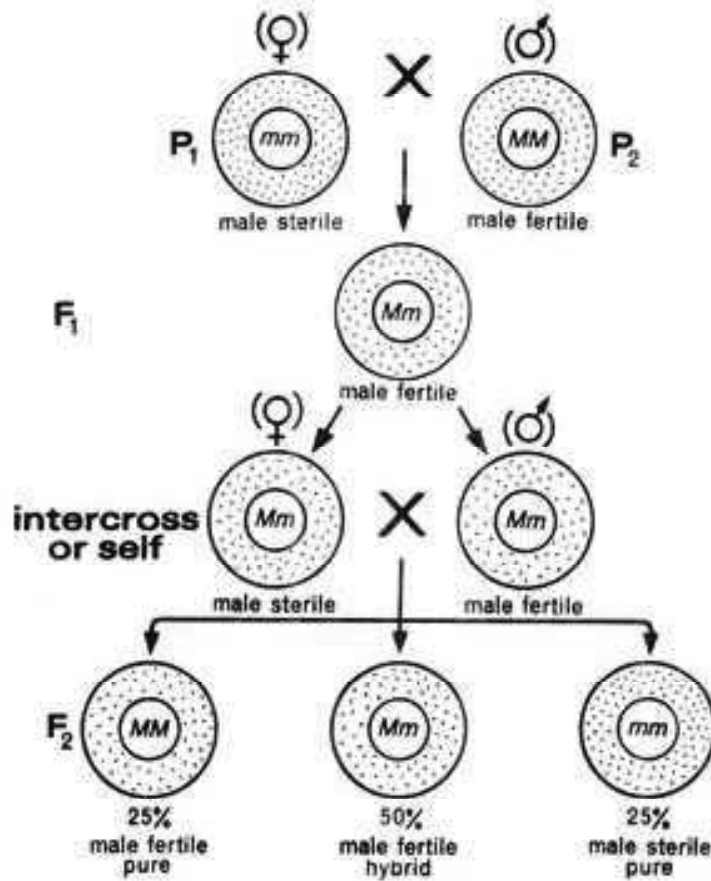


Fig. 9.5 Cytoplasmic Male Sterility

### Check Your Progress

1. Which organisms are termed as homogametic organisms?
2. What is glucose 6 phosphate dehydrogenase?
3. How many genes are assigned to X chromosomes?
4. How does normal green chloroplasts produced?

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## 9.4 CHARACTERISTICS AND DETECTION OF CYTOPLASMIC INHERITANCE

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### NOTES

Cytoplasmic inheritances do not show Mendelian inheritance. They show the following characteristic features:

- Hereditary traits which are transmitted by cytoplasm do not show Mendelian segregation in crosses and in reciprocal crosses with respect to a particular set of characteristics controlled by a set of cytoplasmic genes produce dissimilar hybrids.
- Most of the recorded cytoplasmically inherited characteristics would follow the maternal line, i.e., uniparental mode of transmission. In higher plants and animals, ovum or egg cell is comparatively large and contains large amount of cytoplasm. But male gametes or sperms have very little amount of cytoplasm. So, under this situation, most of cytoplasmic factors are transmitted to the progeny through the ovum of mother.
- It is known as maternal inheritance or trans-ovarian transmission. In this mode of transmission, all the offspring's of the parents have maternal condition and only female progeny can transmit the cytoplasmic characteristics to the succeeding generations.
- Hence the reciprocal crosses yield different or non-Mendelian results.

### Characteristics of Mendelian Inheritance

The inheritance pattern of characters of an organism as proposed by Mendel on the basis of monohybrid and di-hybrid crosses is referred to as Mendelian inheritance. It shows the following characteristic features:

- Contribution of both male and female is equal, hence results from reciprocal crosses are similar.
- Segregation produces the phenotypes ratio 3 : 1 and genotype ratio 1 : 2 : 1 in the F<sub>2</sub> generation of a monohybrid cross and a typical phenotype ratio 9 : 3 : 3 : 1 in di-hybrid crosses.

Mendelian inheritance pattern is regarded as a sufficient evidence for a gene to be located in chromosomes; such genes are called nuclear genes or simply as genes.

### Extra-Nuclear Inheritance in Eukaryotes

Various cases of extra-nuclear inheritance in different eukaryotic organisms have been studied by several scientists.

Few important examples of extra nuclear inheritance in eukaryotes are stated under some classified subheadings:

**(i) Maternal Inheritance**

Maternal inheritance means the inheritance controlled by extra-chromosomal, i.e., cytoplasmic, factors that are transmitted to the succeeding generation through the egg of female organism. They show the following features:

- Reciprocal differences in  $F_1$ ;
- Which in most cases disappears in  $F_2$ ;
- A smaller variation in  $F_2$  as compared to that in  $F_3$ .

Maternal inheritance may be, broadly speaking, of two kinds:

- (i) If some treatments (chemical poison, heat shock etc.) are applied to the female parent, it may affect the egg's cytoplasm. As a result subsequent offspring's are modified in some way. Effects of this kind are called Dauer-modifications or persisting modifications.

It is observed that when protozoa are treated experimentally with chemical poisons or heat shocks, the treatments induce several morphological abnormalities in them. Such abnormalities go on decreasing generation after generation and, eventually, disappear completely through cell division if the treatments are removed.

Further evidences also come from fruit flies subjected to heat treatment and from bacteria treated with chemicals.

- (ii) Other kinds of maternal inheritance are also known which do not depend upon the repeated application of an external stimulus to the cytoplasm. In this case, maternal inheritance is truly controlled by independent cytoplasmic genes.

Maternal effects reflect the influence of the mother's gene on developing tissues. Many important characteristics of both animal and plants show maternal effects of which some examples are described next.

**(ii) Coiling of Snail Shells (*Limnaea Peregra*)**

One of the earliest and classical examples of a maternal effect is that of the direction of coiling in shells of the water snail *Limnaea peregra*. In this snail, the shell is spirally coiled. Usually the direction of coiling of the shell is clock-wise if viewed from the top of the shell. This type of coiling is called dextral. However, in some snails the coiling of shell is anticlockwise. This type of coiling is sinistral.

The direction of shell coiling of both types of snail is governed by genotype of the female parent and not by their own genotype. Further investigation suggests that coiling depends upon the early cleavage in the zygote.

If the mitotic spindle is tilted to left of the median line of zygote, the successive cleavages will produce a spiral to left (sinistral) and if the orientation of spindle is tilted to the right of the median line of zygote, the successive cleavages will produce a spiral to right (dextral). The spindle orientation is controlled by the genotype of oocyte from which the egg develops.

**NOTES**

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## 9.5 EXTRA-NUCLEAR INHERITANCE BY CELLULAR ORGANELLES

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### NOTES

Extra-nuclear inheritance is also associated with certain cytoplasmic organelles (mitochondria, plastids) that contain naked circular DNA and protein synthesizing apparatus. These extra nuclear genetic materials present in the organelles are autonomous and code only for limited number of enzymes and polypeptides. Certain enzymes required for cellular respiration are synthesised in the mitochondria.

Similarly, chlorophyll and other pigments are synthesised in the plastid. Besides the involvement of such biosynthetic activities, these organelle DNAs are directly associated with the inheritance of some phenotypes which are not controlled by the nuclear genes. The genetic material of chloroplasts and mitochondria are transmitted almost exclusively via the egg. The inheritance pattern is well-illustrated by the following examples:

#### Plastid Inheritance in *Mirabilis*

Plastid inheritance means the inheritance of plastid characteristics due to plasma genes located in plastids. Plastid inheritance was first described by C. Correns (1908) in the four o'clock plant, *Mirabilis jalapa*.

Leaves of *Mirabilis jalapa* may be green, white or variegated and some branches may have only green, only white or only variegated leaves. Variegation means the presence of white or yellow spots of variable size on the green background of leaves.

Thus it forms the mosaic pattern of coloration on a leaf. Due to certain inheritable defects chloroplast of all cells or some cells of leaf often are unable to synthesize the chlorophyll pigments. Such cells remain non-green and form white or yellow coloured leaf, or white or yellow patches, interspersed with areas containing normal green cells with healthy chloroplasts. Variegation may be produced by:

- Some Environmental Factors
- Some Nuclear Genes
- Plasma-Genes in Some Cases

Since the first and second causes of leaf variegation do not concern cytoplasmic inheritance, the inheritance of variegation due to plasma-genes will be discussed in this article.

The inheritance of different leaf colours in *Mirabilis jalapa* might be explained if the plastids are somehow autonomous and are never transmitted through male parent. For an organelle to be genetically autonomous, it must be provided with its own genetic determinants that are responsible for its phenotype.

Since the bulk amount of cytoplasm containing many plastids is contributed by the egg and the male gametes contribute negligible amount of cytoplasm, therefore



plastids present in the cytoplasm of egg is responsible for the appearance of maternal colour in the offspring and the failure of male plant to transmit its colour to offspring is reasonable.

In the offspring from variegated female parents, green, white and variegated progeny are recovered in variable proportions. The variegated parent produces three kinds of egg- some with colourless plastids, some contains only green plastids, and some are with both chloroplasts and leucoplasts.

As a result, zygotes derived from these three types of egg cells will develop into green, white and variegated offspring's, respectively.

One of the most striking and spectacular example of cytoplasmic inheritance due to symbiotic bacteria is noted in the most common ciliate protozoan *Paramecium aurelia*. In 1943, T. M. Sonneborn reported that some strains of *P. aurelia* contain kappa particles and are known as killer strain.

Kappa particles are the symbiont bacteria called *Caedobacter taeniospiralis*. The diameter of kappa particles are about  $0.2\mu$ . They are bounded by a membrane and contain a little bit of cytoplasm with DNA. The strain of *Paramecium* in which the kappa particles are absent are called sensitive strain. The sensitive strains are killed by the killer strain.

The destruction of sensitive strain occurs through secretion of a toxic substance called paramecin. This toxic substance is believed to breakdown the food vacuole membrane of the sensitive strain. Paramecin is diffusible in the liquid medium

These kappa bacteria possess a refractile protein containing 'R' body and are called brights because they are infected with a virus that controls the synthesis of a viral protein as well as R protein body in kappa bacterium.

The virus may act as the toxin in the killing response and R body facilitates the penetration of the toxin. The non-bright kappa bacteria may also contain virus but the virus may be in provirus state in them.

The killer character of *Paramoecium* has a nuclear as well as cytoplasmic basis. The existence of kappa particles is determined by presence of a nuclear dominant gene K. Kappa particles, like other bacteria, multiply through fission.

But their multiplication in the cytoplasm of *Paramoecium* depends on the presence of a dominant nuclear gene K which helps to make an environment necessary for the bacteria to reproduce.

When killer strain of *Paramoecium* conjugates with sensitive strain under appropriate condition for brief period and no cytoplasm exchange occurs, two kinds of clones result- one from the original killer cell which contains allele K (Kk) and kappa particles and the other from the original sensitive cell which carries the allele k (kk) and lacks kappa particles.

It indicates that homozygous (either KK or kk) strains become heterozygous following an exchange of K and k genes without cytoplasmic exchange.

## NOTES

## NOTES

Following autogamy (a process of self- fertilisation within one undivided cell resulting in homozygosity), half the progeny (50%) are sensitive Paramecia. But all progenies of sensitives following autogamy will be sensitive's.

In this conjugation, following autogamy of killers, 50% progeny will receive Kk genotype with cytoplasmic kappa particles other 50% progeny will receive kk genotype with cytoplasmic kappa particles. But it will be sensitive, because kappa cannot reproduce in the cells unless a K allele is present in the nucleus and, as a consequence the kappa are eliminated.

On the other hand, in this conjugation the product of autogamy of sensitive strain obtained after conjugation are all sensitive. All through, 50% progeny of autogamy have KK genotype without cytoplasmic kappa particles because no cytoplasm has been transferred in this conjugation. Remaining 50% progeny of autogamy of sensitive's have kk genotype and no cytoplasmic kappa partic

### Check Your Progress

5. What does maternal inheritance mean?
6. What does plastid inheritance mean?
7. What does variegation mean?
8. How is the existence of kappa particles determined?

## 9.6 ORIGIN, INDUCTION AND APPLICATION OF PRIONS

Prion diseases have been found in sheep, cows, humans. The basic phenotype is an ataxia.- a neurodegenerative disorder manifested by inability to remain upright. The name of disease in sheep is scrapie that reflects the phenotype. Scrapie can be perpetuated by inoculating sheep with tissue extracts from infected animals. The human disease Kuru was discovered in New Guinea, where it is perpetuated by cannibalism i.e., eating of brain. When tissue from scrapie infected sheep is inoculated into mice, disease occurs in period ranging from 75 to 150 days. The active component is a protease resistant protein. The protein is encoded by a gene that is expressed in brain. Its conversion to resistant form PrP<sup>Sc</sup> is sensitive to proteases. This is the case of epigenetic inheritance in which there is no change in genetic information.

### Check Your Progress

9. Where have prion diseases been found?
10. What is the form of protein PrPC sensitive to?

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## 9.7 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

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1. In organisms with X and Y chromosomes, the female is termed as the homogametic.
2. The glucose 6 phosphate dehydrogenase is an important enzyme which results from the action of recessive X linked gene that affect 100 million person.
3. Atleast 140 genes are assigned to the X chromosome and 171 more are suspected of being X linked.
4. Normal green chloroplasts are produced in the cell through independent multiplication of proplastids.
5. Maternal inheritance means the inheritance controlled by extra-chromosomal, i.e., cytoplasmic, factors.
6. Plastid inheritance means the inheritance of plastid characteristics due to plasma genes located in plastids.
7. Variegation means the presence of white or yellow spots of variable size on the green background of leaves.
8. The existence of kappa particles is determined by presence of a nuclear dominant gene K.
9. Prion diseases have been found in sheep, cows, humans.
10. The form of protein in normal brain called PrP<sup>C</sup> is sensitive to proteases.

## NOTES

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## 9.8 SUMMARY

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- Extranuclear inheritance includes instances involving DNA in cytoplasm.
- Genes on sex chromosomes are sex linked. A gene that is on only the X chromosome is X linked, one only on Y chromosome is Y linked or holandric.
- The inheritance of most of the characters of an individual is governed by nuclear genes.
- Male sterility in plants implies an inability to produce or to release functional pollen, and is the result of failure of formation or development of functional stamens, microspores or gametes.
- Of the three types of male sterility mentioned, pollen sterility is by far the most common and the only one that has played a major role in plant breeding and hybrid seed production.
- It is believed that sex chromosomes were once ordinary homologous chromosomes and that they carried a number of ordinary genes in addition to certain sex determining genes.

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- An occasional abnormal fertilization give rise to fly with one X but no Y, such an individual though sterile is male phenotypically.
- In organisms with X and Y chromosomes, the female is termed as the homogametic and male as the heterogametic, meaning that as far as sex chromosomes are concerned female produces only X carrying eggs, whereas male produces both X and Y carrying sperms.
- Because off spring that are XY are males, the inheritance of the X chromosome shows a specific pattern.
- When a cross is made between wild type females (+, red eyed) and white eyed males w, all the F1 flies are wild type indicating that w is recessive to normal allele.
- The human X chromosome contain many genes that are required in both sexes, whereas Y chromosome contains only few genes, primarily genes related to maleness.
- The hemizygous male transmits the gene to all its daughter but not to his sons.
- The recessive X linked alleles females express recessive allele only when they are homozygous, whereas males show in hemizygous condition.
- The first to be described is a form of red green colorblindness in which the green sensitive cones are defective and is called deutan colour blindness.
- Even minor injury can cause profuse internal and external bleeding that if not treated may lead to death.
- Haemophilia A is the classic sex linked type of the genetic disease.
- The glucose 6 phosphate dehydrogenase is an important enzyme which results from the action of recessive X linked gene that affect 100 million person.
- During meiosis in males, the X and Y chromosomes don't line up side by side in normal synapsis.
- Males are hemizygous for X linked traits. Dominant X linked genes are always expressed in both sexes just as autosomal traits. Because females have two X chromosomes, the frequency of dominant X linked traits is higher in females than in males.
- The recognition that genes are located on chromosomes led to association of partial colour blindness and the X chromosome both of which show the criss cross inheritance.
- The Y chromosome does bear gene responsible for the testis determining factor, on the short arm.
- This gene on the Y chromosome is responsible for the development of the testis and hence called the Testis Determining Factor (TDF).

- Individuals who are AA produce twice as much of compound as individuals who are Aa.
- The existence of genes as segments of nucleic acid molecules, located in chromosome of nucleus, has been demonstrated by several experiments.
- Carl Correns in 1908 observed a difference in the results of reciprocal crosses and first apparent deviation from Mendelian heredity.
- Many variations in plant colors depend directly or indirectly on nuclear genes. Phenotypes on which leaves are mosaic of pale green or white or dark green areas are dependent on nuclear genes but their occurrence in a particular plant is controlled by the cytoplasm of the maternal line.
- The inheritance of genes of nuclear chromosomes is characterised by the fact that the genes from male and female parents contribute equally to the genetic constitution of the offspring.
- These variations suggest that the genes for the inheritance of certain characters do not occur within the nucleus, but they are present in cytoplasm and play an important role in transmission of certain specific traits, which are not controlled by nuclear genes.
- Evidence for cytoplasmic inheritance was first reported by Correns in *Mirabilis jalapa* and by Bar in *Pelargonium zonule* in 1908. Rhoades described cytoplasmic male sterility in maize in 1933.
- Extra-chromosomal inheritance, extra-nuclear inheritance, somal inheritance and maternal inheritance are all synonyms.
- The sum total of the genes present in cytoplasm of a cell is known as Plasmon. All the genes present in a plastid are known as plastons.
- Hereditary traits which are transmitted by cytoplasm do not show Mendelian segregation in crosses and in reciprocal crosses with respect to a particular set of characteristics controlled by a set of cytoplasmic genes produce dissimilar hybrids.
- The direction of shell coiling of both types of snail is governed by genotype of the female parent and not by their own genotype.
- The basic phenotype is an ataxia a neurodegenerative disorder manifested by inability to remain upright. The name of disease in sheep is scrapie that reflects the phenotype.
- The organellar heredity deals with characters showing inheritance through important organelles such as mitochondria and chloroplast. Examples of chloroplast mutant i.e., coloured leaves in *Mirabilis* and drug resistance in *Chlamydomonas* are associated with the maternal transmission of chloroplast, the phenomenon is controlled by non chromosomal genes.

## NOTES

## NOTES

- In *Drosophila melanogaster*, sex is determined by a balance between genes on the X chromosome and genes on the autosomes, the X:A ratio.
- In humans, sex is determined by the presence or absence of SRY gene located on the Y chromosome.
- Sex linked characteristics are determined by genes on the sex chromosomes, X linked characteristics are encoded by gene on the X chromosomes and Y linked characteristics are encoded by genes on the Y chromosomes.
- A female inherits X linked alleles from both parents, a male inherits X linked alleles from his female parent only.
- Y linked characters are found only in males and are passed from father to all sons.
- The sex chromosomes are evolved from autosomes, crossing over between the X and the Y chromosomes has been suppressed, but palindromic sequences within the Y chromosome allow for recombination on Y chromosome.

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## 9.9 KEY WORDS

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- **Sex linkage:** It is the phenotypic expression of allele that is dependent on the gender of an individual
- **Cytoplasmic inheritance:** It is the inheritance of trait controlled by genes present on the cytoplasm e.g plastids and mitochondrial inheritance
- **Prions:** These are the proteins which characterize neurodegenerative disease in animals and humans.
- **X linked inheritance:** It is a method of inheritance in which a mutation in a gene on the X chromosome causes the phenotype to be expressed in males.
- **Colour blindness:** It is sex linked inheritance where characters are passed from mother to son on the 23<sup>rd</sup> chromosome.

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## 9.10 SELF ASSESSMENT QUESTIONS AND EXERCISES

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### Short Answer Questions

1. What do you mean by plastid inheritance?
2. Describe in detail about sex linkage.
3. Write a short note on sex linked genes in *Drosophila*.
4. What is maternal inheritance? List its kinds.

5. Briefly write about plastid inheritance in mirabilis.
6. What do you mean by red colour blindness?
7. Briefly discuss about X inactivation.

### Long Answer Questions

1. What is the pattern of sex linked gene transmission? Also discuss about x linkage and in humans.
2. Write a detailed note on X linked dominant genes and X linked recessive genes.
3. What are the characteristics of Mendelian inheritance? Discuss about extra-nuclear inheritance in eukaryotes.
4. From your learning of the text, write in detail about extra-nuclear inheritance by cellular organelles.
5. Write a detailed note on the origin, induction and application of prions.
6. Write in detail about the characteristics and detection of cytoplasmic inheritance.
7. What is cytoplasmic inheritance? Also discuss in detail about cytoplasmic male sterility.

### NOTES

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## 9.11 FURTHER READINGS

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## UNIT 10 POLYPLOIDY: TYPES, ORIGIN AND SIGNIFICANCE

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### Structure

- 10.0 Introduction
- 10.1 Objectives
- 10.2 Induction of Polyploidy
- 10.3 Origin of Polyploidy
- 10.4 Types of Polyploidy
- 10.5 Characteristic of Polyploidy
- 10.6 Answers to Check Your Progress
- 10.7 Summary
- 10.8 Key Words
- 10.9 Self Assessment Questions and Exercises
- 10.10 Further Readings

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### 10.0 INTRODUCTION

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Polyploidy was first noted by Lutz (1907) when he discovered that the plant *Oenothera lamarckiana* was actually tetraploid. Blakeslee *et al* (1937) discovered the importance of colchicine in inducing polyploidy. It is widespread in plants and occasional in animals. The frequency of polyploidy is not uniform. It is low in some families (for example, Cucurbitaceae, Moraceae, Fagaceae ) and high in others (e.g. Polygonaceae, Gramineae, Rosaceae, Malvaceae). Similarly Polyploidy is less in annuals and woody plants. It is common in perennial herbs. Majority of the cultivated plants are polyploids.

Polyploidy has played a significant role in the evolution of higher plants (Leitch and Bennett 1997), having helped in tremendously increasing the number of species and sometimes of genera on the planet Earth. It is the most rapid method known of producing radically different but vigorous and well-adapted genotypes (Stebbins 1950); for this reason, it is of great value in plant breeding, more importantly the allopolyploidy. The significance of polyploidy in the plant world can be visualized by considering its high incidence among angiosperms, including the leading commercial crops, such as wheat, sugarcane, oats, cotton, tobacco, apples, pears, plums, *Rubus*, etc. This incidence confers some important advantages, viz. heterosis, gene redundancy and asexual reproduction.

In this unit, you will learn about polyploidy, its significance, type and origin.



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## 10.1 OBJECTIVES

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After going through this unit, you will be able to:

- Discuss spontaneous or natural polyploidy
- Differentiate in gene compositions
- Differ between primary and secondary polyploidy
- Analyse polyploidy and plant evolution

## NOTES

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## 10.2 INDUCTION OF POLYPLOIDY

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Variations in the number of haploid sets are common in many species. Plants such as mosses spend most of their life cycle in a haploid phase and for all sexually reproducing organism, the gametic phase of life cycle is haploid. In bees and wasps, differences in ploidy determine sex: females are diploid, males are haploid. Some fish, salamander and lizard consist entirely of triploid females, no males at all are found in these species.

Endosperm of plant seeds is triploid and human liver cells exhibit many degrees of ploidy- triploid, tetraploid and octaploid. In plants, polyploidy is common. Within a genus, many species have a diploid number that is multiple, greater of the smallest haploid number of that genus. The phenomenon of having more than two sets of chromosomes or genomes. It is defined as genomic mutation which involves an increased dose of one or more genomes. Depending upon the number of genomes present in a polyploidy is known as triploid (3n), tetraploid (4n), pentaploid (5n), hexaploid (6n), heptaploid (7n), octoploid (8n), nonaploid (9n), decaploid (10n). Polyploids with odd number of chromosome sets are sexually sterile because of difficulty of synapsis and disjunction. They are propagated vegetatively, i.e., banana and pineapple.

Polyploidy is uncommon in animals because of the delicate sex balance mechanism. Any upset in this balance such as the one caused by polyploidy, reduces fertility and decreases the chances of individuals to survive and reproduce. Eventually polyploidy will disappear from population. Human skin cells can be diploid or triploid. In mouse, 40% of the liver cells are tetraploid. 5% of the liver cells are octaploid. Mammalian cells grown in tissue culture exhibit polyploidy. Polyploid animals generally multiply through parthenogenesis.

Many common weeds such as dandelion, crabgrass and wild oats are polyploid. Polyploidy originates in two ways. Either from a failure of normal mitotic apparatus where a cell duplicates its chromosomes and complete prophase and metaphase but daughter cell fails to separate. Then parent cell reenters interphase with two diploid sets, it become tetraploid. Polyploid also originates from unreduced gametes. If bivalents don't separate during anaphase I of meiosis, any gametes formed are diploid. Union of a diploid gamete with normal haploid gamete produces

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triploid zygote. Union of two diploid gametes produces a tetraploid zygote. Commonly polyploidy cells and individuals are larger and more vigorous than normal cell. Many domestic fruits and grains originated as polyploids. They are valued highly because they produce fruit which are larger than their diploid progenitors. Food production worldwide has more than kept pace with increasing human population, because of development of new strains of grains like rice, wheat and corn many of which are polyploid.

Sexual reproduction is problematic for polyploids because of difficulty in pairing of homologues. Triploids are not fertile and must be propagated entirely by asexual means. The domestic banana is triploid derived from a wild diploid species and survive by human propagation. In an early experiment, the Russian cytologist G.D Karpechenko synthesized polyploid from crosses between two common vegetables belonging to different genera, the radish *Raphanus sativus* and the cabbage, *Brassica oleracea*. Although these plants were only distantly related, they were enough alike to be crossed successfully with the intervention of the experimenter. Both had 9 pairs of chromosomes.

The diploid hybrid had 18 chromosomes, 9 from each parent but it was sterile and could not perpetuate itself because of the failure in pairing between unlike chromosomes in meiosis. When the chromosomes of F1 hybrid were doubled, a fertile polyploidy named Raphanobrasica was produced with 18 radish and 18 cabbage chromosomes. Because two sets of chromosomes were present from each parental variety, pairing was regular. Normal gametes were produced and high degree of fertility was obtained. This experiment had significance because it demonstrated a method by which fertile interspecific hybrids could be produced. It thus suggests the possibility of incorporating desirable genotypes from two different species into a new polyploidy species.

Sterile hybrid grass made fertile by chromosome doubling : Interesting experiments that made use of induced polyploidy and cytological analysis in grasses have been conducted by W.S Boyle, A.H Holmgren and their associates at Utah State University. On the basis of its sterility and morphological characteristic, grass was identified as natural sterile hybrid between two genera in tribe *hordeae*.

Cytological studies on the two presumed parents and the hybrid supported the view where both *A. Trachycaulum* and *H. jubatum* are tetraploids ( $2n=28$ ). During meiosis of both species, normal pairing of chromosomes occurred to form 14 paired chromosomes. The sterile hybrid is tetraploid with same chromosome number, but its chromosome behavior during meiotic process is highly irregular. 14 unpaired chromosomes and seven bivalents were observed at metaphase in pollen mother cells. Many lagging chromosomes remained in the centre of spindle during anaphase and numerous small micronuclei reflecting chromosome irregularity following division. These observations indicated that meiotic irregularity was a major factor in the sterility.

Polyploidy play role in genetic, evolutionary and systematic studies. The patterns of multiple origin and development of polyploidy in plants have been of

huge interest. It is the presence of multiple sets of genome in same individual. It is the major cause of speciation and evolution. Effects of polyploidy is evident in plant species.

**Diploidization:** Polyploids in their course of evolution have been shown to become diploids both in cytogenetic and genetic make up. It give rise to term paleoploids which indicates diploid species which were polyploid in the past. Examples: Rice, maize, arabidopsis.

**Speciation:** 2-4 % of speciation events in angiosperms is due to speciation. With new sequencing methods, polyploidy is found to be involved in speciation of flowering plants and eukaryotes.

**Conservation of Species:** The recurrence of polyploidization is evident. It counterbalance the local extinction of autopolyploid species.

**Difference in Gene Composition:** Soybean species and its relatives has undergone two polyploidization in their history.

**Variation in Gene Expression:** Gene expression pattern in duplication have shown that polyploidy respond in manner similar to environmental stimuli.

**Epigenetic Changes:** Chromosomal rearrangements and DNA methylation changes are frequent in polyploids and resulted in variation in process of gene expression and regulation

### Spontaneous and Induced Polyploidy

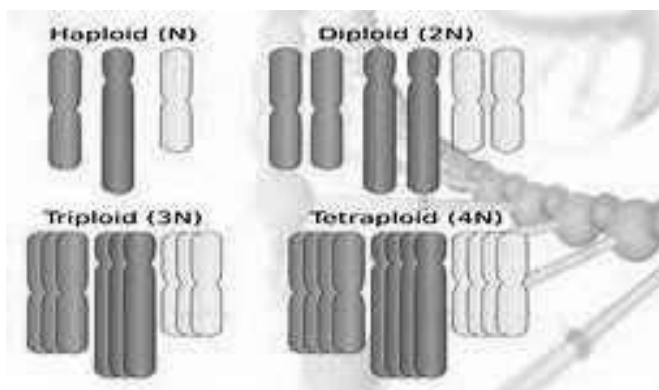
Spontaneous or natural polyploidy is the increase in chromosome sets or genomes naturally without involving any human effort (Refer Figure 10.1). Induced polyploidy is the acquiring of additional chromosome sets through application of special techniques. Polyploidy can be induced by following methods:

- **Decapitation:** Removal of buds or decapitation has been found to induce polyploidy in many cases. In decapitated plant, some of the newly sprouted shoots are polyploidy. Application of IAA to cut end of non meristematic region enhances the chances if polyploidy shoots.
- **Irradiation:** X rays and other radiations interfere with the normal replication and distribution of chromosomes. They bring about polyploidy.
- In many cases, the cells surrounding the area of infection show doubling of chromosomes.
- At times, tissue culture induces the formation of polyploid plants, for example, *Datura*, *Oryza sativa*.
- A heating pad around the ear shoot region of maize, wheat, barley and rye induces doubling of chromosomes. A sudden low temperature is observed to cause polyploidy in *Drosophila*.
- A number of chemicals are known to cause polyploidy. Examples are chloral hydrate, ethyl mercuric chloride, sulphanilamide, indole acetic acid, coumarin, benomyl, colchicine. Out of these colchicines is the most effective. It was

## NOTES

first used for induction of polyploidy by Blakeslee. Colchicine is mitotic poison. It inhibits spindle formation and distribution of replicated chromosomes into two nuclei. As a result cells become polyploidy.

## NOTES



*Fig. 10.1 Types of Polyploidy*

## 10.3 ORIGIN OF POLYPLOIDY

The origin of polyploidy is due to several reasons, a few of which are listed below:

- Failure of spindle formation so that replicated chromosomes remain together in the same nucleus.
- Failure of meiosis or reduction division and give rise to diploid gametes.
- Fusion of two spindles at the time of meiosis II.
- Fertilization of an egg by more than one sperm.
- Fusion between two unreduced or diploid gametes or a diploid gamete and a normal gamete.
- Cross fertilization between two different species usually produces sterile hybrids due to difficulty in synapsis between non homologous chromosomes.

Because of the increased productivity of many polyploids, horticulturists use chemicals to increase the frequency of polyploidization. Application of colchicines to growing tissue of plants stops mitotic cycle prior to separation of daughter cells, producing tetraploidy. Successive colchicines treatment can produce octaploid and higher level of ploidy. The domestic strawberry is an octaploid. Polyploidy is mostly fatal in humans. A few triploids and tetraploids even survived a few months but suffered malformations. About 12 % of spontaneously aborted foetus are triploid or tetraploid. In fact more than 50% of all conceptions are inviable due to genetic causes.

Polyploidy in humans is due to two causes: unreduced gametes and dispermy. The fertilization of an unreduced diploid egg by a haploid sperm give rise to triploid zygote while union of two diploid gametes produce tetraploid. Dispermy, the fertilization of an egg by two sperm, produces a triploid foetus.

Polyploidy is the change in the basic number of chromosome set in any individual species. These are autopolyploids and allopolyploids. It ranges from triploid to octaploids. It may be induced in plants for economic benefit of mankind and environment.

Polyploidy in plants is about one and half of total plant species. These plants are commercially more valuable than the corresponding diploids or haploids.

- Polyploid plants produce larger sized flower and fruits.
- Flower more brightly coloured showy than the diploids.
- Polyploids are of high nutrient content high content of is usually found in fruits of tetraploid fleshy fruits.
- Tetraploids shows tendency towards perenniality rather than annual.

By induction of polyploidy economic value of crops and vegetables are enhanced and evolved varieties of crops are formed. Polyploid plants are better in quality, taste, yield, nutritional value and sugar content. Polyploids are disease resistance.

### **Primary Polyploidy**

The condition of having one or more extra chromosome sets or genomes of the original basic number of a species is known as primary polyploidy. The extra chromosome sets may belong to the same species, another species or both. So primary polyploidy is known as autopolyploidy, allopolyploidy and autoallopolyploidy.

### **Secondary Polyploidy**

It is the increase in chromosome complement in an aneuploid. Apple, pear, Rice are developed through secondary polyploidy. The basic chromosome number of 17 has developed from a triple trisomic ( $2N+1+1+1$ ). Polyploidy developed through hybridization is also considered as secondary polyploidy

Knowledge of polyploidy is of great help in plant breeding to originate new evolved and economically important varieties.

- In Nicotiana resistant variety is formed by using polyploidy.
- Use of polyploidy in wheat breeding results in the production of einkorn, emmer and Vulgare varieties of wheat which are polyploids are better of better quality.
- Intergeneric and interspecific breeds evolved by polyploidy are of great economic value.

Among ornamental plants various polyploid variety of rose dahlia and others are of outstanding attraction in comparison to other normal diploids.

Induced polyploidy has not been exploited to a great extent. Practical applications become more common as additional data are accumulated. By

## **NOTES**

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artificially induced polyploidy, disease resistance and other desirable qualities have been incorporated into some commercial crop plants. Tobacco, is susceptible to the tobacco mosaic virus whereas *N. glutinosa* appeared to be resistant. In *N. glutinosa* the virus killed the cells that were invaded and the virus particle became isolated in the dead cells. The apparent resistance is due to hypersensitivity. When the two tobacco species were crossed, hybrid was resistant to the virus but totally sterile. Some varieties of plants that serve man's purpose more effectively than others, are identified as polyploids. Many polyploids were selected and cultivated because of their large size, vigour and ornamental values. The texture of giant apples is as fine as that of diploids but yield is inferior. Tetraploid maize (4n) is more vigorous than the ordinary diploid and produce 20 percent more vitamin A. Polyploid watermelons have been developed by colchicines treatment. The tetraploid with 44 chromosomes is large and has practical value. Triploid watermelons with 33 chromosomes are desirable because they are sterile and have no seeds. The history of plant breeding is characterized by improvement in many polyploidy crops. This is accomplished by developing disease resistant strains and breeding for increased hardiness and great efficiency under various environmental conditions available in wheat growing regions.

### **Polyploidy in Animals**

Organisms with three or more genomes are polyploids. Fully one half of all known plant genera contain polyploids and about two third of all the grasses are polyploids but it is rarely seen in animals. One reason is that the sex balance in animals is much more delicate than that of plants. The addition of chromosomes above the diploid number give rise to intersex that don't reproduce. Sterility in animals is always associated with a departure from the diploid number. The few animals that show evidence of polyploidy utilize parthenogenesis to escape the hazard of anomalous gametes. Female salamander of this particular group having large erythrocytes and erythrocyte nuclei produced some triploid larvae with 42 chromosomes whereas those with small erythrocytes and erythrocyte nuclei produced diploid larvae with 28 chromosomes.

Field observations and laboratory studies indicated that distinct, persisting populations of triploid females had become established in parts of the range occupied by this species complex. Although animals composed entirely of polyploidy cells are rare, many diploid animals have polyploidy cells within certain tissues of their bodies. In teleostean embryos, for example, giant nuclei, have been observed in many species. Giant polyploid nuclei occur in particular tissue of wide range of diploid animals, for example, liver and kidney in man.

Somatic and germ cell doubling as a cause of polyploidy in plants

Two basic irregular processes discovered by which polyploids may evolve from diploid plants and establish in nature

- **Somatic doubling:** Cells sometimes undergo irregularities at mitosis and give rise to meristematic cells that perpetuate these irregularities in new generation of plants.
- Reproductive cells may have an irregular reduction or equational division in which sets of chromosomes fail to separate completely to the poles at anaphase. Both sets thus become incorporated in the same restitution nucleus, which doubles the chromosome number in the gamete. Once polyploidy is established, intercrossing among plants with different chromosome numbers may give rise to numerous chromosome combinations that are under the influence of natural selection.

Two main kinds of polyploids – autopolyploid and allopolyploids are distinguished on the basis of their source of chromosomes. Autopolyploids occur when the same genome is duplicated. This occurs frequently in single cells of many plants but these cells don't survive. Allopolyploids result when different genomes come together through hybridization. Usually it is impossible to determine whether genomes are alike and whether the polyploids are autopolyploids or allopolyploids. Some plant groups have a series of chromosome numbers based on a multiple of a basic number. In genus *Chrysanthemum*, the basic number is 9 and the species are known that have 18, 36, 54, 72 and 90 chromosomes. Inviability and sterility seem to preclude the perpetuation of true autopolyploids in nature. Autopolyploid combined with allopolyploid produces autoallopolyploid and has been an important process in the evolution of some plants. Triploids (3n) with three genomes can occur when unreduced (2n) and normal (n) gametes unite. Reduction is missed in many diploid plants resulting in gametes with more than single genome. Triploids (3n) with three genomes can occur when unreduced (2n) and normal gametes unite (n). Reduction is missed in many diploid plants, resulting in gametes with more than single genome. Triploids don't become established because of irregularity during meiosis which results in sterility and low survival. Plants that can be vegetatively reproduced, may be preserved as triploids. Triploids occurring in grasses, vegetables and flower gardens varieties are less stable and less fertile than their corresponding diploids.

### **Polyploidy and Plant evolution**

Some seeds of the American saltmarsh grass were transported by ship to Bayonne, France and England. The American species became established in the same localities where a European saltmarsh grass was growing. A new saltmarsh grass commonly called Townsend's grass was later identified in these localities. Townsend's grass was more vigorous and aggressive than either American or the European species. It was introduced into Holland to support the likes and imported into other localities for similar purposes. The European grass had  $2n=60$ , American species  $2n=62$  and Townsends grass  $2n=122$ . Townsends grass was an amphidiploids with the sum of the diploid chromosomes carried by the two species. This indicated that the new plant arose from natural hybridization and doubling of

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chromosomes. The chromosome doubling had given the hybrid its fertility and ability to survive.

## NOTES

### Check Your Progress

1. Why is polyploidy uncommon in animals?
2. Why is sexual reproduction problematic for polyploids?
3. How many chromosomes did the diploid hybrid have?
4. What is the cause of polyploidy in humans?

## 10.4 TYPES OF POLYPLOIDY

Polyploidy or increase in chromosome sets is of three types, autopolyploidy, allopolyploidy and autoallopolyploidy, (Refer Figure 10.2). These are described below:

**Autopolyploidy:** It is a type of polyploidy in which there is a numerical increase of the same genome, for example, AAA,AAAA. It is also termed as intraspecific polyploidy. Muntzing (1936) thought autopolyploidy to be more prevalent than other forms. He listed 58 natural autopolyploid plants, for example, rice, gram, maize, guava

Autopolyploidy increase the gene dosage. Depending upon the gene complement present in the stock, it may be harmful or useful to the plants. It manifests gigas effect where each cell tissue or organ shows increase in size. There is increased vitamin, alkaloid and sugar content. The plants are better fitted to adapt to new environment and have wide range of distribution. It is observed that beyond tetraploidy, a number of abnormalities begin to appear. The plants become weak and dwarf with wrinkled foliage. Autopolyploidy is of commercial importance due to its gigas effect and production of seedless fruits. It is however of little importance in evolution of new species because mutations have lesser chances of finding expression, low fertility and chromosomal irregularities during meiosis. It is of two types, triploidy and tetraploidy. Pentaploidy and hexaploidy are also known.

### Autopolyploidy

- Polyploidy arising WITHIN a species
- Results from a mutation in chromosome number

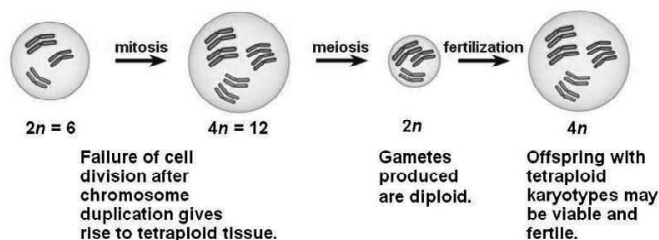


Fig. 10.2 Mechanisms of Polyploidy



**Allopolyploidy:** It is the phenomenon of having a multiple of genomes which are dissimilar from each other due to their being derived from different species. They are also called as interspecific polyploidy. Allopolyploids have characters of both the parents along with some specific characters of their own, (Refer Figure 10.3). The most common is allotetraploids where two chromosome sets are derived from one species and other sets from another species. They are also known as amphidiploids.

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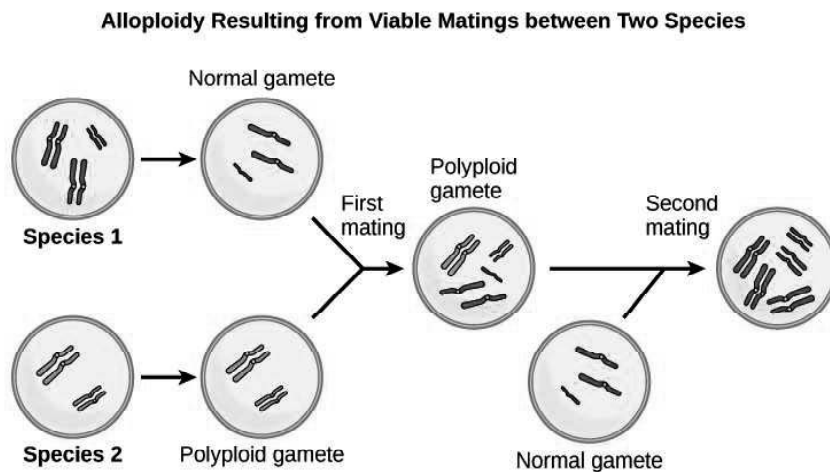


Fig. 10.3 Diagrammatic Representation of an Allopolyploidy

**Autoallopolyploidy:** It is a type of allopolyploid in which at least one genome is represented in more than the diploid state. It arises by three methods: Doubling of chromosomes in an amphidiploids or allotetraploid, hybridization between two different autotetraploids followed by doubling of chromosomes, fusion between a diploid gamete and a haploid gamete of two different species alongwith doubling of chromosomes. Autoallopolyploidy is known in *Helianthus tuberosus* ( $6n=102$ ). Autoallopolyploid can be recognized from chromosomal association in the haploids. They are both bivalents and univalents.

## 10.5 CHARACTERISTIC OF POLYPLOIDY

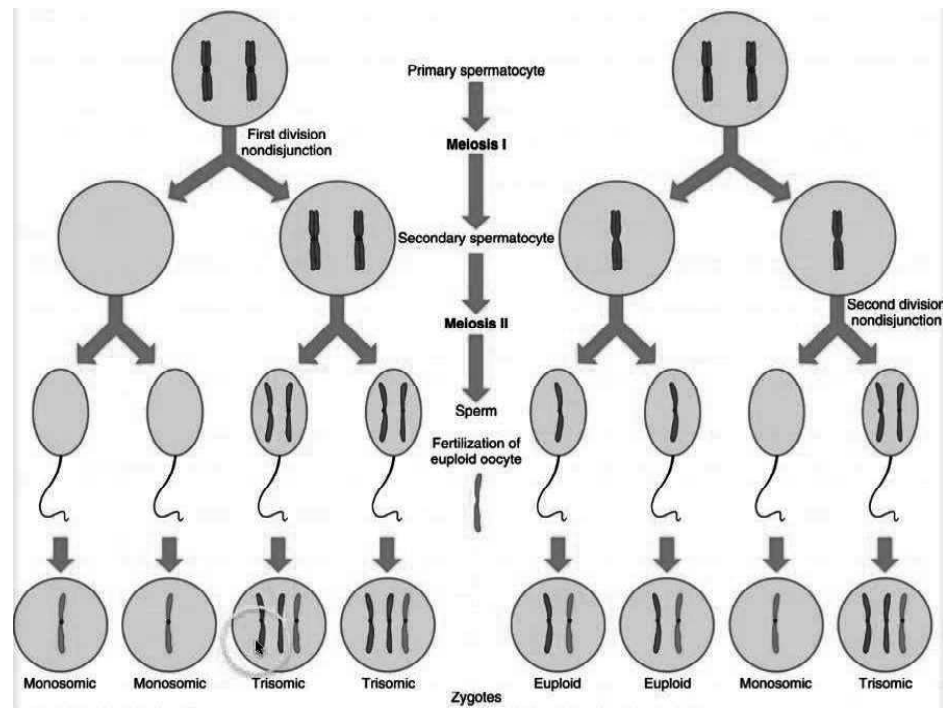
- There is increase in cell size, stomata and guard cells are larger in size, flowers and pollen grains are large sized, nuclei are larger.
- Stem is thicker and stouter, leaves are thick and broad, dark green, fruits become seedless.
- Triploid sugarbeet possess more sugar than the diploid.
- Nicotine content of tetraploid tobacco has been found to be double than that of diploid plant.
- There is increase in salt content in polyploids and decreased production of some enzymes like catalase, diastase.

**NOTES**

- Polyploids possess higher amount of gene dosage, one or more alleles in excess than the normal gene complement.
- Annual herbs have tendency to change to perennial herbs in polyploidy state.
- Polyploid show higher degree of aberration and other types of chromosomal irregularities as compared to diploids.

**Importance of Polyploidy in Plant Breeding**

**Aneuploidy:** The condition of having few or extra chromosomes than the exact multiple of the genome or whole chromosome set, gain or loss of individual chromosomes from normal chromosome complement of an individual is called aneuploidy. It is a variation in chromosome number which does not involve a whole set of chromosomes but only a part of set. It is of two types hypoploidy or loss of chromosomes and hyperploidy or addition of chromosomes. The organisms showing aneuploids may be monosomic, nullisomic, trisomic, tetrasomic, pentasomics, (Refer Figure 10.4). They are mainly originated by various methods:



*Fig. 10.4 Diagrammatic Representation of Aneuploidy*

**Non disjunction:** It brings about abnormal distribution of chromosomes. Some gametes will come to have an extra chromosome  $n+1$  while other would become deficient of chromosome  $n-1$ .

**Polyploidy:** They are genetically balanced. They show number of irregularities during synapsis and disjunction. The abnormalities result in gain or loss of chromosomes in the gametes.

**Translocation Heterozygotes:** Chromosomes with translocation often form ring like arrangements where adjacent chromosomes move to the same pole giving rise to  $n+1$  and  $n-1$  gametes

**Mitotic Non-separation:** Aneuploidy arise due to abnormality during separation of chromosomes in mitosis. Aneuploid patches may be morphologically distinct from the cells having normal chromosome number. Individuals with such cell patches are known as mosaics.

## NOTES

### Check Your Progress

5. What are the types of polyploidy?
6. What is genomatic mutation?
7. When does autopolyploid occur?
8. What does polyploid show?

## 10.6 ANSWERS TO CHECK YOUR PROGRESS

1. Polyploidy is uncommon in animals because of the delicatated sex balance mechanism.
2. Sexual reproduction is problematic for polyploids because of difficulty in pairing of homologues.
3. The diploid hybrid had 18 chromosomes, 9 from each parent.
4. Polyploidy in humans is due to two causes: unreduced gametes and dispermy.
5. Polyploidy or increase in chromosome sets is of three types autopolyploidy, allopolyploidy and autoallopolyploidy.
6. The phenomenon of having more than two sets of chromosomes or genomes is defined as genomatic mutation.
7. Autopolyploid occurs when an individual has more than two sets of chromosomes, both of which comes from same parental species while allopolyploid occurs when the individual has more than two copies but each copy comes from different species
8. Polyploid show higher degree of aberration and other types of chromosomal irregularities as compared to diploids.

## 10.7 SUMMARY

- Polyploidy was first noted by Lutz (1907) when he discovered that the plant *Oenothera lamarckiana* was actually tetraploid.
- Polyploidy has played a significant role in the evolution of higher plants (Leitch and Bennett 1997), having helped in tremendously increasing the number of species and sometimes of genera on the planet Earth.

## NOTES

- Polyploidy denotes a condition where more than two complete sets of chromosomes are present.
- Euploid plants have a multiple of a basic chromosome number for the species
- Aneuploids deviate from a base number by one, two or few chromosomes ( $2n+1$ ,  $2n-1$ ).
- Polyploidy is induced in plants to increase in alkaloid content, size of plant in terms of morphology and for various other genetic defects.
- In plants, polyploidy has been an important factor in evolution, induced polyploidy has a practical value in plants like grapes, apple and tobacco.
- Variations in the number of haploid sets are common in many species. Plants such as mosses spend most of their life cycle in a haploid phase and for all sexually reproducing organism, the gametic phase of life cycle is haploid.
- Endosperm of plant seeds is triploid and human liver cells exhibit many degrees of ploidy- triploid, tetraploid and octaploid.
- Polyploidy is uncommon in animals because of the delicated sex balance mechanism.
- Many common weeds such as dandelion, crabgrass and wild oats are polyploid. Polyploidy originates in two ways.
- Union of two diploid gametes produces a tetraploid zygote.
- . Food production worldwide has more than kept pace with increasing human population, because of development of new strains of grains like rice, wheat and corn many of which are polyploid.
- Sexual reproduction is problematic for polyploids because of difficulty in pairing of homologues.
- The diploid hybrid had 18 chromosomes, 9 from each parent but it was sterile and could not perpetuate itself because of the failure in pairing between unlike chromosomes in meiosis.
- Polyploidy play role in genetic, evolutionary and systematic studies. The patterns of multiple origin and development of polyploidy in plants have been of huge interest.
- Polyploids in their course of evolution have been shown to become diploids both in cytogenetic and genetic make up.
- 2-4 % of speciation events in angiosperms is due to speciation. With new sequencing methods, polyploidy is found to be involved in speciation of flowering plants and eukaryotes.
- The recurrence of polyploidization is evident. It counterbalance the local extinction of autopolyploid species.

- Gene expression pattern in duplication have shown that polyploidy respond in manner similar to environmental stimuli.
- A number of chemicals are known to cause polyploidy. E.g chloral hydrate, ethyl mercuric chloride, sulphanilamide, indole acetic acid, coumarin, benomyl, colchicine.
- Polyploidy in humans is due to two causes: unreduced gametes and dispermy. The fertilization of an unreduced diploid egg by a haploid sperm give rise to triploid zygote while union of two diploid gametes produce tetraploid.
- Polyploidy is the change in the basic number of chromosome set in any individual species.
- The condition of having one or more extra chromosome sets or genomes of the original basic number of a species is known as primary polyploidy.
- Organisms with three or more genomes are polyploids. Fully one half of all known plant genera contain polyploids and about two third of all the grasses are polyploids but it is rarely seen in animals.
- Field observations and laboratory studies indicated that distinct, persisting populations of triploid females had become established in parts of the range occupied by this species complex.
- The condition of having few or extra chromosomes than the exact multiple of the genome or whole chromosome set, gain or loss of individual chromosomes from normal chromosome complement of an individual is called aneuploidy.

## NOTES

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### 10.8 KEY WORDS

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- **Polyploidy:** It is a condition where organism have more than two paired sets of chromosomes.
- **Euploid:** It is a complete set of chromosomes.
- **Haploid:** It refers to half of the total chromosome set in a cell.
- **Tetraploid:** It is the cell containing four homologous sets of chromosomes.
- **Aneuploid:** It is the presence of abnormal number of chromosomes in a cell.
- **Autopolyploid:** It occurs when individual has more than two sets of chromosomes, all derived from the same species.
- **Allopolyploid:** It occurs when chromosomes are composed of more than two genomes each of which has been derived but modified from one of two or more species.

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## 10.9 SELF ASSESSMENT QUESTIONS AND EXERCISES

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### NOTES

#### Short Answer Questions

1. What is the importance of polyploid?
2. Give a brief introduction of polyploidy.
3. Write a short note on diploidization.
4. What do you understand by primary polyploidy?
5. How is secondary polyploidy different from primary polyploidy?
6. What is polyploidy in animals?
7. Differentiate between allopolyploidy and autoallopolyploidy.

#### Long Answer Questions

1. What is autopolyploidy? Give an example of autopolyploid and explain it in detail.
2. Write a detailed note on spontaneous and induced polyploidy.
3. From your learning of the text state the difference in gene composition.
4. What is spontaneous or natural polyploidy? Discuss.
5. How did polyploidy originate? Explain.
6. Write in detail about polyploidy and plant evolution.

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## 10.10 FURTHER READINGS

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# UNIT 11 BASIC ACCOUNT ON MUTATION AND POPULATION GENETICS

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## NOTES

### Structure

- 11.0 Introduction
- 11.1 Objectives
- 11.2 Basic Account on Mutation
- 11.3 Practical Applications of Mutations
- 11.4 Gene Mutations and its Types
- 11.5 Basic Account of Population Genetics (Hardy-Weinsberg Law)
- 11.6 Calculating Genotypic Frequencies
- 11.7 Implications of the Hardy-Weinberg Law
- 11.8 Extensions of the Hardy-Weinberg Law
- 11.9 Answers to Check Your Progress Question
- 11.10 Summary
- 11.11 Key Words
- 11.12 Self Assessment Questions and Exercises
- 11.13 Further Readings

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## 11.0 INTRODUCTION

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At the simplest level, a *mutation* is a change or transformation. In biology, mutations refer to changes in chromosomes and genes, which typically manifest physically. The effect of a mutation can depend on the region in which the sequence of genetic material has been changed. The simplest and the most harmless are substitutions of a single base pair with another, with no effect on protein sequence. At the other end are insertion or deletion mutations that lead to non-functional gene products. Mutations can also occur on a large scale, with long stretches of DNA (or RNA when it is the genetic material) being inverted, inserted, duplicated, deleted, transposed or translocated.

The result of a mutation could be harmful, beneficial, neutral or even silent. Mutation can lead to the loss or gain of a specific function, to changes to the expression levels, or in extreme cases, even embryonic lethality. Mutations can be classified in various ways depending on the cause of the mutation, its effect on the function of the gene product or the kind of changes to the structure of the gene itself.

In this unit, you will learn about mutation, its types, and population genetics in detail.

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## 11.1 OBJECTIVES

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### NOTES

After going through this unit, you will be able to:

- Discuss the basic account on mutation
- Explain radiation induced mutations
- Analyze practical applications of mutations
- Describe gene mutations and its types
- Calculate allelic frequencies
- Discuss the implications of the Hardy-Weinberg Law

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## 11.2 BASIC ACCOUNT ON MUTATION

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The term mutation refers both to the change in the genetic material and to the process by which the change occurs. An organism exhibiting a novel phenotype as a result of the presence of mutation is referred to as mutant. Mutation refers to any sudden, heritable change in the genotype of an organism not explainable by recombination of preexisting genetic variability. Such genotypic changes includes change in chromosome number (euploidy and aneuploidy), gross changes in the structure of chromosomes (chromosomes aberration) and changes in individual genes. The term mutation refers to changes in individual genes.

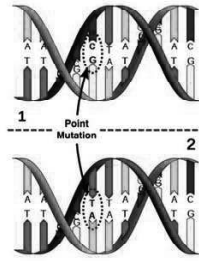
Many mutations involve changes in single base pairs, the substitution of one basepair for another, or duplication or deletion of single base pairs, such mutation is referred as point mutation.

Mutation is the ultimate source of all genetic variation, it provides the raw material for evolution,(Refer Figure 11.1). Recombination (independent assortment plus recombination of genetic variability present in individual chromosomes) rearranges this genetic variability into new combinations and natural selection preserves the combinations adapted to existing environmental conditions. Without mutation, all genes would exist on only one form. Alleles would not exist and genetic analysis would not be possible. Organisms would not be able to evolve and adapt to environmental changes.



## Mutations

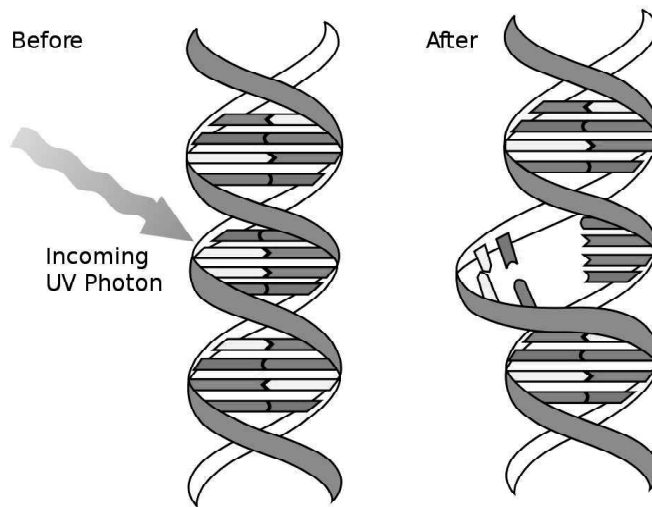
- Any unpredictable change in the structure or amount of DNA of an organism is called a **mutation**.
- Most mutations occur in **somatic (body) cells** and are not passed from one generation to the next.
- Only those mutations which occur in the formation of **gametes** can be inherited.



## NOTES

*Fig. 11.1 Diagrammatic Representation of Mutations*

Mutations are inherited alterations in the DNA sequence. DNA is a highly stable molecule that is replicated with amazing accuracy but changes in DNA structure and errors of replication do occur, (Refer Figure 11.2). A mutation is defined as an inherited change in genetic information, the descendants may be cells or organisms.



*Fig. 11.2 DNA Incoming UV Structure Before and After Incoming UV Photon*

The appearance of a new mutation is a rare event. Most mutations that were originally studied occurred spontaneously. This class of mutation is termed spontaneous mutations. Historically, geneticists recognized these in nature. The mutations were collected, and the inheritance of these mutations were analyzed. But these mutations clearly represent only a small number of all possible mutations. To genetically dissect a biological system further, new mutations were created by scientists by treating an organism with a mutagenizing agent. These mutations are called induced mutations.

## NOTES

Mutagens are chemical compounds or forms of radiation (such as ultraviolet (UV) light or X-rays) that cause irreversible and heritable changes (mutations) in the cellular genetic material, deoxyribonucleic acid (DNA). In this way, mutagenesis becomes a cumulative process, stretching over the lifetime of an organism.

The biological consequences of a mutation depend upon many critical factors such as the target loci, size of the mutation, timing during the cell cycle, and compounding effects of preexisting mutations. Mutagenic lesions persist when they escape detection by protective cellular DNA repair mechanisms, when mistakes occur in the repair process, or when repair mechanisms are overwhelmed by extensive damage. Upon subsequent cellular replication, these mutations become fixed in the genome and are inherited by all daughter cells. Thus, a mutagenic event occurring in a nonfunctional area of DNA will have no effect (*silent mutation*), whereas a similar change in an actively transcribed region may profoundly affect gene expression and phenotype or even lead to cell death (*lethal mutation*).

There are many chemical mutagens which can react with nucleus and the DNA, e.g., base analogues which are derivatives of normal bases. This can cause direct mutagenesis. Similarly alkylating agents like methylmethanesulphonate, ethylmethanesulphonate and ethylnitrosourea are capable of producing DNA lesions which are repaired to prevent any disruption to process of transcription and replication. Nitrous acid deaminates cytosine to produce uracil which can base pair with adenine creating transitions. Similarly, 5 bromouracil can lead to mutations. Transition is where a pyrimidine changes to another pyrimidine or purine to another purine while in transversion change involves purine to pyrimidine or pyrimidine to purine.

The spontaneous mutation rate varies. Large gene provide a large target and tend to mutate more frequently. A study of the five coat color loci in mice showed that the rate of mutation ranged from  $2 \times 10^{-6}$  to  $40 \times 10^{-6}$  mutations per gamete per gene. Data from several studies on eukaryotic organisms shows that in general the spontaneous mutation rate is  $2-12 \times 10^{-6}$  mutations per gamete per gene. Given that the human genome contains 100,000 genes, we can conclude that we would predict that 1-5 human gametes would contain a mutation in some gene. Spontaneous mutations occur infrequently although the observed frequencies vary from gene to gene and from organism to organism.

### Phenotypic Effects of Mutation

Mutations cause some detectable phenotypic change for their presence to be recognized. The effects of mutations on phenotype range from alterations so minor that they can be detected only by special genetic or biochemical techniques to gross modifications of morphology. A gene is a specific sequence of nucleotide pairs coding for a particular polypeptide. Any mutation occurring within a given gene will produce a new form. Because of the degeneracy of the genetic code, some base pair changes don't change the protein products encoded by the genes in any way. Genes containing mutations with small effects can be recognized by

special techniques called isoalleles. Mutations may be either recessive or dominant. In haploid organisms like viruses or bacteria, both recessive and dominant mutations can be recognized by their effects on the phenotype of the organism in which they originated. In diploid organisms, recessive mutation will be recognized only when present in the homozygous condition. Most recessive mutations in diploids will not be recognized at the time of their occurrence, since they are present in heterozygous state. Sex linked recessive mutations are an exception, since they are expressed in hemizygous state in the heterogametic sex. The most useful mutations for genetic analyses of many biological processes are conditional lethal mutations.

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### **Somatic and Germinal Mutations**

Mutation can occur in any cell and at any state in the cell cycle. The immediate effect of mutation and its ability to produce phenotypic change are determined by its dominance, the type of cell in which it occurs and when it happens relative to life cycle of the organism.

If the mutation occurs in a somatic cell, which can produce cell like itself but not the whole organism, the mutant change will be perpetuated only in somatic cells that descend from original cell in which mutation occurred. The *Delicious apple and the navel orange* were mosaics in somatic tissues. Changes that give these two fruits their desirable qualities followed spontaneous mutation in single cells. If dominant mutation occur in germ cells, their effects may be expressed immediately in progeny. If mutations are recessive, their effects are obscured in diploids. Germinal mutations may occur at any stage in the reproductive cycle of the organism, but they are common during some stages than others.

### **Back Mutation and Suppressor Mutation**

The mutation of a wild type gene to form that results in a mutant phenotype is referred as forward mutation. In a population composed entirely of brown eyed individual, the allele for blue eyes is thought as mutant allele. Mutation events are reversible. Mutation may occur that restores the original wild type phenotype. This is referred to as back mutation, reverse mutation or reversion. Reversion may occur by true back mutation at the same site in the gene as the original mutation, restoring the wild type nucleotide sequence or by the occurrence of a second mutation at a different location in the genome, which compensates for first mutation, these mutations are called suppressor mutations. It may occur at distinct sites in the same gene as the original mutation or in different genes, even in different chromosomes.

### **Radiation Induced Mutations**

That portion of the electromagnetic spectrum containing wavelengths that are shorter and of higher energy than the visible light can be divided into ionizing radiation(X rays, gamma rays , cosmic rays) and non-ionizing radiations (UV light). Ionizing radiations are of high energy and are useful for medical diagnosis because

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they penetrate the living tissue. UV rays having lower energy penetrate only the surface layer of cells in higher plants and animals and don't induce ionizations. UV rays dissipate their energy to atoms that they encounter raising the electrons in the outer orbitals to higher energy levels referred as excitation. Molecules containing atoms in their ionic or excited forms are chemically more reactive than those containing atoms in their normal stable state. The increasing reactivity of atoms present in DNA molecules is the basis of the mutagenic effects of UV light and ionizing radiations. UV radiations are readily absorbed by substances like purines and pyrimidines which enter a more reactive or excited state. The two major products of UV absorption by pyrimidines appear to be pyrimidine hydrates and pyrimidine dimers.

### Chemically Induced Mutations

The first chemical mutagen discovered was mustard gas. When C. Auerbach and her associates discovered the mutagenic effects of mustard gas and related compounds during world war II. These compounds are examples of large class of chemical mutagens that transfer alkyl group to the bases in DNA, called as alkylating agents.

Chemical mutagens are divided into two classes: those that are mutagenic to both replicating and non-replicating DNA such as alkylating agent and nitrous acid., those that are mutagenic only to replicating DNA, which mainly includes acridine dyes, which bind to DNA and increase the probability of mistakes during replication and base analogs, purines and pyrimidines with structure similar to normal bases of DNA. The base analogs must be incorporated into DNA chains in place of normal bases during replication to exert their mutagenic effects.

**Base Analogs:** The base analogs that are mutagenic have structures similar to the normal bases so that they are incorporated into DNA during replication. The two most commonly used base analogs are 5 bromo uracil and 2 amino purine.

**Nitrous Acid:** It is a very potent mutagen that acts directly on either replicating or non-replicating DNA by oxidative deamination of the bases that contain amino groups- adenine, guanine and cytosine. Conversion of the amino groups to keto groups changes the hydrogen bonding potential of the bases.

**Acridines:** The positively charged acridine intercalate between the stacked base pairs in DNA. They increase the rigidity and alter the conformation of the double helix, causing kinks in the molecule. When DNA molecules containing intercalated acridines replicate, addition and deletions of one or few base pairs occur. These small additions and deletions of single base pair result in reading frame shifts for the portion of the gene distal to the mutation

**Alkylating and Hydroxylating Agents:** Alkylating agents such as Nitrogen and sulphur mustards, methyl and ethyl methanesulfonate have several effects on DNA. Nitrosoguanidine, one of the most potent chemical mutagens has been found to induce clusters of closely linked mutations in the segment of the chromosome that

is replicating during mutagenic treatment. The hydroxylamine has a very specific mutagenic effects. It induces only GC- AT transitions.

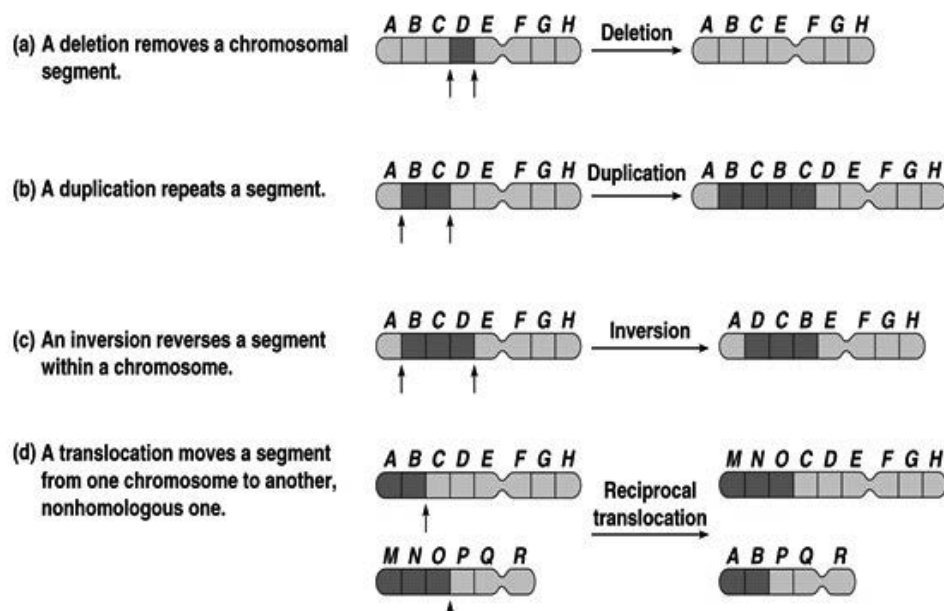


Fig. 11.3 Chromosome Segments

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### 11.3 PRACTICAL APPLICATIONS OF MUTATIONS

Mutations are invaluable to the process of evolution since they provide the raw material required for its occurrence. Mutation provide the alleles required for various types of genetic analysis, from Mendel’s two factor crosses to chromosome mapping to studies of genetic structures of populations. Even though most mutations make the organism less efficient and disadvantageous, the possibility of developing new desirable traits through induced mutations has intrigued many plant breeders. Plant breeders have reported induced mutants in barley, wheat, oat, soyabeans, tomatoes, fruit trees that improve cultivated strains. Barley mutants have been obtained that provide increased protein content and hull less seeds. One application of induced mutations came from efforts to improve the yield of penicillin by the mold penicillium. When penicillin was first discovered, yield was low and production was limited. Then millions of spores were irradiated and few of the surviving colonies produced more penicillin than the average. Such mutant overproducers of penicillin have proven invaluable in the commercial production of this important antibiotic.

#### Mutations and Humans

Purposeful artificial selection is not practiced in humans and therefore the possible advantages cited for domestic animals and plants do not apply to humankind. Variations do exist in populations, however and presumably they originated through past mutations. Since mutations are detrimental, it would seem advantageous from

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the stand point of short term effects for humans to avoid excessive exposure to mutagenic agents. In case of acute irradiation, two types of dangers are considered, i.e., the immediate damage to the exposed person, more damage to the DNA in his or her reproductive cells, which would affect future generations. The immediate damage is indicated by burns and other direct or secondary effects on body tissues. When doses are on the order of 50 mr or lower, no immediate damage can be detected, although some harmful effects such as induction of leukemia and shortening of life span may occur. Effects of the second type of damage will be observed in only future generations. This is a reason to believe that exposure to high energy radiations at any dosage level is potentially harmful. In the few results to date, mutations have been proportional to the dosage and the effects have been cumulative.

The relation between dosage and effects cannot be accurately measured in humans at present because of the complexity of the subject and difficulties of dealing with the genetics of humans. Preliminary reports including data on children born to parents who survived the Nagasaki bombing have revealed a significant increase in incidence of leukemia. The normal sex ratio has been altered, and changes have been interpreted as resulting from induced sex linked lethals. Most of the data available on mutation rates and the nature of mutations have come from other organisms and only by inference are they applied to humans. However the genetic material in humans is DNA, just as it is in most other organisms. It should not be surprising that the effects of irradiation are comparable to most organisms.

Mutations can be induced by several methods. The three general approaches used to generate mutations are radiation, chemical and transposon insertion. The first induced mutations were created by treating *Drosophila* with X-rays. Using this approach Mueller to induce lethal mutations. In addition to X-rays, other types of radiation treatments that have proven useful include gamma rays and fast neutron bombardment. These treatments can induce point mutations (changes in a single nucleotide) or deletions (loss of a chromosomal segment).

Chemical mutagens work mostly by inducing point mutations. Point mutations occur when a single base pair of a gene is changed. These changes are classified as transitions or transversions. Transitions occur when a purine is converted to a purine (A to G or G to A) or a pyrimide is converted to a pyrimidine (T to C or C to T). A transversion results when a purine is converted to a pyrimidine or a pyrimidine is converted to a purine. A transversion example is adenine being converted to a cytosine. You can determine other examples.

Two major classes of chemical mutagens are routinely used. These are alkylating agents and base analogs. Each has a specific effect on DNA. Alkylating agents (such as Ethyl Methane Sulphonate (EMS), Ethyl Ethane Sulphonate (EES) and mustard gas) can mutate both replicating and non-replicating DNA. By contrast, a base analog (5-bromouracil and 2-aminopurine) only mutate

DNA when the analog is incorporated into replicating DNA. Each class of chemical mutagen has specific effects that can lead to transitions, transversions or deletions.

Scientists are now using the power of transposable elements to create new mutations. Transposable elements are mobile pieces of DNA that can move from one location in a genome to another. Often when they move to a new location, the result is a new mutant. The mutant arises because the presence of a piece of DNA in a wild type gene disrupts the normal function of that gene. As more and more is being learned about genes and genomes, it is becoming apparent that transposable elements are a power source of or creating insertional mutants.

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### 11.4 GENE MUTATIONS AND ITS TYPES

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Germ line mutations arise in cells that ultimately produce gametes. A germ line mutation can be passed to future generations producing individual organisms that carry the mutation in all their somatic and germ line cells. Mutations have been partitioned into those that affect a single gene, called gene mutations and those that affect the number or structure of chromosomes called chromosome mutations.

**Base Substitutions:** The simplest type of gene mutation is a base substitution, the alteration of a single nucleotide in the DNA. Base substitution are of two types. In transition, a purine is replaced by different purine or pyrimidine is replaced by a different pyrimidine. In transversion, a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine.

**Insertions and Deletions:** It mainly includes the addition or the removal of one or more nucleotide pairs. Insertions and deletions within sequences that encode proteins may lead to frameshift mutations, changes in the reading frame of the gene. It alter all amino acids encoded by nucleotides following mutation and have drastic effects on the phenotype.

**Expanding Nucleotide Repeats:** Mutations in which the number of copies of a set of nucleotides increases in number are called expanding nucleotide repeats. This type of mutation was observed in 1991 in a gene called FMR-1 which causes fragile X syndrome, the most common cause heredity cause of mental retardation. Expanding nucleotide repeats are found in almost 30 human diseases. The number of copies of the nucleotide repeat correlates with the severity or age of onset of disease.

The detailed knowledge of the structure and function of transposable elements is now being applied in the pursuit of new mutations. Stocks are created in which a specific type of element is present. This stock is then crossed to a genetic stock that does not contain the element. Once that element enters the virgin stock, it can begin to move around that genome. By carefully observing the offspring, new mutants can be discovered. This approach to developing mutants is termed insertional mutagenesis.

## NOTES

### Check Your Progress

1. What are the chances of appearance of a new mutation?
2. What is a gene?
3. What is forward mutation?
4. Why can the relation between dosage and effects be not measured accurately?

## 11.5 BASIC ACCOUNT OF POPULATION GENETICS (HARDY-WEINSBERG LAW)

The big horn sheep at the National Bison Range illustrate an important principle of genetics, i.e., small populations lose genetic variation with passage of time through genetic drift often with catastrophic results for survival and reproduction. The introduction of new genetic variation into an inbred population is called genetic rescue which improves the health of population and can ensure its long term survival. These effects have important implications for wildlife management along with it for organisms evolve in the natural world

Humans are not unique in their extensive variability, almost all organisms exhibit variation in phenotype. Much of the phenotypic variation is hereditary. Genetic variation is the basis of all evolution and the extent of genetic variation within population affects its potential to adapt to environment change. Much variation exists at the molecular level owing in part to the redundancy of the genetic code, which allows different codons to specify the same amino acid. Two members of a population can produce the same protein even if their DNA sequences are different. DNA sequences between the genes and introns within genes don't encode proteins much of the variation in these sequences has little effect on the phenotype.

An important tool in population genetics is the mathematical model. A mathematical model describes a process as an equation. The equation defines the way in which the variables influence the process. At first model might consider only one factor but after their effects are understood, the model can be improved by addition of more details. Before we explore the evolutionary processes that shape genetic variation, we must be able to describe the genetic structure of a population. The way of describing this structure is to enumerate the types and frequencies of genotypes and alleles in a population.

## 11.6 CALCULATING GENOTYPIC FREQUENCIES

A frequency is a proportion or percentage usually expressed as a decimal fraction. For example, if 20% of the alleles at a particular locus in a population are A, we would say that the frequency of the A allele is 0.20. For large populations, for



which determination of the genes of all individual members is impractical, a sample of population is taken and genotypic and allelic frequencies are calculated for this sample. The genotypic and allelic frequencies of the sample are used to represent the gene pool of the population.

To calculate a genotypic frequency, we add up the number of individuals possessing the genotype and divide by the total number of individuals in the sample (N). For a locus with three genotypes, AA, Aa and aa, the frequency of each genotype is

$$f(AA) = \frac{\text{No. of AA individuals}}{N}$$

$$f(Aa) = \frac{\text{No. of Aa individuals}}{N}$$

$$f(aa) = \frac{\text{No. of aa individuals}}{N}$$

The sum of all the genotypic frequencies always equal to 1.

### Calculating Allelic Frequencies

The gene pool of a population is described in terms of allelic frequencies. There are always fewer alleles than the genotypes, so the genepool of a population can be described in terms when allelic frequencies are used. In a sexually reproducing population, the genotypes are temporary assemblages of the alleles. The genotypes break down each generation when individual alleles are passed to the next generation through the gametes and so the types and number of alleles, rather than genotypes from one generation to the next and make up the gene pool of a population.

Allelic frequencies can be calculated from the numbers or the frequencies of the genotypes. To calculate the allelic frequency from the numbers of genotypes, we count the number of copies of a particular allele present in a sample and divide by the total number of all alleles in the sample

$$\text{Frequency of an allele} = \frac{\text{No. of copies of the allele}}{\text{No. of copies of all alleles at the locus}}$$

The Hardy – Weinberg law which describes the effect of reproduction on genotypic and allelic frequencies

- The main goal of population genetics is to understand the processes that shape a population gene pool. We must know what effects reproduction and Mendelian principles have on the genotypic and allelic frequencies, how do segregation of alleles in gamete formation and the combining of alleles in fertilization influence the gene pool. Answer lies in the Hardy – Weinberg law which is the most important principle of population genetics.

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- The Hardy-Weinberg law was formulated independently by both **GH Hardy and Wilhelm Weinberg** in 1908. The law is a mathematical model that evaluates the effect of reproduction on the genotypic and allelic frequencies of a population. It makes several simplifying assumptions about the population and provides two key predictions of these assumptions are met.
- If a population is large, randomly mating, and not affected by mutation, migration or natural selection then either the allelic frequencies of a population don't change or the genotypic frequencies stabilize after one generation in the proportions  $p^2$  (frequency of AA),  $2pq$  (frequency of Aa) and  $q^2$  (frequency of aa), where  $p$  equals the frequency of allele A and  $q$  equals the frequency of allele a.
- The Hardy-Weinberg law indicates that when assumptions are met, reproduction alone does not alter allelic or genotypic frequencies and the allelic frequencies determine the frequencies of genotypes.
- The statement that genotypic frequencies stabilize after one generation means that they may change in the first generation after random mating, because one generation of random mating is required to produce Hardy Weinberg proportions of genotypes. The genotypic frequencies don't change as long as the population continues to meet the assumptions of Hardy Weinberg law. When genotypes are in the expected proportions of  $p^2$ ,  $2pq$  and  $q^2$ , population is said to be in Hardy Weinberg equilibrium.
- The science of population genetics is based on this principle, which may be stated as follows: in a large, random-mating population, the proportion of dominant and recessive genes present tends to remain constant from generation to generation unless outside forces act to change it. In such a way even the rarest forms of genes, which one would assume would disappear, are preserved. The outside forces that can disrupt this natural equilibrium are selection, mutation, and migration. The discovery of this law was especially significant in affirming natural selection as the primary mechanism of evolution. If the proportions of gene forms in a population do not change, the rate of evolution will be zero. Individual variations occur because of the various genetic combinations that result from random mating of individuals, but nonrandom, or selective, mating must occur for natural selection to take place.
- Certain gene-controlled traits are selected for or selected against by the partners involved. Over a long period of time, this selective pressure will change the frequency of appearance of certain gene forms, and the traits they control will become commoner or rarer in the population.

- Medical geneticists can use the Hardy-Weinberg law to calculate the probability of human matings that may result in defective offspring. The law is also useful in determining whether the number of harmful mutations in a population is increasing as a result of radiation from industrial processes, medical techniques, and fallout.

## NOTES

### Check Your Progress

5. Which is an important tool in population genetics?
6. What is a frequency?
7. What is called genetic rescue?

## 11.7 IMPLICATIONS OF THE HARDY-WEINBERG LAW

The implications of the Hardy-Weinberg law are listed below:

- The Hardy-Weinberg law has several important implications for the genetic structure of a population. One implication is that a population can't evolve if it meets the Hardy-Weinberg assumptions, because evolution consists of change in the allelic frequencies of a population. Therefore the Hardy-Weinberg law tells us that reproduction alone will not bring about evolution. Other processes such as natural selection, mutation, migration or chance are required for populations to evolve.
- A second important implication is that when a population is in Hardy-Weinberg equilibrium, the genotypic frequencies are determined by the allelic frequencies. When a population is not in Hardy-Weinberg equilibrium, we have no basis for predicting the genotypic frequencies. Although we determine the allelic frequencies from the genotypic frequencies, the reverse is possible only when the population is in Hardy-Weinberg equilibrium.
- For a locus with two alleles, the frequency of the heterozygotes is greatest when allelic frequencies are between 0.33 and 0.66 and is at maximum when allelic frequencies are each 0.5. The heterozygote frequency never exceeds 0.5 when population is in Hardy-Weinberg equilibrium. When frequency of one allele is low, homozygotes for that allele will be rare and most of the copies of rare allele will be present in heterozygotes.
- Third implication of Hardy-Weinberg law is that a single generation of random mating produces the equilibrium frequencies of  $p^2$ ,  $2pq$  and  $q^2$ . The genotypes are in Hardy-Weinberg proportion does not prove that the population is free from natural selection, mutation and migration. It means only that these forces have not acted since the last time random mating took place.

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## 11.8 EXTENSIONS OF THE HARDY-WEINBERG LAW

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The Hardy-Weinberg proportions can be applied to multiple alleles and X-linked genes. With multiple alleles, the genotypic frequencies expected at equilibrium are the square of the allelic frequencies. For an autosomal locus with three alleles, the equilibrium genotypic frequencies will  $(p+q+r)^2 = p^2 + 2pq + q^2 + 2pr + 2qr + r^2$ . For an X-linked locus with two alleles  $X^a$  and  $X^A$ . Males have only a single X-linked allele and so the frequencies of male genotypes are  $p$  and  $q$ .  $p^2$  is the expected proportion of females with genotype  $X^A X^A$ , if females make up 50% of the population, then the expected proportion of this genotype in the entire population is  $0.5 \times p^2$ . The frequency of an X-linked recessive trait among males is  $q$ , whereas the frequency among females is  $q^2$ . When an X-linked allele is uncommon, the trait will be much more frequent in males than in females.

### Check Your Progress

8. The science of population genetics is based on which principle?
9. What happens if the proportions of gene forms in a population do not change?

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## 11.9 ANSWERS TO CHECK YOUR PROGRESS QUESTION

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1. The appearance of a new mutation is a rare event.
2. A gene is a specific sequence of nucleotide pairs coding for a particular polypeptide.
3. The mutation of a wild type gene to form that results in a mutant phenotype is referred to as forward mutation.
4. The relation between dosage and effects cannot be accurately measured in humans at present because of the complexity of the subject and difficulties of dealing with the genetics of humans.
5. An important tool in population genetics is the mathematical model.
6. A frequency is a proportion or percentage usually expressed as a decimal fraction.
7. The introduction of new genetic variation into an inbred population is called genetic rescue.
8. The science of population genetics is based on this principle that in a large, random-mating population, the proportion of dominant and recessive genes present tends to remain constant from generation to generation unless outside forces act to change it.

9. If the proportions of gene forms in a population do not change, the rate of evolution will be zero.

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## 11.10 SUMMARY

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- Sudden, heritable changes in the genetic material are called mutations.
- It refers to the process by which such changes are produced.
- It may occur spontaneously or may be induced by agents that interact with DNA and RNA.
- At the simplest level, a *mutation* is a change or transformation. In biology, mutations refer to changes in chromosomes and genes, which typically manifest physically.
- The result of a mutation could be harmful, beneficial, neutral or even silent.
- Mutation can lead to the loss or gain of a specific function, to changes to the expression levels, or in extreme cases, even embryonic lethality.
- Mutations can be classified in various ways depending on the cause of the mutation, its effect on the function of the gene product or the kind of changes to the structure of the gene itself.
- A mutation is defined as an inherited change in genetic information, the descendants may be cells or organisms.
- The appearance of a new mutation is a rare event. Most mutations that were originally studied occurred spontaneously.
- Mutagens are chemical compounds or forms of radiation (such as ultraviolet (UV) light or X-rays) that cause irreversible and heritable changes (mutations) in the cellular genetic material, deoxyribonucleic acid (DNA).
- The biological consequences of a mutation depend upon many critical factors such as the target loci, size of the mutation, timing during the cell cycle, and compounding effects of preexisting mutations.
- Mutations cause some detectable phenotypic change for their presence to be recognized.
- Mutation occur in any cell and at any state in the cell cycle. The immediate effect of mutation and its ability to produce phenotypic change are determined by its dominance, the type of cell in which it occurs and when it happens relative to life cycle of the organism.
- That portion of the electromagnetic spectrum containing wavelengths that are shorter and of higher energy than the visible light can be divided into ionizing radiation (X rays, gamma rays, cosmic rays) and non-ionizing radiations (UV light).

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## NOTES

- The first chemical mutagen discovered was mustard gas. When C. auerbach and her associates discovered the mutagenic effects of mustard gas and related compounds during world war II.
- The positively charged acridine intercalate between the stacked base pairs in DNA. They increase the rigidity and alter the conformation of the double helix, causing kinks in the molecule.
- Mutations are invaluable to the process of evolution since they provide the raw material required for its occurrence.
- Mutation provide the alleles required for various types of genetic analysis, from Mendel's two factor crosses to chromosome mapping to studies of genetic structures of populations.
- The relation between dosage and effects cannot be accurately measured in humans at present because of the complexity of the subject and difficulties of dealing with the genetics of humans.
- Germ line mutations arise in cells that ultimately produce gametes. A germ line mutation can be passed to future generations producing individual organisms that carry the mutation in all their somatic and germ line cells.
- The detailed knowledge of the structure and function of transposable elements is now being applied in the pursuit of new mutations. Stocks are created in which a specific type of element is present.
- When a population is not in Hardy Weinberg equilibrium, we have no basis for predicting the genotypic frequencies.
- Various kinds of irradiation and many chemicals that react with DNA and RNA are very potent mutagenic agents
- New mutations provide the genetic variability that fuels evolution. Some level of mutation is required to provide the raw material for evolution however most mutations are detrimental. High frequencies of mutation would be disadvantageous to a species except in a rapidly changing environment.
- The potential benefits of the use of irradiation must be weighed against the known and estimated potential risks.
- Precautions must be taken to prevent the continued pollution of environment with mutagenic chemicals
- The Hardy Weinberg law indicates that when assumptions are met, reproduction alone doesnot alter allelic or genotypic frequencies and the allelic frequencies determine the frequencies of genotypes.

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### 11.11 KEY WORDS

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- **Mutation:** It is a permanent alteration of the nucleotide sequence of a genome.

- **Allelic frequency:** It is a relative frequency of allele at a particular locus in a population expressed as a fraction or percentage.
- **Hardy Weinberg law:** It states that allele and genotype frequency in a population will remain constant from generation to generation.
- **Ionizing radiation:** These are radiations that consist of X-rays, gamma rays with sufficient energy to cause ionization in a medium.
- **Mutagens:** These are either physical or chemical agents that causes genetic mutation.
- **Somatic mutations:** It is a change that appears in somatic cells and it shows mutant change.
- **Germinal mutations:** If mutations originate in a gamete, if dominant mutations occur in germ cells, effect is expressed immediately in the progeny.
- **Spontaneous mutations:** It is a kind of mutations that occurs without any cause.
- **Induced mutations:** It arise due to exposure of mutagenic agents such as ionizing radiations or various chemicals.

## NOTES

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### 11.12 SELF ASSESSMENT QUESTIONS AND EXERCISES

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#### Short Answer Questions

1. What are the phenotypic effects of mutation?
2. Write a note on somatic and germinal mutations.
3. Discuss back mutation and suppressor mutation.
4. What are chemically induced mutations?
5. Write a note on mutations and humans.
6. What is radiation induced mutations?
7. List a few practical applications of mutations.
8. Discuss gene mutations and its types.

#### Long Answer Questions

1. What do you mean by somatic and germinal mutations? Also discuss the effects of mutation.
2. From your learning of the text, differentiate between back mutation and suppressor mutation.
3. How are radiation induced mutations different from chemical induced mutation? Discuss.

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4. "Purposeful artificial selection is not practiced in humans and therefore the possible advantages cited for domestic animals and plants don't apply to humankind." Discuss.
5. What is the Hardy-Weinberg Law? Write the implications and extensions of the Hardy-Weinberg Law?
6. What are the various mutagens which causes mutation? Also explain how are genotypic frequencies calculated?

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**BLOCK IV**  
**PLANT BREEDING**

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*Genetic Variability and Its  
Role in Plant Breeding*

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**UNIT 12 GENETIC VARIABILITY  
AND ITS ROLE IN PLANT  
BREEDING**

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**NOTES**

**Structure**

- 12.0 Introduction
- 12.1 Objectives
- 12.2 Plant Breeding
- 12.3 Objectives of Plant Breeding
- 12.4 Genetic Variability and Its Role in Plant Breeding
- 12.5 Factors Limiting Genetic Improvement of Crop Plants
- 12.6 Answers to Check Your Progress Questions
- 12.7 Summary
- 12.8 Key Words
- 12.9 Self Assessment Questions and Exercises
- 12.10 Further Readings

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**12.0 INTRODUCTION**

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Plant Breeding is the process by which humans change certain aspects of plants over time in order to introduce desired characteristics. Plant breeding activities began at least 10000 years ago in the Fertile Crescent with plant domestication. Plant breeding is an election made by man of the best plants within a variable population as a potential cultivar. It is the purposeful manipulation of plant species in order to create desired genotypes and phenotypes for specific purposes. This manipulation involves either controlled pollination, genetic engineering, or both, followed by artificial selection of progeny.

Plant breeding often, but not always, leads to plant domestication. Plant breeding has been practiced for thousands of years, since near the beginning of human civilization. It is now practiced worldwide by government institutions and commercial enterprises. International development agencies believe that breeding new crops is important for ensuring food security and developing practices through the development of crops suitable for their environment.

Classical plant breeding uses deliberate interbreeding (crossing) of closely or distantly related individuals to produce new crop varieties or lines with desirable properties. Plants are crossbred to introduce traits/genes from one variety or line into a new genetic background. For example, a mildew-resistant pea may be

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crossed with a high-yielding but susceptible pea, the goal of the cross being to introduce mildew resistance without losing the high-yield characteristics. Progeny from the cross would then be crossed with the high-yielding parent to ensure that the progeny were most like the high-yielding parent, (backcrossing). The progeny from that cross would then be tested for yield and mildew resistance and high-yielding resistant plants would be further developed. Plants may also be crossed with themselves to produce inbred varieties for breeding

Genetic variability is the cornerstone of wheat breeding. Variability can be sourced from bread wheat or its near and distant relatives. Traits can be introgressed into bread wheat from these sources by using conventional hybridization techniques. In interspecific or intergeneric hybridizations the use of chromosome doubling may be required to get a balanced chromosome complement for successful cell division of the embryo and the resultant plant.

In this unit, you will learn about plant breeding and its objectives. The unit will also discuss genetic variability and its role in plant breeding.

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### 12.1 OBJECTIVES

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After going through this unit, you will be able to:

- Explain plant breeding and its objectives
- Discuss the short cuts in plant breeding
- List the types of plant breeding
- Differentiate between backcrossing and introgression breeding
- Explain genomic engineering

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### 12.2 PLANT BREEDING

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Plant breeding is a selection made possible by the existence of variability. The main objective has been to increase yield by improvement of the potential productivity followed by the successful expression of that potential. The potential productivity of a plant has been increased by modifying its morphological characteristics such as number of kernels per year in a cereal or the weight of individual seeds within a pod of pulse, and by modifying physiological traits such as harvest index, the utilization of nutrients or tolerance to stress.

With changing demands on agriculture, objectives are altered to take account of new requirements. Quality and nutritive value are of increasing importance, in association with improved efficiency of production. Modern agriculture is highly mechanized and for this reason some breeding programmes include objectives to make the crop more amenable to mechanical handling.

The extension of a crop to an area to which it was not previously adapted necessitate the production of new cultivars. The introduction of stone fruits to

warmer regions was possible by selective breeding, eliminating the cold requirement for fruiting.

Plant breeding is a unique science in two ways as it uses knowledge and technique from many basic science areas and second it contributes to agricultural progress, measured not only by information it imparts but by material products such as crop varieties, hybrids, synthetic populations. Before the rediscovery of Mendelism, plant improvement was the result of visual selection of material practiced on a trial and error basis by untrained people. Thus selection being an intrinsic part of plant breeding, came into operation with the practice of plant breeding itself.

Almost all the varieties developed in different crops before the advent of 20<sup>th</sup> century were the products of plant breeding. An adequate scientific knowledge of botany, genetics, cytogenetics, physiology, pathology, agronomy of crop plants has provided the necessary background to a creative breeder for manipulating and guiding the inheritance of plant characters in a desired direction. Though plant breeding supported by appropriate genetical principles, the new plants are created to exploit the available resources of ecological conditions.

Thus plant breeding is hazardous if either genetics or ecology is ignored. The success of plant breeding depends not only upon the adequate understanding of scientific principles that are germane to a plants performance, but upon the employment of art consummated by breeders skill and judgement. Modern plant breeding is neither an art nor a science but combination of both. It is regarded as technology of crop improvement and its success is attendant upon mutual cooperation of diverse disciplines of plant sciences.

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### **12.3 OBJECTIVES OF PLANT BREEDING**

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Among the agencies responsible for the evolution of crop plants, natural selection is exclusively a random process which can be guided, though it can be contained or rendered ineffective under controlled conditions, but other agencies like mutation, polyploidy, hybridization and recombination can be directed at the will and fancy of man for crop improvement. Domestication of these latter evolutionary processes for crop improvement is called plant breeding. It is a deliberate process so is destined for goals to bring about improvement in crops in respect of following aspects.

- **Disease Resistance:** Plant diseases and pests are natural enemies which levy heavy taxation on the crop productivity. In contrast to costly chemical control of diseases/ pests, genetic resistance conferred by resistant crop varieties is stable, safest, and the cheapest. But the fine adjustment of the individuals to specific ecological niche is the ultimate job of polygenes which constitute the genetic background of the individuals. The breeders have become much interested in taming these minor genes for future strides in crop improvement. The polygenic complexes are the backbone of continuous variation and improvement in these traits is economically much viable.

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- **Productive Capacity:** The first and foremost objective of plant breeding programme that can be realized by developing efficient crop varieties. In order to attain such variety, redesigning of plants could lead to perfection. Plant breeding aims depending upon the crop at improving crop frame, comprising greater response to fertilizers combined with lodging resistance, high chlorophyll content, desirable growth habit with proper leaf canopy and slow leaf senescence.
- **Quality Considerations:** Along with the increment in the quantum of yield, quality characters also catch the breeders attention. The emphasis is laid on the following aspects:
  - o **Market value:** High amino acid profile and high protein, high vitamin rations in food crops, fine and long staple in fibre crops, high starch for malting in barley, etc.
  - o **Feeding quality:** Such as palatability, leafiness, texture, nutritive value in fodder crops.
  - o **Seed quality:** Large and bold grains, pleasing and acceptable grain colour and better setting, etc.
- **Suitability for New Crop Husbandry:** New varieties are to be tailored to suit new agronomy such as multiple and relay cropping system.
- **Adaptive Ability:** The yield of crop plants under diverse environmental conditions of their habitat are influenced and altered by genotype. Substantial genotypic differences do exist among crop varieties in their capacity to adapt themselves in the immediate environment or over wide range of agro ecological conditions. To exploit this adaptive capability of crop varieties is goal of plant breeders. This would help extend the range of cultivation of improved cultivars under variable ecological conditions involving varying edaphic and climatic factors and changing latitudes.
- **Horizontal Resistance:** The concept of resistance needs to be interpreted in terms of carry home yields obtained in the field that is subjected to various diseases and pathogens. In contrast to vertical resistance with a limited impact on final yield, horizontal resistance offers a fair degree of protection to crops from a no of physiological races of pathogens. The latter ensures satisfactory yield of crops under existing field conditions. Breeding for horizontal resistance is an example of the genetic manipulation of polygenic complexes.
- **Short Cuts in Plant Breeding:** Faced with a mounting pressure to achieve rapid crop improvement, many short cuts in plant breeding have been identified. Exploitation of offseason nursery for quick multiplication and advanced breeding materials, deploying green house facilities to affect advanced crossing, etc.

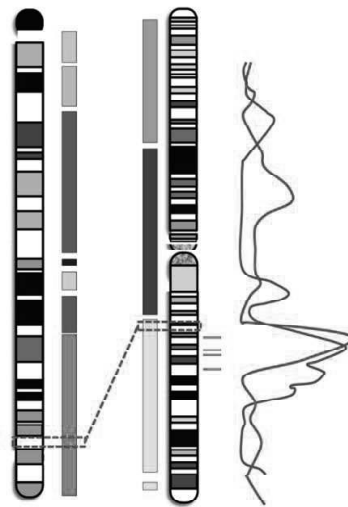
- **Radical Changes:** Some of the ultramodern biological techniques designed for a gigantic upsurge in farm production such as transfer through genetic engineering of nitrogen fixing capacity from haves to have nots enhancing the photosynthetic efficiency of plants to above 20 percent, unusual hybridization above species level by somatic hybridization technique and control over photorespiration to restrict the losses of assimilates are not only distant dreams but changing into reality. These concepts of crop improvement through genetic manipulation are responsible for the substantial elevation of yield levels in many crop plants.

The success of a breeding programme in meeting the various objectives is dependent on two factors. First having the necessary variation and second being able to manipulate it to produce a stable new cultivar. The variation exploited in most breeding programme is derived from naturally occurring variants and the wild relatives of our main crop species. The distribution of variability among species was found by Vavilov.

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*Fig. 12.1 Illustrative Example of Invitro Culture*



*Fig. 12.2 Illustrative Example of Genomic Tools*

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*Fig. 12.3 Illustrative Example of Genomic Engineering, Cotton Plant*

### Check Your Progress

1. When is plant breeding hazardous?
2. What is an example of the genetic manipulation of polygenic complexes?
3. What is the success of a breeding programme dependent on?
4. Where is the variation exploited in most breeding programme derived from?

## 12.4 GENETIC VARIABILITY AND ITS ROLE IN PLANT BREEDING

There are four processes which have been responsible for bringing about profound changes in the wild species and rendering them suitable for domestication.

- Generation of novel variability
- Recombination
- Differential reproduction, i.e., selection
- Isolation

Each of these processes is essential for the success of the whole in accelerating the speed of change, which is higher in domesticated species than in natural species.

The intraspecific variability so created is reflected at two levels:

- Lower level, i.e., intra population variation between individuals or interallelic variation arising from direct mutation and recombination of major gene which can be accounted for
- Higher level, i.e., interpopulation variation with respect to the gene frequency which is the product of accumulated variation at the lower level. New variability between populations for gene frequency doesn't originate through mutation alone. There are two other agencies known for producing interpopulation variability, the chance fixation of random variation and the directive action of natural selection.

While interpopulation variation involving changes in gene frequencies is most important for evolution, intrapopulation variation by itself is of little significance in evolution, but of great use in plant breeding. Maintenance of this variability depends either on the perpetuation of heterozygosity or on the occasional crossing between homozygotes bearing different genotypes. The intrapopulation variability is supplemented by the local evolution of new variation and the contribution of foreign genes to locally adapted variability. Simmonds (1962) suggested three kinds of variability:

- Variability of adapted combination: (i) exposed, for example, phenotypic differences among inbreds or clones (ii) concealed, for example, heterozygosity which is stabilized in clones.
- Variability developed insitu i.e the product of mutation, recombination and selection.
- Variability developed from foreign genes, i.e., introduction from other populations.

These kinds of variability are mutually inclusive since a recombination of migrant genes into adaptive combinations may take place. He indicated as to how much variability occurs and how quickly it does so depend upon more than one factor such as breeding system, reproductive habit, population size, selection, mutation rates, seed and pollen dispersal. Owing to these, a tremendous amount of genetic variability, both at individual and population levels can be channelized under plant breeding is available in all the species. The wild species when brought under domestication based on their property for immediate adaptation and attractiveness are called crops.

Three kinds of genetic resources have been identified for exploitation in plant breeding:

- The wild or weedy relatives of crop species together with alien species
- The land races or primitive cultivars which were prevalent under primitive agriculture about century ago
- The advanced cultivars of scientific agriculture produced during the last 100 years or so

All cultivars including weedy relatives have evolved under domestication, i.e., under conditions of cultivation. The principal creative agencies were natural selection for land races and wild relatives and deliberate human selection for advanced cultivars under domestication

The intraspecific variability is regulated mainly by two corresponding processes which are i) chromosomal behavior during meiosis and ii) breeding system. There exists a perfect coordination in nature between these two processes- the coordination which confers requisite capacity to the population for a compromise between immediate fitness and resilience for future changes. This coordination is achieved through the control of crossing over within the chromosome during cellular

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processes. Thus the meiotic mechanism provides recombination within the genotype and the breeding system extend the same to the level of population.

It is known that while restriction of recombination increases fitness, its release due to frequent crossing over turns population into dynamic state. A change in breeding structure of the population exerts major influence on the chromosome behavior during meiosis, hence the frequency of crossing over and release of recombinations. This regulation of variability, i.e., release of recombinations is called genetic system which is specific to the population. Genetic system promotes greater flexibility by frequent outcrossing which is ancestral one. The importance of genetic system lies in the recombination of variability which influences the adaptability of the crop either to the same environment or to newly created one. This recombinational variability is adapted and augmented by new gene combinations and migrant gene combinations. Both of these tend to be ill adapted until incorporated in the local genetical milieu. By contrast, the rate of such augmentation is high in unselected outbreeders, hence they vary much more widely and rapidly. We can identify particular changes in morphology and physiology whereby they become adapted to particular conditions of growth and management. The casual basis of variation was made available, that is exposed to the action of selection. In plants there are many causes, they include nuclear and cytoplasmic mutation, their combination and recombination, modification in breeding behavior, structural and numerical changes in chromosomes.

Whatever the nature of selection pressure, its effectiveness will depend upon the nature and extent of the variation in plant form and function to which it is directed. In nature, changes in the constituent elements of genetic systems are matters of chance. To the plant breeder they are not. They may be imposed and the genetic system thereby manipulated. For early breeders, the variability from which varieties were constructed derived from two main sources, the first was from farm crops at home and abroad, the second was generated from crosses. The vast majority of new varieties are bred from selection practiced upon variability deriving from the very same sources. The conscious manipulation of elements of genetic system have contributed more to the practice of plant breeding than has been the case to date. The possibilities and the prospects for such manipulations are considered with respect to recombination.

The recombination of genes, be they genes of major effect or component of polygenic complexes is of paramount importance in plant breeding is undeniable. The breeders aim is to construct by selection among hybrids and their progenies those combination of genes most suited to his purposes. The speed and the efficiency with which they are built up will depend on the number of genes involved and whether or not genes are linked. If as is likely, there is linkage, recombination will depend upon the frequency with which chiasmata form between them. The greater the likelihood of the formation of such chiasmata the more effective and expeditious the selection.



The breeder is selecting for recombinants, he would unconsciously select plant genotypes with high chiasmata frequencies, these being the most likely to yield most rapidly the recombinants. The frequency and distribution of chiasmata are under genotypic control. So one would expect that the progenies of these plants constitute the bred varieties that would have higher chiasma frequencies than the original populations from which they derive. Breeding in itself is instrumental in effecting change in genetic systems especially the recombination component.

Much of the variation, in chiasma formation is under polygenic control and is difficult to manipulate than would be the case where control exercised by major genes. Many of our most valuable crop plants are allopolyploids. They are endowed with the advantages of hybridity without the handicap of infertility. Apart from polyploidy, there is also possibility of utilizing genes to boost recombination in intervarietal diploid hybrids such as produced in breeding programme. Except for instances of asynapsis, most of variation in chiasma frequency is controlled by polygenic complexes and is difficult to handle. In many species they carry determinants affecting chiasma frequencies and they influence the variability of progenies

During recent years, a battery of new techniques become available to the plant breeder like transformation following the isolation and cloning of genes, use of transposons, clonal propagation of somatic cells and tissues followed by regeneration to produce mature plants, regeneration from pollen, protoplast fusion, cybrid formation involving fusion between nuclei and alien cytoplasm. All these methods will be deployed to advantage in some instances Transformation offers opportunities for incorporating useful genes into alien species without accompanying unwanted genetic material. Transposons provide new opportunities for inducing mutation.

For transformation the generation and incorporation into chromosomes of alien genes are achieved by variation on the theme of recombination. Protoplast fusion, cybrid formation, regeneration from pollen, from clones of somatic cells and tissue are exotic, unusual modes of hybridization, reproduction and unorthodox breeding systems. The somaclonal variation associated with regenerated products of cell and tissue culture represent additional source of mutation. The transfer of alien genes by vectors or other means in the course of transformation represents a form of hybridization. The new techniques provide opportunities for manipulating more effectively different components of genetic systems.

According to an estimate of the committee on the threatened plants of IUCN about 10 percent of the world's flowering plants are getting to be dangerously rare or under threat of extinction. There are two processes responsible for genetic erosion and loss of variability in crop plants, breeding methods employed for crop improvement and advanced agricultural system. Two main processes of plant breeding which are of major consequences are inbreeding and selection. It was pointed that most important cause of genetic variation within unselected populations

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is inbreeding. It diminishes the genetic variance contributed by segregating loci. When a population is subjected to inbreeding, it undergoes a fractionation into a series of inbred lines. The desirable ones are selected for further use while undesirable are eliminated.

A sizeable no of alleles are wasted in the inbreeding process. It is remarkable that inbreeding reduces variability within the line, it increases the variability between the lines, thereby making rejection or selection of appropriate lines possible. On similar grounds, in the absence of provision for proper maintenance, a large amount of genetic variability is lost in exercising selection in selected population specially in locally adapted varieties which are genetically most exploited for crop improvement.

The modern agriculture which mainly thrives on new crop varieties developed through plant breeding methods, reduces genetic variability by replacing inefficient land races of primitive agricultural systems by improved crop varieties and by invading the marginal areas where primitive cultivars are maintained under natural conditions. Advanced agriculture is characterized by a great increase in adaptation associated with a decline of the locally adapted variability. Such highly specialized adaptations result in narrow genetic base and lead to evolutionary allele and lead to ultimate extinction. Variability is lost by many ways pure lines tend to displace not only primitive cultivars but also local cultivars, advanced propagation techniques permit multiplication in place of seedlings. Wild relatives tend to disappear fast and face extinction as their natural habitats are destroyed by agricultural extension and other forces in marginal lands and primitive areas.

Plant breeding is able to contribute both to a more secure world food supply and to sustainable agricultural system, in future plant breeders will have to increase crop yields and product quality, develop varieties with wide adaptation as well as varieties for local and specific environments, produce varieties which have improved resistance to various abiotic stress conditions and which make better use of crop inputs, develop varieties with better resistance to pests and diseases and less dependence on agrochemicals, provide crops and varieties with wide range of uses. The main breeding objective has to be increased yield and quality of products, or to be more precise is to produce maximum yield of saleable products at an economical level of input and with a minimum of negative environmental effects. Although the other breeding objectives are major or dominant features of a breeding programme.

### **Type of Plant Breeding**

- **Backcrossing or Introgression Breeding:** Crop breeders sometimes use a process called backcrossing. A plant that has the desirable trait—let's say mildew resistance—is crossed with a plant that doesn't have that trait, but is desirable in all other traits. There is a quality control step to make sure

that the only change to the original variety is the desired trait. For example, a high-yielding pea can be crossed with a mildew-resistant pea. The next generation plant is called the progeny. All progeny that are still mildew resistant are then crossed to their high-yielding parent. This is repeated a few more times, always crossing back to the high-yielding parent, and selecting the mildew-resistant progeny. This process ensures the next generation is in most ways similar to the high-yielding parent while adding the mildew-resistant quality from the other parent.

- **Inbreeding:** Depending on the species, some plants may be fertilized by themselves. This is done to produce an inbred variety, which is exactly the same generation after generation. Because it preserves the original traits, it is useful in three ways: for research; as new, true-breeding cultivars; and as the parents of hybrids.
- **Hybrid Breeding:** In this situation, two different inbred varieties are crossed to produce an offspring with stable characteristics and hybrid vigor, where the offspring is much more productive than either parent.
- **Mutation Breeding:** Naturally occurring genetic mutations exist throughout the world. If these random examples are found and seen as an improvement, they can be used to create new varieties. Alternatively, mutations can be artificially encouraged by exposing plants to chemicals or radiation.
- **Molecular Marker-assisted Selection:** This uses classical, backcrossing, or inbreeding and hybridization methods, with an important difference. Instead of selecting desirable plants based on the way they look or grow, breeders select plants after confirming the information on the genes the plants inherited from their parents. Just like having a map to an unfamiliar city, this takes some of the guesswork out of breeding. Researchers can confirm the gene is present, not just assume it is, before they move forward with breeding the plant.
- **Genetic Engineering:** Engineers who design bridges or skyscrapers insert strong building design into their plans. Similarly, modern genetics techniques can insert desirable traits into plants. The resulting plants are called transgenic or Genetically Modified Organisms (GMOs).
- **Gene Editing:** These Cutting-Edge Genetic Techniques, Including CRISPR-Cas9, enable breeders to modify specific genes directly. It targets very specific plant characteristics with razor-like precision.

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### 12.5 FACTORS LIMITING GENETIC IMPROVEMENT OF CROP PLANTS

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The breakthrough in a few cereal crops need not instantly warrant a similar success in other cereal and non-cereal crops. Many obstacles that a breeder confronts

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with in his breeding programmes, tend to restrict the pace of progress under crop improvement.

- **Narrow Genetic Base:** The presence of genetic variability in the breeding material is the prerequisite, particularly at the initial stage, for a breeding programme to succeed. Almost all the cultivars are developed by exploiting the variation. Variability is depleted rapidly due to genetic drift and genetic erosion in limited material. The most important is the use of a limited number of genes and kind of cytoplasm for crop improvement.
- **Absence of Suitable Plant Type:** The concept of ideotype envisages a plant which commands the physiological efficiency to exploit most usefully all the available resources including soil nutrients, agronomic management and solar energy so as to realize the maximum theoretical yield. Such a capacity is conferred to the plant by exploitation of developmental traits.
- **Unpredictable Genotype Environment Interaction:** The efficiency of breeding procedures in terms of speed of advance is influenced by presence of gene interaction. Inadequate choice of environment for exercising selection in segregating generations lead to genetic slippage due to effect of environmental agencies during selection process.
- **Small Population Size and Indiscriminate Experimental Design:** The small size of F<sub>2</sub> population and the low number of F<sub>3</sub> families grown and retained, lack of sensitive design fail to eliminate the effects of plant competition and soil heterozygosity.
- **Inherent Genetic Barriers:** Serious genetic bottlenecks inherent in the crop material itself restrict its rapid improvement by ordinary breeding methods.

### Evolutionary Forces and Crop Evolution

The forces responsible for the creation of genetic variability in sexual organisms are:

- Revolutionary forces which produce genetic novelty by creating Mendelian variation due to mutation and recombination after hybridization
- Conservative forces which preserve certain constellations of genes over a large number of generations by
  - o Selection pressure through natural selection, human selection and genetic drift.
  - o Genotypic factors which tend to produce homogeneity among individuals such as limited number of chromosomes.
  - o Phenotypic factors which conserve variability as devices favouring inbreeding, sterility mechanism, mating discrimination, etc.

While mutation including polyploidy give rise to new species at a single stroke, genetic drift, selection and hybridization followed by recombination which alter gene frequency of populations are slow processes and hence speciation proceeds gradually.

The important variation within and between natural and artificial populations is for the most part continuous, differences in expression being of degree rather than absolute. Such quantitative variation is displayed by all characters of an organism affecting growth, development and reproduction including chromosome form and function. So far as individual genic differences between genotypes affect growth and development their effects on the phenotype can vary from the gross to those so slight that they can be detected under strictly controlled conditions. Since the breeding system and chromosomal linkage are integral elements of the machinery governing the flow of genetic variability. Compared with the haploid condition of primitive organisms, the diploid state and segregation has allowed the evolution of a sophisticated genetic system for the expression and storage. Higher levels of polyploidy are common in plant taxa but are rarely found in conjunction with potentially associated levels of polysomic segregation. Autopolyploids with polysomic segregation are rare and confined to long lived species with wide faculty for asexual reproduction.

The conservation of plant genetic resources goes far beyond the preservation of a species. Plant genetic resources can be conserved in situ as well as ex situ and two system must be complementary and not antagonistic. In situ conservation consists in the legal protection of the area and habitat in which the species grow. This is preferred technique for wild plants. The great advantage is that the evolutionary dynamics of the species are maintained. Ex situ conservation implies the collection of representative samples of genetic variability of a population and their maintenance in germplasm banks or botanical gardens as seeds, shoots, in vitro culture, plants etc. The major advantage is the control of the material in a small space under intensive care. The material is easily accessible to plant breeders. The major drawback is that germplasm ceases to evolve and natural processes of selection and continuous adaptation to local habitat are halted. Further drawbacks are genetic drift and selection pressure. Both phenomenon produce a cumulative genetic erosion which sometimes exceed the genetic erosion taking place in the field.

#### **Check Your Progress**

5. From where have weedy relatives evolved?
6. What does exsitu conservation imply?
7. What is the efficiency of breeding procedures in terms of speed of advance influenced by?
8. What is the prerequisite for a breeding programme to succeed?

#### **NOTES**

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## 12.6 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

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### NOTES

1. Plant breeding is hazardous if either genetics or ecology is ignored.
2. Breeding for horizontal resistance is an example of the genetic manipulation of polygenic complexes.
3. The success of a breeding programme is dependent on two factors- having the necessary variation and being able to manipulate it to produce a stable new cultivar.
4. The variation exploited in most breeding programme is derived from naturally occurring variants and the wild relatives of our main crop species
5. Weedy relatives have evolved under domestication.
6. Ex situ conservation implies the collection of representative samples of genetic variability of a population and their maintenance in germplasm banks or botanical gardens as seeds, shoots, invitro culture, plants, etc.
7. The efficiency of breeding procedures in terms of speed of advance is influenced by presence of gene interaction.
8. The presence of genetic variability in the breeding material is the prerequisite for a breeding programme to succeed.

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## 12.7 SUMMARY

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- Plant breeding activities began at least 10,000 years ago in the Fertile Crescent with plant domestication.
- The main objective has been to increase yield by improvement of the potential productivity followed by the successful expression of that potential.
- Plant Breeding is the process by which humans change certain aspects of plants over time in order to introduce desired characteristics.
- Plant breeding often, but not always, leads to plant domestication. Plant breeding has been practiced for thousands of years, since near the beginning of human civilization. It is now practiced worldwide by government institutions and commercial enterprises.
- Classical plant breeding uses deliberate interbreeding (crossing) of closely or distantly related individuals to produce new crop varieties or lines with desirable properties. Plants are crossbred to introduce traits/genes from one variety or line into a new genetic background.
- Genetic variability is the cornerstone of wheat breeding. Variability can be sourced from bread wheat or its near and distant relatives.

- Plant breeding is a selection made possible by the existence of variability. The main objective has been to increase yield by improvement of the potential productivity followed by the successful expression of that potential.
- Among the agencies responsible for the evolution of crop plants, natural selection is exclusively a random process which can be guided, though it can be contained or rendered ineffective under controlled conditions, but other agencies like mutation, polyploidy, hybridization and recombination can be directed at the will and fancy of man for crop improvement.
- The first and foremost objective of plant breeding programme that can be realized by developing efficient crop varieties. In order to attain such variety, redesigning of plants could lead to perfection.
- In contrast to vertical resistance with a limited impact on final yield, horizontal resistance offers a fair degree of protection to crops from a no of physiological races of pathogens.
- While interpopulation variation involving changes in gene frequencies is most important for evolution, intrapopulation variation by itself is of little significance in evolution, but of great use in plant breeding.
- The intraspecific variability is regulated mainly by two corresponding processes which are i) chromosomal behavior during meiosis and ii) breeding system.
- In plants there are many causes, they include nuclear and cytoplasmic mutation, their combination and recombination, modification in breeding behavior, structural and numerical changes in chromosomes.
- For early breeders, the variability from which varieties were constructed derived from two main sources, the first was from farm crops at home and abroad, the second was generated from crosses.
- The recombination of genes, be they genes of major effect or component of polygenic complexes is of paramount importance in plant breeding is undeniable.
- The breeder is selecting for recombinants, he would unconsciously select plant genotypes with high chiasmata frequencies, these being the most likely to yield most rapidly the recombinants. The frequency and distribution of chiasmata are under genotypic control.
- During recent years, a battery of new techniques become available to the plant breeder like transformation following the isolation and cloning of genes, use of transposons, clonal propagation of somatic cells and tissues followed by regeneration to produce mature plants, regeneration from pollen, protoplast fusion, cybrid formation involving fusion between nuclei and alien cytoplasm.

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## NOTES

- According to an estimate of the committee on the threatened plants of IUCN about 10 percent of the worlds flowering plants are getting to be dangerously rare or under threat of extinction. There are two processes responsible for genetic erosion and loss of variability in crop plants, breeding methods employed for crop improvement and advanced agricultural system.
- A sizeable no of alleles are wasted in the inbreeding process. It is remarkable that inbreeding reduces variability within the line, it increases the variability between the lines, thereby making rejection or selection of appropriate lines possible.
- The presence of genetic variability in the breeding material is the prerequisite, particularly at the initial stage, for a breeding programme to succeed.
- The important variation within and between natural and artificial populations is for the most part continous, differences in expression being of degree rather than absolute. Such quantitative variation is displayed by all characters of an organism affecting growth, development and reproduction including chromosome form and function.
- The conservation of plant genetic resources goes far beyond the preservation of a species. Plant genetic resources can be conserved in situ as well as ex situ and two system must be complementary and not antagonistic.
- Quality and nutritive value are of increasing importance, in association with improved efficiency of production.
- Plant breeding is hazardous if either genetics or ecology is ignored. The success of plant breeding depends not only upon the adequate understanding of scientific principles that are germane to a plants performance.
- The success of a breeding programme in meeting the various objectives is dependent on two factors. First having the necessary variation and second being able to manipulate it to produce a stable new cultivar.
- Maintenance of this variability depends either on the perpetuation of heterozygosity or on the occasional crossing between homozygotes bearing different genotypes.

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## 12.8 KEYWORDS

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- **Selection pressure:** It refers to an agent of differential mortality or fertility that tends to make a population change genetically. Their range of variation is constrained by natural selection pressures imposed by their environment.
- **Genetic variation:** It is a term used to describe the variation in the DNA sequence in each of our genomes. This is what makes us all unique.



- **Genetic systems:** It is the organization of genetic material in a given species, and its method of transmission from the parental generation to its filial generations.
- **Recombinations:** It is the exchange of genetic material between different organisms which leads to production of offspring with combination of traits that differ from those found in either parent.
- **Allopolyploids:** It refers to an individual or strain whose chromosomes are composed of more than two genomes each of which has been derived more or less complete but modified from one of two or more species

## NOTES

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## 12.9 SELF ASSESSMENT QUESTIONS AND EXERCISES

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### Short Answer Questions

1. Write a short note on plant breeding.
2. What are the objectives of plant breeding?
3. What do you mean by disease resistance?
4. Briefly discuss productive capacity.
5. What are the short cuts in plant breeding?
6. List the types of plant breeding.
7. Differentiate between backcrossing and introgression breeding.
8. How is whin breeding different from hybrid breeding?

### Long Answer Questions

1. Write a detailed note on the factors limiting genetic improvement of crop plants.
2. What do you mean by narrow genetic base? Also explain the absence of suitable plant type.
3. "The conservation of plant genetic resources goes far beyond the preservation of a species." Explain.
4. "Autopolyploids with polysomic segregation are rare and confined to long lived species with wide faculty for asexual reproduction." Discuss.
5. What is plant breeding? Discuss its significance and list the common factors that govern plant breeding.

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## 12.10 FURTHER READINGS

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### NOTES

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# UNIT 13 BREEDING METHODS IN POLLINATED, VEGETATIVELY PROPAGATED AND APOMICTIC PLANTS

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*Breeding Methods in  
Pollinated, Vegetatively  
Propagated and Apomictic  
Plants*

## NOTES

### Structure

- 13.0 Introduction
- 13.1 Objectives
- 13.2 Breeding Methods in Self Pollinated Crops
- 13.3 Breeding Methods in Cross Pollinated Crops
- 13.4 Methods for Breeding of Vegetative Propagated Crops
- 13.5 Breeding Methods in Apomictic Plants
- 13.6 Answers to Check Your Progress Questions
- 13.7 Summary
- 13.8 Key Words
- 13.9 Self Assessment Questions and Exercises
- 13.10 Further Readings

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## 13.0 INTRODUCTION

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The results of genetic breeding have been excellent for most self-pollinated species. It should be however emphasized that the evaluation of the breeding success should not only be based on the yield increase, but, mainly on the improved sustainability of self-pollinated species. This increase in sustainability can be measured by the availability of cultivars adapted to new growing conditions, more tolerant to biotic and abiotic stresses.

Among the self-pollinated species there are some as soybean that attract the attention of large multinational seed companies. The case of tomato is also very peculiar, because attention is focused on the production of hybrid seeds and although there are public institutions dedicated to tomato improvement, most of the seed stock is produced by multinational companies. Contrastingly, in the case of some species such as common bean, the development of new cultivars is controlled by the public sector. In essence, the improvement process in different species is rather similar, but there are differences in the resources invested in breeding, especially in terms of the crosses number, progenies evaluated annually and the number of test sites of new lines.

The commonly used breeding methods of self-pollinated plants were developed over a century ago in Europe. No great subsequent changes were

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made. Basically, the selection procedure of the best progenies was optimized, particularly with the development of agricultural experimentation based on the publications of Fisher. In the past 25 years, the techniques of molecular biology opened up the prospect of the possibility of direct selection of a genotype. The emphasis of global research was focused mainly on the use of molecular DNA markers. Abundant basic information was compiled, but evidently the phenotypic analysis in the field, as accurately as possible, is still indispensable.

Information was accumulated that indicated that the traits the breeders work on are mostly complex, in other words, probably controlled by several genes. This observation became even patent because the improvement consists, in summary, of an accumulation of advantages through successive selection cycles, recurrent selection, that involves intercrosses of the best lines/cultivars available in each cycle. The objective is therefore to identify the plants/progenies with the highest genotypic merit in each selection cycle. Another goal is to develop cultivars that can be used for several years.

Numerous studies have focused on improving breeding efficiency. This presentation will comment some of these studies targeting an increased breeding efficiency of self-pollinated plants by the identification of the progenies/lines with highest genotypic merit and comment about strategies to obtain cultivars with a longer lifetime.

In this unit, you will learn about breeding methods in self-pollinated, cross pollinated, vegetative propagated and apomictic plants.

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### 13.1 OBJECTIVES

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After going through this unit, you will be able to:

- Discuss breeding methods in cross pollinated crops
- Explain apomixis in amphimictic crops
- Analyse hybridisation of facultative apomicts
- Describe breeding methods in apomictic plants

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### 13.2 BREEDING METHODS IN SELF POLLINATED CROPS

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In sexual organisms like crop plants, a greater and appropriate control over pollination or breeding system is the avowed mission of the plant breeder. The breeding system in plants is considered to be the sum of all genetic, physiological and morphological traits of a species that determines its optimum fitness, i.e., adaptation to the immediate environment or to future changes. The very genetic system which regulates the release of variability in population, specific for each

species is sustained and directed by the breeding system prevailing in that species. It helps maintain a delicately balanced system of coadapted gene complexes in a population through an efficient control over the genetic system. A sudden or deliberate shift in the breeding system tends to alter the constellation of characters. Since the development of new and productive crop plants through appropriate genetic manipulation of variability is the prerogative of plant breeding and so great is the impact of breeding system on nature and rate of release of the variability in crops. The general pattern of breeding programme suitable for a particular species is influenced by breeding system. The significance of mating systems with respect to crop improvement can be summarized in following points:

- The mating system by and large determines the nature of response of populations to different breeding procedures.
- It indicates the limitations and possibilities of different methods.
- It signifies the efficient way to regulate pollination in crop plants as required under different breeding programmes. Out of all the processes involved in the reproductive system of sexual crop plants, only pollination can be modified, to large extent at breeders will.

The accomplishment of pollination in sexual plants is dependent mainly upon two factors:

- **Sources of Pollen:** Whether the receptive stigma receives pollen grains from the same or from different flower, or in other words, from individuals like or unlike at both genotypic and phenotypic levels.
- **Contrivances of Pollination:** Apparently, there are certain mechanical and genetical devices in floral organs which encourage and enforce a particular type of pollination in some species but not necessarily in all.

Three basic or natural systems of pollination that are germane to plant breeding are Natural self-pollination, Natural cross pollination and cross pollination. Cross pollination is ancestral one. That wild species are nearly all allogamous is a testimony of this fact. Self-pollination was evolved much later out of necessity of the plants for immediate fitness under domestication.

### **Natural Self-Pollination (Autogamy)**

Transference of pollen grains from the anther of a flower to the stigma of the same flower borne on the same plant is termed self-pollination or natural inbreeding. No external agencies are instrumental in achieving autogamy. But the flower structure itself is provided with a mechanism which encourages self-pollination. What is needed is the synchronous maturity of both the anthers and the stigma of the hermaphrodite flowers favouring homogamy. Autogamy occurs naturally in almost all the legumes, in many cereals, minor millets, etc. In this system of mating, crosses between unrelated plants are either excluded or they occur at a very low frequency than would be expected on the basis of random mating. The intensity of inbreeding

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determines the rate at which the consequences of inbreeding are felt in descendants. The natural self-pollination concerns with only one parent plant for producing progenies. Therefore no new recombinations can be anticipated. Self-pollination does not require control over pollination when subject to plant breeding. Deliberate or occasional crossing between two parents in selfers creates a genetic variation on which selection can operate. The purpose of deliberate crossing is to isolate superior recombinations in segregating generations rather than to develop better F1 hybrids.

### Consequences

Continuous inbreeding results in progressive loss of genetic variability until each individual has become homozygous for all or almost all the genes. In other words, it leads to fixation of alleles and hence fixation of genotypes. The results of inbreeding in self-pollinated crops are:

- Each variety is a highly inbred line distinctly differentiated for gene frequency.
- Intra varietal genetic variation is absent.
- Intervarietal genetic variation is large due to random fixation of different alleles in different lines. This ensures a prompt success of selection for superior genotypes.
- Genetic correlations between relatives are gradually enhanced with the progress of inbreeding till it approaches unity.
- In the absence of directional selection, intense and prolonged inbreeding promotes discrete groupings of non-interbreeding individuals within a population, since it tremendously increases the total genetic variance due to random alignment of gene frequencies. Therefore inbreeding can proceed only with appropriate selection pressure directed to purge the population of some of the non-interbreeding groups in each generation so as to keep the population size optimum, or selection of a new variety from a segregating population after hybridization the methods followed in self-pollinated crop are of three types: pedigree method, bulk method, and back cross method.

### Pedigree Method

Individual plant progeny is selected from F<sub>2</sub> and subsequent generations, and their progenies are tested. During this process the record of parents as well as offsprings is kept, for which it is known as pedigree method.

The pedigree is defined as the description of the ancestors of an individual and it is generally helpful in finding out the amount of relatedness among two individuals, i.e., whether they are related by common parent in their descent ancestry or not.

## Procedure

The procedure for this method is elaborately detailed below:

- **First Year:** The hybridization is done among two selected parents, after emasculation one become female parent and another male parent. After seed set and maturation, the  $F_1$  seeds are harvested separately from each plant individually. On the basis of choice of parents, the type of cross will be of two types — it will be simple cross or complex cross.
- **Second Year:**  $F_1$  generation seeds are space planted and selfing is allowed, each  $F_1$  will produce more  $F_2$  seeds. From 15-30 selected  $F_1$  plants, the  $F_2$  seeds are collected to get a reasonable size of  $F_2$  population and variation.
- **Third Year:** In  $F_2$  generation, 2000-10000 plants are space planted, 100-500 plants are selected and their seeds are harvested separately. If the parent plants are closely related varieties then the number of selected  $F_3$  plants would be smaller whereas in case of distantly related varieties the number of  $F_3$  progenies will be of relatively larger numbers.
- **Fourth Year:** In  $F_3$  generation also, the individual plant progenies are space planted. Each progeny should have about 30 or more plants. Individual plants with desirable characteristics are selected; disease and lodging susceptible progenies to be eliminated, and also the progenies with undesirable characters are rejected even from the selected plants. During this selection if the number of superior progenies is very small then the whole cross programme may be rejected.
- **Fifth Year:** The selection procedure is same as previous year, only if two or more progenies coming from the same  $F_3$  progeny are similar and comparable, then only one may be saved and others may be rejected. The emphasis is given on the selection of desirable plants from superior progenies.
- **Sixth Year:** Individual plant progenies of  $F_5$  generation are planted according to recommended commercial seed rate. Three or more rows for each progeny will help in comparison among progenies. Many progenies may have become reasonably homozygous genotype and may be harvested in bulk. If the progenies show variation then the individual plants are selected. The number of selected progenies should be reasonable so that preliminary yield trial with 25-100 progenies can be done.
- **Seventh Year:** Individual plant progenies of  $F_6$  generation are planted in multi-row plots and evaluated visually. Progenies harvested in bulk since they become homozygous. The segregating progenies may be discarded and the preliminary yield trial may be done for the progenies which are reasonably homozygous and have enough seeds.
- **Eighth Year:** Preliminary yield trial with three or more replications is conducted to identify few superior lines. The progenies are evaluated for

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plant height, lodging, disease resistance, flowering time, maturity time, etc. Quality test is done to serve as an additional basis for selection.

- **Ninth to Tenth or Thirteenth Year:** The superior lines are tested in replicated yield trials at several locations. The above mentioned criteria are evaluated for these lines. The line which is superior to the best commercial variety may be released as new variety.
- **Eleventh or Fourteenth Year:** The selected strain should get multiplied to release as a new variety.

### Merits

- This method is most useful as transgressive segregation for yield and other quantitative characters may be recovered in addition to improvement of specific characters.
- This method is well suited for improvement of characters which can be easily identified and simply inherited.
- Through the maintenance of pedigree record the breeder may be able to obtain the information about inheritance of characters.
- Plants or progenies with weaker and visible defects are eliminated at an early stage in the breeding programme.
- This method gives maximum importance on the breeder to use his/her skill and judgement about the selection of plants and progenies.
- This method takes less time than bulk method to release a new variety.

### Demerits

- The success of the method is mainly dependent on the skill of the breeder.
- To keep the individual pedigree record is laborious and time consuming, it may be the limiting factor for large breeding programme.
- Selection of large number of progenies in every generation is also laborious and time consuming.
- In F<sub>2</sub> and F<sub>3</sub>, the selection for yield is not effective. If sufficient number of progenies is not retained, valuable genotypes may be lost in early segregating generations.

### Achievements

Pedigree method is useful in selection of new superior recombinant types from a hybridization programme. This method is suitable for improving specific characteristics, such as disease resistance, plant height, maturity time, etc., as well as yield and quality characters.

Many improved varieties have been developed through pedigree method in many crops like wheat, rice, barley, pulses, oil seeds, cotton, tobacco, jowar, vegetables, etc.



Three different advantages may be achieved from this method

- Isolation of homozygous lines,
- Waiting for selection by environmental disaster,
- The long period of bulking may be helpful for natural selection to change the composition of population.

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### 13.3 BREEDING METHODS IN CROSS POLLINATED CROPS

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Cross pollination is the movement of pollen grains from one flower to another borne on the same or different plant of the same or allied species, regardless of whether the flower is bisexual or unisexual. This is accomplished by external agencies since floral structure and physiological factors in this group of crops pose a great barrier to the occurrence of self-pollination. These pollinating agencies are insects, some animals, wind and water. The only thing needed is the production of an abundance of pollen grains, particularly in anemophilous plants. In cross pollination, two parents are involved and a mingling of the two sets of parental characters produces better progenies.

#### Consequences

Frequent cross pollination tends to create and maintain a tremendous amount of genetic variability chiefly because of the high degree of heterozygosity in populations. The heterozygosity shelters a large number of recessives which may be wiped out or promptly eliminated by selection when exposed by inbreeding. That is why cross fertilized heterozygous populations carry a great deal of balanced genetic load. The occurrence of heterozygosity due to allogamy prevents genetic stagnation. It renders the population fairly dynamic which can be manipulated in a desirable direction. Because of these reasons in cross pollinated crops:

Each variety is a highly random pollinated population usually maintained at genic equilibrium in absence of selection. An immense amount of genetic variability floats in crops at both intra and inter population levels due to frequent random gene flow among genotypes.

Role of dominance is potential. Release of recessive alleles on selfing lends credibility to this fact. Too high a degree of panmixia among outbreeding populations may lead to rapid non-discrimination of population characteristics or in other words, loss of genetic identity.

The mating of phenotypically dissimilar individuals tends to reduce genetic correlation among progenies. Such a mating tends to preserve genetic variation in small open pollinated populations where allelic erosion is imminent due to genetic drift under domestication.

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Inbreeding of cross pollinated crops leads to a rapid loss of vigour. However conclusive evidences indicate differential tolerance to inbreeding in different outbred varieties. Where it is tolerated, varieties of maize, inbred lines can be maintained by regular inbreeding coupled with selection

Hybridization between two inbreds usually leads to the recovery of the vigour lost by inbreeding. These consequences make outbreeders most exploitable for crop improvement. In contrast to selfers, improvement in outbreeders proceeds with inbreeding first for few generations followed by hybridization of inbred stocks.

In crops like cotton, sorghum and sudan grass, the amount of cross pollination that occurs varies considerably. Stigma remains exposed to both self and cross pollination but self-pollination is predominant. Outcrossing does occur by wind blown pollen grains or when suitable pollen vectors are present. Such crops enjoy the advantages of both self and cross pollination. The breeding methods include:

The methods are:

- **Inbreeding:** The mating of individuals more closely related than individuals mating at random is known as inbreeding. The lines produced by continued inbreeding are known as inbred lines. Self-fertilization is the most intense form of inbreeding.

In plant breeding nearly homozygous lines are produced by continued self-fertilization accompanied by selection for five to six generations. This can be used as the method of breeding only in those crops, which do not show any loss of vigour due to inbreeding, like cucurbits.

The three important uses of inbreeding in cross-pollinated crops are as follows:

- o To attain uniformity in plant characters.
  - o To improve yield etc. by individual plant selection as in cucurbits in which there is no inbreeding depression.
  - o To develop suitable inbred lines in production of hybrids and synthetics.
- **Synthetic Variety:** The term 'synthetic variety' has come to be used to designate a variety that is maintained from open pollinated seed following its synthesis by hybridization in all combinations among a number of selected genotypes, which have been tested for combining ability.

The components of a synthetic variety could be inbred (usually), clones, mass selected populations or various other materials. The component units are maintained so that the synthetic may be reconstituted at regular intervals.

The inbreds to be used as component lines are chosen on the basis of combining ability tests. The component inbred are crossed in all possible combinations. This inter-crossed seed is called as Syn 0.

Equal quantity of seed from all crosses is composited and the mixture is allowed open-pollination in isolation and seed is harvested. This becomes Syn 1 generation. In absence of reconstitution of a synthetic at regular intervals, the population becomes an open-pollinated variety.

The testing for combining ability is the decisive criterion for a synthetic variety by which it can be distinguished from a conventional variety of a cross-pollinating species, which originates in a continuous selection of individuals and subsequent progeny tests. The greater variability caused by crossing several components with high general combining ability makes the synthetic varieties more adaptable compared to conventional varieties.

- **Mass Pedigree Method:** In this method of breeding, the best individuals with desired characters are selected on the basis of phenotypic performance in a source population. Open-pollinated seeds of the selected individual plants are divided into two halves. Second year replicated progeny row trial is conducted using one set of half seeds from each plant.

On the basis of the progeny performance, the best parental individuals are identified. The remnant half seeds from the superior parental plants are mixed and grown in isolation for random mating during the third year.

This method of breeding is equivalent to ear-to-row selection in context of maize originally proposed by C.G. Hopkins at the Illinois Agricultural Experiment Station in 1896 to improve protein and oil content of maize. This method has been named as mass-pedigree method by S.S. Rajan in India. This very method is called line breeding when selection is based on progeny tests and a group of progeny lines is composited.

- **Recurrent Selection:** Recurrent selection is a method of breeding designed to concentrate favourable genes scattered among a number of individuals by selecting in each generation among progeny produced by mating inter-se of the selected individuals (or their selfed progeny) of the previous generation.

Based on the ways in which plants with desirable characters are identified, recurrent selection has been divided into four types.

These types are:

- Simple recurrent selection or recurrent selection for phenotype
- Recurrent selection for general combining ability
- Recurrent selection for specific combining ability
- Reciprocal recurrent selection

In simple recurrent selection a number of plants are self-pollinated in a source population in first year. At maturity superior plants based on phenotypic performance are selected. In second year, seeds produced by self-fertilization of the selected

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plants are planted and crossed in all possible combinations and the produce is bulked.

This completes original selection cycle. Since selection is based on the phenotype of the plant, it is useful only for characters with high heritability. In those cases, where it is possible to identify the desired selections before flowering as in case of cauliflower, cabbage, etc., inter-crosses of selections may be made in the first year of each cycle and the second year may be eliminated from each cycle.

Thus, strictly speaking, selfing is not an integral component of simple recurrent selection, rather it is done only to prevent crossing from the inferior pollen grains before the plants reach to selection stage.

In recurrent selection for general combining ability, a three year cycle is involved. In first year a number of plants are self-pollinated and crossed to a broad based heterozygous tester stock to identify the  $S_0$  plants with good general combining ability. In second year, the crosses are evaluated to identify those that are superior. Self's of first year are kept in reserve.

In third year, the reserve self seeds are grown out, inter-crossed in all combinations, and a composite of inter-crossed seeds is used to establish an improved population for further selection. This procedure developed as a direct outgrowth of studies of early testing first proposed by M.T. Jenkins in 1935.

Recurrent selection for specific combining ability was proposed by F.H. Hull in 1945. This method of selection is same as that of recurrent selection for general combining ability except that the tester selected is a narrow base an inbred line. The recurrent selection for general and specific combining ability is equivalent to half sib progeny test.

Reciprocal recurrent selection proposed by R.E. Comstock, H.F. Robinson and P.H. Harvey in 1949 aims at simultaneous improvement of two heterozygous and heterogenous populations (designated as A and B).

A serves as tester for B and B serves as tester for A. This method is as effective as recurrent selection for GCA when additive gene action predominates, and is as effective as recurrent selection for sea when non-additive effects are of major importance.

The steps are as follows:

- Selected plants of population A are self-pollinated and crossed to plants of population B. Likewise plants are selected and self-pollinated in B and outcrossed to plants of population A.
- Test cross progenies of both the populations are evaluated in replicated trial. Superior progenies are identified on the basis of performance in this trial.
- Self seed from plants with superior test cross progenies are grown population wise separately and inter-crossed to reconstitute two populations which will be now called as A' and B'. This completes one cycle and additional cycle (s) may be initiated.

### **Check Your Progress**

1. What is the general pattern of breeding programme suitable for a particular species influenced by?
2. Does self-pollination require control over pollination?
3. How are nearly homozygous lines are produced in plant breeding?
4. What does hybridization between two inbreds lead to?
5. What does the mating of phenotypically dissimilar individuals reduce?

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## **13.4 METHODS FOR BREEDING OF VEGETATIVE PROPAGATED CROPS**

- **Domestication:** This is not majorly involved in vegetative propagated crops breeding, but is involved in the process. It is the process of cultivating vegetative crops and keeping them under human care and management.
- **Collection of Germ Plasm:** Germ plasm is the sum total of all genes present in a crop. The entire collection of vegetative propagated crops having all the diverse alleles for all genes in a given crop is known as Germ plasm collection. The germ plasm of any crop species consist of the following types of materials:
  - o Cultivated improved varieties
  - o All the wild species related to the crop species
  - o Improved varieties that are no more cultivated
  - o Old local varieties.

The collection of germ plasm from different sources id an essential first step in any breeding work, and germ plasm is usually stored at a low temperature, and it is cone within country or from other countries.

- **Evaluation and Selection of Parents:** The parents are evaluated to identify plants with desirable combination of characters.
- **Plant Evaluation:** This is the process of introducing plants of germ plasm either from a foreign country or introducing plants or germ plasm from one region to other region of the same country. After plant introduction, an adaptation period is followed. Plant introduction is done for the purpose of genetically improvement of economical crops and also for studying the origin, distribution, classification and evolution of plants.
- **Hybridization:** This is the mating or crossing of two plants or lines of dissimilar genotype. The objective of hybridization is to create genetic variation. When two dissimilar genotypical plants are crossed, the genes from both the parents are brought together in F1 generation. Segregation and recombination

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produce many new gene combination in F<sub>2</sub> and further generation. The aim of hybridization may be transfer of one or few qualitative characters, improvement of one or more quantitative traits or use of the F<sub>1</sub> as a hybrid variety.

- Selection of parents from available material possessing desired characters.
- Selfing of plants to obtain homozygosity in desired traits.
- Emasculation In this the anthers are removed before they mature and have shed their pollen.
- Bagging, tagging and labeling of males as well as females to be used in crossing.
- Crossing, in which the pollen from bagged males are spread on the bagged female plant.

### Hybridization in Vegetative Propagated Crops

We have two types of hybridization

- Interspecific.
- Inter – generic.

In interspecific hybridization: The plants of two different species belonging to the same genus are crossed together. It is also known as intra- generic hybridization. Disease, insect, drought and frost resistant varieties in wheat, tomato, sugarcane, etc. have been evolved by this method.

Gene introgression also known as introgressive hybridization in genetics is the movement of a gene from one species into the gene pool of another by the repeated backcrossing of an inter specific hybrid with one of its parent species.

*Solanum rybinii* is a wild diploid species of potato and is resistant to frost and virus infection. Another species *Solanum tuberosum* is cultivated and tetraploid species. The potato probably has the wildest genetic diversity among related wild species than any other cultivated plant, with the vast majority (74%) of the diploid species, and the rest are triploid, tetraploid, pentaploid and hexaploid (Hawkes, 1990).

- In most *Solanum* species, the germ plasm can not all be directly used for breeding due to a combination of diploid level and endoplasm balance number (EBN) incompatibility (Hewkes and Jackson, 1992).
- The majority of the diploid wild species can be directly crossed with dihaploids ( $2n = 2x = 24$ ) of cultivated potato. Dihaploid occur as result of parthenogenesis (haploid pollination technique) or another culture (Hermsen, 1994).

- Dihaploid x wild species can be crossed with cultivated potato through unilateral sexual polyploidization ( $4x - 2x$  crosses) using  $2n$  gametes. Also, two dihaploid x wild species hybrids that produce  $2n$  pollen and  $2n$  eggs can be crossed in a bilateral sexual polyploidization ( $2x - 2x$  crosses) to create novel tetraploids (Hermsen, 1994; Hanneman, 1999). The wild parents are selected for useful traits as tuberization, tuber quality traits, tolerance to environmental stresses and resistance to diseases and sexual fertility and  $2n$  gamete production before crossing with dihaploids.

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### Importance of Gene Introgression to Cultivated Germ Plasm

The importance of gene introgression to cultivated germ plasma is detailed below:

- The gene introgression was used for the introgression of late blight (*Phytophthora infestans*) resistance from the wild diploid species *S. microdontum* ( $2n = 4x = 24e$  2EBN) to cultivated potato ( $2n = 4x = 48e$  4EBN). A total of 175 clones from six accessions of *Solanum microdontum* were evaluated against the use of genotype.
- AZ matting type of *P. infestans* and 27 highly resistant ones were selected (Oouches et al., 2001).
- The quantitative trait loci conferring late blight resistance in *S. microdontum* could be followed through polyploidization and identified microsatellite used in a marker assisted selection program to introgress the resistance gene from a wild species to cultivated potato (Bisognin et al., 2005).
- Besides, gene introgression, interspecific hybridization was also involved in the origin of a new horticultural crop. *Solanum rybinii*, a wild diploid species of potato and is resistant to frost and viral infection and *Solanum tuberosum* are not crossed directly because *S. tuberosum* is tetraploid and the ploidy levels are different. So *Solanum rybinii* is first raised to tetraploid level by auto- polyploidy one then crosses with *Solanum tuberosum* to introduce the characters of the wild species.

### Inter-Generic Hybridization

Inter-generic hybridization is a crossing between plants belonging to two different genera. Inter generic hybridization is observed when sugar cane and sorghum are crossed. Sugar cane takes about 9 months to ripen and so no other crop can be grown. Sorghum is a short duration crop (3 to 4 months). So early maturing sugar cane varieties have been evolved by crossing with sorghum and since sorghum has less sugar content, the crossing results to a low sugar content hybrid, but by repeated back crossing of the hybrid with sugar cane early maturing varieties having normal sugar contents have been evolved.

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## 13.5 BREEDING METHODS IN APOMICTIC PLANTS

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Most plants reproduce sexually through seeds. A zygote is formed by fusion of reduced female and male gametes (amphimixis) and develops into an embryo. Plants of some species reproduce through seeds having an embryo which is formed without reduction of the chromosome number and fertilization. Such vegetative or asexual reproduction by means of seeds is called apomixis. Apomixis is found mostly in polyploidy species of the families Gramineae, Rosaceae and Asteraceae and confers fertility to hybrid genotypes which otherwise would have been sterile. Most successful apomictic species are facultative with sexual reproduction and apomixis being in equilibrium. Such species comprise sexual and apomictic entities, with several ploidy levels and are called agamospecies or agamocomplexes. Clausen (1961) explained the evolutionary adaptability and multitude of microspecies recognizable in agamocomplexes by their dual ability to sidestep sexual reproduction and to multiply the successful combinations asexually.

Apomixis occurs in numerous species of agricultural value. Among the grasses, it prevails in polyploidy species of *Panicum*, *Poa*, *Dichanthium*, *Pennisetum* and *Cenchrus*.

Apomixis is potentially powerful breeding tool to fix heterosis. But it has mostly been regarded as a barrier for the breeder because recombination by means of crossing is very difficult or even impossible. Transfer of apomixis to wheat, rice, maize, sorghum and millets is currently attempted. New techniques such as tissue culture, somatic hybridization, protoplast regeneration and genetic manipulation may contribute to the breeders ability to handle apomixis.

Apomixis may include asexual reproduction through vegetative organs such as rhizomes, stolons, bulbils as well as many modern biotechnological methods of asexual reproduction. Apomixis is synonymous with agamospermy, i.e., seed formation without fertilization of the egg cell. The routes are summarized as follows:

- **Diplospory:** A non-reduced embryo sac develops from an archespore cell through omission or restitution of meiosis; the egg cell develops parthenogenetically into an embryo, or another cell of the embryo sac divides and develops into embryo. The latter route is called apogamety.
- **Apospory:** The non-reduced embryo sac develops from a somatic cell of the nucellus or the integument instead of the embryo sac mother cell.
- **Adventitious or Nucellar Embryony:** The embryo develops directly from the sporophytic tissue, without formation of a gametophyte. This equals pure asexual reproduction. In many apomictic species, pollination is obligatory for the formation of endosperm and the development of egg cell; this is called psuedogamy. Although the egg is not fertilized, the central nucleus or one or two of the unreduced polar nuclei are. Pollen development



in apomictic plants can be as regular as in sexual plants giving haploid pollen. A special form of apomixis is haploid parthenogenesis. A normal, reduced egg cell or other nucleus in the embryo sac develops parthenogenetically into an embryo and seed, giving a plant with half the chromosome number of the parent. Apomixis offers many advantages to the breeder. A single outstanding plant of an obligate apomict is sufficient for a cultivar and cultivars are uniform, stable and easy to describe. Heterosis and epistatic gene effects can be fixed. For seed production the isolation distances between cultivars can be reduced. Finally, viral infections can be eliminated from the plants. These advantages weigh most heavily in less developed countries where breeding and seed production lack technological sophistication with high levels of variation in the species cultivars can be developed by collection and ecotype screening. Collecting new genotypes is easy where ecotypes have evolved that are adapted to various natural and man-made environments, The production of cultivars from ecotypes comes to its natural end when the best types have been collected. Some successful ecotypes have wide distribution and reappear regularly in breeders collection.

- **Hybridisation of obligate apomicts:** Fertilization of an unreduced egg sometimes occur in obligate apomicts but recently it was considered to occur too infrequently for practical plant breeding. Obligate apomixis precludes hybridization until exceptional sexual types as discovered.
- **Hybridisation of facultative apomicts:** A facultative apomict with a very low tendency to sexuality approaches the obligates type. Guayule is a facultative apomictic tetraploid species producing rubber. Breeding for increased rubber content has proved to be very difficult and breeding at diploid level is advocated as an attractive way to make quick progress as in potato. Doubling the chromosome number of selected diploids should yield elite tetraploids, which should be apomictic.
- **Induction of mutation:** Induction of variation by mutagenic treatment appears at first very promising, as useful mutations could be fixed by apomixis. Treating with X rays, dry seeds of the cultivar increased the number of aberrant from 2% to 14 %. The aberrant were weaker than the original cultivar, but some were atleast as vigorous. It was found that sexuality could be induced by X rays, make crosses and in later generations select new apomictic types with favorable characters.

### Tissue culture

The application of tissue culture techniques as a source of useful genetic variation. In *Poa pratensis* Wu and Jampates (1986) used tissue culture as a source of novel genetic variation. They initiated callus on shoot tip pieces of cultivars, after callus transfer, isolated regenerated shoots which were established in soil. In vitro

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regeneration of plants from immature endosperm could release variation in pseudogamous apomicts as the endosperm is the product of fertilization. Tissue culture of interspecific hybrids can provide for the introgression of desirable genes from the wild species into the crop due to chromosomal rearrangements. Somatic hybridization could be used in a different way of breeding apomicts.

### **Introducing Apomixis in Amphimictic Crops**

This could be achieved by interspecific hybridization between the amphimictic crop and related apomicts. Synthesis would involve combining different mutation for elements of apomixis e.g. unreduced egg cell development, suppression of recombination and parthenogenesis of the egg cell. Meiotic mutants are common in plant kingdom and have almost always incomplete penetrance. Such mutants could be employed to synthesize apomixis. Apomixis would be very useful for the production of true breeding heterozygous cultivars in self-fertilizing crops. New breeding tools and biotechnology may be useful for manipulating apomixis, both in apomictic crops and for introducing apomixis into sexual crops. Somatic hybridization could be an ideal tool to combine two elite genotypes into a new apomictic polyploidy which is expected to express the good characters of both the parents. When a symmetric protoplast fusion becomes possible between distantly related species, novel combinations of apomictic and sexual species may be obtained. Molecular analysis of the process of apomixis identify the genes responsible and enable their isolation, cloning and use in genetic transformation. Eventually breeders might make crosses and selections and transform the elite selections into apomictic cultivars. The ultimate breeders goal, complete mastery over meiosis and fertilization will be reached.

#### **Check Your Progress**

6. How is a zygote formed?
7. Where does apomixis occur?
8. From where does a non-reduced embryo sac develop?
9. What does apomixis include?

### **13.6 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS**

1. The general pattern of breeding programme suitable for a particular species is influenced by breeding system.
2. No, self-pollination does not require control over pollination.
3. In plant breeding nearly homozygous lines are produced by continued self-fertilization.

4. Hybridization between two inbreds usually leads to the recovery of the vigour lost by inbreeding.
5. The mating of phenotypically dissimilar individuals tends to reduce genetic correlation among progenies.
6. A zygote is formed by fusion of reduced female and male gametes.
7. Apomixis occurs in numerous species of agricultural value.
8. A non-reduced embryo sac develops from an archespore cell through omission or restitution of meiosis.
9. Apomixis may include asexual reproduction through vegetative organs such as rhizomes, stolons, bulbils.

## NOTES

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### 13.7 SUMMARY

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- The breeding system in plants is considered to be the sum of all genetic, physiological and morphological traits of a species that determines its optimum fitness i.e. adaptation to the immediate environment or to future changes.
- The commonly used breeding methods of self-pollinated plants were developed over a century ago in Europe.
- Numerous studies have focused on improving breeding efficiency. This presentation will comment some of these studies targeting an increased breeding efficiency of self-pollinated plants by the identification of the progenies/lines with highest genotypic merit and comment about strategies to obtain cultivars with a longer lifetime.
- In sexual organisms like crop plants, a greater and appropriate control over pollination or breeding system is the avowed mission of the plant breeder.
- A sudden or deliberate shift in the breeding system tends to alter the constellation of characters. Since the development of new and productive crop plants through appropriate genetic manipulation of variability is the prerogative of plant breeding and so great is the impact of breeding system on nature and rate of release of the variability in crops.
- Individual plant progeny is selected from  $F_2$  and subsequent generations, and their progenies are tested.
- Continuous inbreeding results in progressive loss of genetic variability until each individual has become homozygous for all or almost all the genes.
- Transference of pollen grains from the anther of a flower to the stigma of the same flower borne on the same plant is termed self-pollination or natural inbreeding. No external agencies are instrumental in achieving autogamy.
- The hybridization is done among two selected parents, after emasculation one become female parent and another male parent.

## NOTES

- $F_1$  generation seeds are space planted and selfing is allowed, each  $F_1$  will produce more  $F_2$  seeds.
- The selection procedure is same as previous year, only if two or more progenies coming from the same  $F_3$  progeny are similar and comparable, then only one may be saved and others may be rejected.
- Individual plant progenies of  $F_6$  generation are planted in multi-row plots and evaluated visually.
- Preliminary yield trial with three or more replications is conducted to identify few superior lines.
- The superior lines are tested in replicated yield trials at several locations. The above mentioned criteria are evaluated for these lines. The line which is superior to the best commercial variety may be released as new variety.
- Pedigree method is useful in selection of new superior recombinant types from a hybridization programme.
- Cross pollination is the movement of pollen grains from one flower to another borne on the same or different plant of the same or allied species, regardless of whether the flower is bisexual or unisexual.
- The only thing needed is the production of an abundance of pollen grains, particularly in anemophilous plants. In cross pollination, two parents are involved and a mingling of the two sets of parental characters produces better progenies.
- Frequent cross pollination tends to create and maintain a tremendous amount of genetic variability chiefly because of the high degree of heterozygosity in populations.
- Role of dominance is potential. Release of recessive alleles on selfing lends credibility to this fact.
- The mating of phenotypically dissimilar individuals tends to reduce genetic correlation among progenies.
- Inbreeding of cross pollinated crops leads to a rapid loss of vigour.
- Hybridization between two inbreds usually leads to the recovery of the vigour lost by inbreeding. These consequences make outbreeders most exploitable for crop improvement.
- In crops like cotton, sorghum and sudan grass, the amount of cross pollination that occurs varies considerably.
- The mating of individuals more closely related than individuals mating at random is known as inbreeding.
- In plant breeding nearly homozygous lines are produced by continued self-fertilization accompanied by selection for five to six generations.

- The term ‘synthetic variety’ has come to be used to designate a variety that is maintained from open pollinated seed following its synthesis by hybridization in all combinations among a number of selected genotypes, which have been tested for combining ability.
- Inter – generic hybridization is a crossing between plants belonging to two different genera.
- Apomixis may include asexual reproduction through vegetative organs such as rhizomes, stolons, bulbils as well as many modern biotechnological methods of asexual reproduction.
- In autogamy system of mating, crosses between unrelated plants are either excluded or they occur at a very low frequency than would be expected on the basis of random mating. The intensity of inbreeding determines the rate at which the consequences of inbreeding are felt in descendants.
- Cross pollination is the movement of pollen grains from one flower to another borne on the same or different plant of the same or allied species, regardless of whether the flower is bisexual or unisexual.
- Apomixis is found mostly in polyploidy species of the families Gramineae, Rosaceae and Asteraceae and confers fertility to hybrid genotypes which otherwise would have been sterile.

## NOTES

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### 13.8 KEY WORDS

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- **Cross pollination:** It is the pollination of a flower by pollen from another flower of different plant.
- **Self-pollination:** It refers to the pollination of a flower by pollen from the same flower or from another flower on the same plant.
- **Vegetative propagation:** It is a form of asexual reproduction occurring in plants in which new plant grows from a fragment of the parent plant.
- **Breeding methods:** It is a method of altering the genetic pattern of plants to increase their value and utility for human welfare.
- **Apomixis:** It is the replacement of the normal sexual reproduction by asexual reproduction without fertilization

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### 13.9 SELF ASSESSMENT QUESTIONS AND EXERCISES

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#### Short Answer Questions

1. What are the breeding methods in self-pollinated plants?
2. Discuss the methods of breeding in cross pollinated plants.

## NOTES

3. Write a note on vegetative propagated and apomictic plants.
4. What do you mean by Sources of pollen?
5. Describe contrivances of pollination.

### Long Answer Questions

1. Write a detailed note on the breeding methods required for self-pollinated crops.
2. From your learning of the text, discuss in detail about natural self-pollination.
3. What are the breeding methods in apomictic plants? Also discuss about the pedigree method.
4. What do you believe is the importance of gene introgression to cultivated germ plasm? Explain.
5. “The breeding system in plants is considered to be the sum of all genetic, physiological and morphological traits of a species that determines its optimum fitness” Discuss.

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### 13.10 FURTHER READINGS

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# UNIT 14 INBREEDING DEPRESSION THEORIES, MUTATION BREEDING AND BREEDING FOR DISEASE RESISTANCE AND STRESS TOLERANCE

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*Inbreeding Depression  
Theories, Mutation Breeding  
and Breeding for Disease  
Resistance and Stress  
Tolerance*

## NOTES

### Structure

- 14.0 Introduction
- 14.1 Objectives
- 14.2 Inbreeding Depression Theories
- 14.3 Limits and Prospects of Disease Control by Genetic Means
- 14.4 Answers to Check Your Progress Questions
- 14.5 Summary
- 14.6 Key Words
- 14.7 Self Assessment Questions and Exercises
- 14.8 Further Readings

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## 14.0 INTRODUCTION

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Inbreeding depression is the reduced biological fitness in a given population as a result of inbreeding, or breeding of related individuals. Population biological fitness refers to an organism's ability to survive and perpetuate its genetic material. Inbreeding depression is often the result of a population bottleneck. In general, the higher the genetic variation or gene pool within a breeding population, the less likely it is to suffer from inbreeding depression.

Inbreeding depression seems to be present in most groups of organisms, but varies across mating systems. Hermaphroditic species often exhibit lower degrees of inbreeding depression than outcrossing species, as repeated generations of selfing is thought to purge deleterious alleles from populations. For example, the outcrossing nematode (roundworm) *Caenorhabditis remanei* has been demonstrated to suffer severely from inbreeding depression, unlike its hermaphroditic relative *C. elegans*, which experiences outbreeding depression.

Hybrid vigour is of interest to the plant breeder because of its beneficial effects on the yield and quality of domestic plants. These benefits may be produced in various ways. This is an expensive process and one which is a serious obstacle to the commercial utilization of hybrid vigour, particularly with agricultural crops. More favourable economic conditions are found in some horticultural crops, notably tomatoes, egg plants, cucurbits and onions. Here the flowers are relatively large, easily handled and one pollination produces many seeds. In addition, the produce

## NOTES

from one plant has a significant commercial value when viewed against the background of the total crop.

Mutation breeding, in the case of self-pollinated crops that are disseminated by seed, is based on the self-fertilization – or selfing – of mutants until the induced desired character is stably expressed in advanced mutant generations. Often backcrossing to the original non-mutated genotype (part of the DNA sequence of a cell that determines its specific characteristic) is necessary to retain its favourable characteristics.

Mutation breeding is built on mutation induction and mutation detection. It has many comparative advantages: it is cost effective, quick, proven and robust. It is also transferrable, ubiquitously applicable, non-hazardous and environmentally friendly. More than 3,200 mutant varieties – including numerous crops, ornamentals and trees – have officially been released for commercial use in more than 210 plant species from over 70 countries.

In this unit, you will learn about inbreeding depression theories, hybrid vigour in plant breeding, its mutation breeding. The unit will also discuss breeding for disease resistance and stress tolerance.

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### 14.1 OBJECTIVES

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After going through this unit, you will be able to:

- Discuss the theories of inbreeding depression
- Explain hybrid vigour in plant breeding
- Describe mutation breeding and breeding for disease resistance and stress tolerance
- Understand the impact of inbreeding
- Analyse genetic basis of inbreeding in allogamous crops
- Understand inbreds and variation within inbreds

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### 14.2 INBREEDING DEPRESSION THEORIES

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Hybrids are usually very vigorous in comparison with their parents. This vigour called hybrid vigour is associated with unusually high levels of heterozygosity found in hybrids, termed as heterosis. Biometrical genetic analysis shows that heterosis is commonly the result of dispersion of dominant alleles. Thus if one parent was AABbCcdd and the other was aabbCCDD, F1 hybrid would be AaBbCcDd. If all dominants additively and equally donated vigour effects, hybrid would be more vigorous than the parents. Increased fitness associated with dominance and heterozygosity at single locus is called heterozygote advantage and this may be expressed through increased vigour in comparison with homozygotes. The corollary of hybrid vigour or heterosis is inbreeding depression, for by definition inbred and



relatively homozygous individuals will be less vigorous than their heterozygous counterparts. The first major investigation of inbreeding depression and by far the most complete is by Darwin (1876). He compared the performance of lines that had been artificially selfed and artificially crossed for ten generations or more, in more than 40 species. From it, major conclusions that can be drawn are:

Repeated selfing renders the majority of species lines relatively less vigorous, when measured as height, weight or reproductive capacity. This is not true of all species lines investigated and some show little response to repeated selfing when compared to repeated crossing, inbreeding depression manifests itself in the first generation, but may increase for ten inbred generations, even a single outcross will tend to nullify inbreeding depression, especially when made to another inbred line. The amount of inbreeding depression expressed after ten generations differ between species and between lines of the same species. It is typical in an outbreeder to find inbred lines with about 70% vigour of outcrossed lines.

Vigour in inbred lines appear to be inherited and selection of vigorous inbred lines in successive generations may yield lines that are more vigorous than the most outbred. On the whole, normally outbred species show more response to selfing than normally inbred species. This is shown for cowslip for which normal heterostyled lines show marked inbreeding depression. Homostyle lines of horticultural origin show little if any inbreeding depression when selfed.

Inbreeding depression may express itself at different stages in the life cycle., In *Spergula arvensis*, a cornfield weed in which outcrossing is estimated as below 3%, heterozygote for seed coat character germinate faster than either selfed homozygotes. In the wild oat, *Avena fatua*, Allard et al. (1968) showed that survival after germination, the number of tillers produced per plant and the time to heading were all superior in the progeny of open pollination in comparison with progeny of artificial selfs This pertains despite the low level of outcrossing estimated in open pollinated progeny.

It cannot be assumed that the lower vigour of inbred lines confers less fitness on those lines in comparison with out crossed lines. For instance, slow growth rates or small stature might be favourable in suboptimal conditions. Fitness is not only an attribute of vigour but also of reproductive capacity and the lower production of reproductive units in less vigorous inbred line might be more than compensated for by its greater reproductive efficiency. In a very interesting recent study, Waller showed that the offspring of allogamous flowers outcompete the offspring of cleistogamous flowers from the same parent plant of the balsam *Impatiens capensis*.

Some competitive effects were heritable and density dependent and seedlings from allogamous flowers tended to be more variable than those from cleistogamous flowers for a number of attributes. This variability was considered to form an important component in competitive success. That inbreeding depression may be allied to most obvious genetic feature of selfing, scarcity of heterozygotes is not entirely self-evident. From this result, two important deduction can be made:

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- Heterozygote advantage and the heterosis may be frequency dependent, and therefore they may be more marked vigour response resulting from an occasional outcross in a predominantly selfed population
- Selfers with high levels of homozygosity may be able to maintain vigour by retaining heterozygosity at few loci with marked heterozygote advantage, in a selfing condition heterozygosity at these loci will be strongly selected for the tendency to homozygosity at these loci.

There is relationship between selfing, homozygosity and inbreeding depression is beyond dispute. The most useful concept at present is that of alternative metabolic pathways or shunts. The most vigorous individual is the one that is metabolically most efficient. Rates of metabolic reactions depend on each other, showing positive and negative feedback and thus dependent on metabolic bottlenecks. It is possible that inbreeding leads to reduced male fertility as a result of homozygosity in recessive partial male sterility genes and such infertility automatically selects for outbreeding events. Levin shows greater levels of female sterility after selfs rather than crosses.

Inbreeding is a form of mating system in sexual organisms. It implies mating together of individuals that are close to each other by pedigree relationship. Actually inbreeding mating promotes the union of gametes from related plants so that crosses between unrelated plants are either disfavoured or their frequency of occurrence is dampened compared with that expected on the basis of random mating. Any mating system which involves fewer number of ancestors than random mating entails some degree of inbreeding. Thus self fertilization is an extreme form of inbreeding carrying the implication that any line of descent has only single ancestor in each generation. Apart from this, preferential crossing between more or less distant but related individuals may occur. This is the weak form of inbreeding. Both intense and weak forms of inbreeding are useful for development of inbreds in outbreeding crop plants.

### **Impact of Inbreeding**

The most revealing impact of inbreeding is the loss of vigour and physiological efficiency of the organisms, characterized by reduction in size and fecundity, etc. A number of weak and lethal segregants and staglers appear in the population which has undergone inbreeding. This loss of fitness in the progenies or decline in trait expression with decreased heterozygosity arising from mating is known as inbreeding depression or inbreeding decline. Since the maximum decline is reflected in F<sub>2</sub> generation, the inbreeding depression can be computed by data on F<sub>1</sub> and F<sub>2</sub> for any character. The greater the intensity of inbreeding, the larger is the inbreeding decline. The effect of inbreeding is pretty variable in different crops belonging to diverse breeding system such as self and cross pollinated ones. The inbreeding depression is most pronounced in allogamous crops (like pearl millet, maize, alfalfa etc.) while it is tolerated in autogamous crops (like wheat, rice and barley etc.) For instance, some outbreeders such as cucurbits, sunflower and hemp don't exhibit loss of vigour on inbreeding. The decline in vigour is associated with number of heterozygous loci which is exposed on selfing. Cross pollinated crops carry heavy load of lethal recessives under the shelter of heterozygosity. The resulting

weak inbred lines in these crops are eliminated by natural selection and the surviving breeding populations are derived almost exclusively as a result of natural outcrossing. Therefore heterozygosity in self fertile outbreeding crops forms as a joint venture of breeding system and selection. On the other hand, in self-pollinated crops, inbreeding is of no consequence in so far as the loss of vigour is concerned, this is because almost all the genes are maintained in the homozygous state. Based on differences in the breeding behavior and its influence on the genetic system, the crop pollinated crop plants can be grouped for inbreeding depression as:

**Crops intolerant to Inbreeding**, i.e., a regular inbreeding tends to wipe out the lines concerned, since loss of vigour is drastic and damaging or may not be tolerated much. Thus two types of crops can be delineated i.e. where inbreeding is regularly deleterious not more than two selfings are tolerated, as in hayfield and where inbreeding is deleterious where three or more selfings are tolerated as in carrot

Though inbreeding results in a substantial loss of vigour, lines can be maintained as inbreds by regular selfing as long as desired. E.g. pearl millet, onion, maize, rye, orchard grass. The outbreeding cucurbits, hemsps and sunflower are as good as self-pollinated crops.

Characters that form an important component of fitness show a reduction on inbreeding, whereas characters that contribute little to fitness show little or no change in vigour. Therefore inbreeding is associated with the relative fitness of traits. Merit traits such as yield, height, variability are more influenced by inbreeding than meristematic traits such as floral parts, number of internodes and quantitative traits such as colour, shape, size of flowers and seeds etc. Inbreeding decline is the property of quantitative traits as against qualitative traits. Metric variability accrued from inbreeding is greater in the inbreds of naturally outbreeding species than in the heterozygous outbreeding populations of same species. In contrast, no such difference in the variance of metric traits between homozygotes and heterozygotes exist in inbreeders. Selection favouring only one type of homozygotes during the course of inbreeding tends to reduce genetic variance to zero as the types become fixed. Under high selection pressure (high selection intensity favouring only strongest plants during each generation of selfing), the rate at which homozygosis is achieved is slowed down.

### **Genetic Basis of Inbreeding in Allogamous Crops**

Inbreeding particularly in outbreeders leads to rapid homozygosity and marked depression of vigour. Let us discuss these in detail.

#### **Homozygosity**

Outbreeding species carry a substantial genetic load. Subjected to selfing, the heterozygous loci segregate and heterozygosity diminishes at the rate of 50 percent in each successive generation. Conversely, homozygosity increases with the same rate. For example, if a line heterozygous for one locus (Aa) is successively selfed, the increase in homozygosity is found. Homozygosity increased from 50 percent in S1 generation to 99.1 percent and heterozygosity reduced to 0.39 percent. Thus

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6-7 generations of selfing are enough to bring about almost perfect homozygosity for a single locus and cause differentiation between two homozygotes.

### Loss of Vigour

The loss of vigour on inbreeding in allogamous crops is essentially the consequence of mendelian segregation of heterozygous loci present in the original population, but not of the process of inbreeding itself. The sudden exposition of recessive homozygotes to natural and human selection, operating against them, results in the loss of fitness in recessive inbreds. The genetic basis of inbreeding depression in outbreeders can be explained by following mechanism

- **Heterozygote Superiority:** Heterozygotes are naturally superior to homozygotes due to overdominance. In contrast, homozygotes are weak and show depression
- **Directional Dominance:** Dominant alleles at major loci tend to act in the positive direction by favouring increase in size, number and viability of traits of an individual. Recessive alleles act in the opposite direction. In other words, dominance is directional towards greater vigour and luxuriance. In absence of such a directional dominance effect, i.e., if dominance is equally distributed among positive and negative alleles, then the loss of vigour is the natural consequence. Some degree of dominance is the common attribute of alleles by which change in heterozygosity would effect the level of performance. Thus larger the frequency of dominant alleles, greater is the inbreeding depression. If epistatic factors were involved, inbreeding would fail to change the mean level of performance without dominance.
- **Physiological Limit:** Inbreeding depression may occur due to physiological limit imposed by negative correlation between unit characters and the complex character. Such association tends to restrict the expression of the full potential of complex traits because of metabolic limits beyond which plant cease to develop normally.

### Inbreds and Variation within Inbreds

The rapid increase in homozygosity on inbreeding promotes genetic differentiation. As a result, numerous genetically different inbred lines are evolved. The number of such lines is governed by the number of heterozygous loci and by the variability of the inbred lines. A single diploid plant heterozygous for one loci can give rise to two genetically different inbreds. (AA and aa); that for five loci will get 32 inbreds; for 10 loci 1024 inbreds and thus plant heterozygous for n loci will produce  $2^n$  inbreds, provided no limitation of viability is imposed. Thus there lies a tremendous potential even in a single cross pollinated plant showing heterozygosity for a few or more loci to produce genetically diverse inbred stocks on selfing. Variation within inbred lines is purely of environmental nature, since a high degree of homozygosity would freeze genetic variation. Therefore, average phenotypic variance between highly inbred lines and their hybrids provide the best measure of environmental variance.

## **Inbreeding for Crop Improvement**

Darwin in 1868 concluded that free crossing is a danger on one side, which every one can see too close inbreeding is a hidden danger on the other. However inbreeding depression is not produced by the process of inbreeding itself, it is the consequence of the segregation of heterozygous loci in the original population. The consequence of inbreeding is of great significance in plant breeding as under:

- Inbreeding tends to increase the genetic correlation between relatives and in turn brings about an increase in the prepotency. The latter determines precisely the success of pure line breeding for improvement of the self-pollinated crops.
- Since inbreeding splits the population into genetically divergent families with little additive variance at intra family level but ample variability at inter family level, selection is effective only for between family and not for within family
- Inbreeding is useful for progeny testing, since close inbreeding is the only effective method of differentiating heritable differences from non heritable differences.
- Inbreeding is used to develop inbreds in cross pollinated crops for different purposes. Evidences are available that the frequency of haploidy is increased by homozygosity resulting from self-pollination in sweet corn. This is of great importance for the rapid development of inbred stocks.

Thus the role of inbreeding in plant breeding is to provide the basic material for breeding procedures. If two inbreds obtained from inbreeding are inter se mated, a tremendous gain in vigour in resulting hybrid progeny. This phenomenon is called heterosis and the gain in vigour is called hybrid vigour.

### **Heterosis- The concept**

The impulse of progress in crop improvement through plant breeding was propelled by exploitation of heterosis, the gain in vigour on crossing two inbreds. Actually, a decrease in vigour due to inbreeding and increase in vigour due to crossing are manifestations of the same phenomenon, i.e., heterozygosis. Thus heterosis which is one aspect of heterozygosis, another being inbreeding depression is the result of hybridization. Heterosis implies the excellence of F1 over strictly homozygous parents. Since inbred lines are weak, in outbreeding species, hybrids are likely to be heterotic and better than inbreds. A considerable degree of heterosis is encountered in both self and cross pollinated crops, but it is smaller in former than in latter group. The hybrid vigour is increased on crossing genetically diverse inbreds more than on crossing closely related ones. Thus heterosis springs mostly from genetic diversity among the parents involved.

### **Categories of Heterosis**

Heterosis can be classified into three classes, these are discussed below:

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- **Intrapopulation Heterosis:** All cross fertilizing species carry a substantial genetic load. This burden of unfavourable alleles in cross breeders is not a liability. It provides the requisite genetic resiliency to its carrier for changing environmental conditions. Most of the loci show heterozygosity, a situation that is akin to F1 hybrids, and which confers considerable amount of hybrid vigour on the population. This situation is not warranted by hybridization but by occurrence of mutation. Cross pollination maintains it effectively. This heterosis arises from mutation.
- **Intervarietal Heterosis:** On crossing two homozygous varieties, the resulting F1 hybrid manifests a considerable degree of hybrid vigour. Heterosis is the function of a particular type of gene action manifested by parents in the heterozygote. A heterozygote is phenotypically more extreme than either of homozygotes and more viable and productive.
- **Interspecific Heterosis:** The heterosis resulting from hybridization between two races or species tends to cause an excessive increase in size, weight and growth rate in interspecific or interracial hybrids. It is also known as luxuriance, which is evolutionary accident brought about by the complementary action of parental genes. Both the extremes of vigour are equally disadvantageous, atleast to the organism. Luxuriance is pseudoheterosis. The luxuriant hybrids are poor competitors and are more frequently in domesticated than in wild species. Normal perpetuation of generation is restricted due to poor or no seed setting in luxuriant hybrids.

Three approaches have been traded to control pests and diseases, i.e., avoidance through quarantine, good husbandry, crop rotation, hygiene or sanitation of seeds and soil, control of vectors and preventive chemicals etc., direct control measures through various pesticides and fungicides and biological control through hyperparasites or predators and resistant crop varieties. Integrated approach involving an appropriate combination of two or more control measures and broad spectrum of pesticides. It should be noted that except for resistant varieties, all other measures of disease and pest control are recurring, hence costly and cumbersome. Use of chemicals against pest and pathogens is fraught with dangers of pollution and health hazards due to lack of expertise in application of chemicals. Therefore, resistant crop varieties are the most effective, most economical and least hazardous measure of disease control. Resistant cultivars evolved through directed plant breeding practices have contributed to the welfare of mankind by restricting or eliminating the pathogenic invasion on crop plants. They have stabilizing effect on crop production.

The committee of National Academy of Science in its report on Genetic vulnerability of major crops pointed out: To be unprepared is to maximize the impact of the epidemic. The more devastating epidemics are sudden. They catch us with no defence, no reserves and no back up potential. They hurt most when there are no alterations. There are numerous instances, across the world of tremendous crop losses and human hardship caused by sudden outbreak of epidemics. Potato famine of 1840 in Ireland, coffee rust of 1870 in Ceylon, sugarcane red rot of

1838-39, Bengal rice famine 1942, downy mildew epidemic of pearl millet of 1970-71. The recurrent epidemics of wheat rust in different parts of India

Resistant cultivars can be developed by breeding either for vertical resistance, or for horizontal resistance or for both combined. This decision has to be arrived at prior to the commencement of a breeding programme. It must be remembered that epidemiologically, no two plant diseases are alike. Every disease is unique in itself and must be treated on its own merit. From an economic view point, deployment of genetic resistance is more fruitful for epidemic diseases rather than for endemic diseases since losses due to latter are not significant. Near immune (vertical resistance) varieties promote a great directional selection pressure favouring resistance breaking variants of parasites. Hence development of partially resistant (horizontal resistance) varieties which minimize the effects of parasitism to an acceptable level by and large more sensible than to develop near immune cultivars.

- Vertical resistance is unlikely to be valuable in perennial crops or in crop that is difficult to breed, because replacement of variety is difficult with such crops.
- Vertical resistance is likely to be more valuable against a simple interest disease than against a compound interest disease because the infection rate is slower in the former than in the latter.
- Vertical resistance is unlikely to be valuable against a pathogen with a higher vertical mortality since frequent mutability of the pathogen jeopardizes the variety with vertical resistance.
- Against facultative parasites one strong gene is adequate for the exploitation of stabilizing pressure, against obligate parasites at least two strong genes are necessary.
- Vertical resistance is likely to be less valuable against diseases transmitted by the propagating material of the host.
- Vertical resistance will breakdown more quickly if the protection it confers is incomplete.
- Crop and plant patterns of vertical resistance in space are valuable against compound interest disease. The crop pattern tend to contain the infection rate hence is useful in controlling the compound interest disease.
- Vertical resistance is likely to be more valuable if there is a closed season (winter or long tropical dry season unfavourable for pathogen).
- Vertical resistance is likely to be more valuable when legislative control is possible, (by compelling or forbidding the planting of a particular vertical host population, etc.).
- Vertical resistance us likely to be more valuable when it is reinforced with useful levels of horizontal resistance for epidemiological reasons.
- The breakdown of a complex vertical resistance may result in the less disease from the breakdown of simple vertical resistance probably because of an inverse relationship between vertical and horizontal pathogenicity.

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## NOTES

- It is within the framework of these guiding principles that a sound strategy for control of diseases on crop plants can be envisaged.

### Check Your Progress

1. What is inbreeding depression?
2. What is hybrid vigour?

## 14.3 LIMITS AND PROSPECTS OF DISEASE CONTROL BY GENETIC MEANS

Breeding for disease resistance is precisely a purposeful manipulation of interorganism genetics. Therefore, limits to disease control by genetic means are set by nature and the number of resistance genes in the host, and by that of pathogenicity genes in the parasite. Nelson in 1973 listed a number of factors that restrict the success of a breeding programme aimed at evolving disease resistant crops cultivars. These are:

- **Lack of Resistance Gene:** When a cultivated species and its wild relatives apparently have no resistant genes, then genetic improvement is not possible. Such instances are very rare. If present, they are possibly due either to an incomplete survey of genetic variability or to improper screening techniques used. For instance, genotypes resistant to the ergot disease in the existing germplasm of pearl millet were reported to be non-existent. But scientist at ICRISAT (Hyderabad) could detect some resistant genotypes in the African germplasm
- **Passively Responsive Plant Organs and Poorly Specialized Pathogen:** Some plant organs, particularly storage organs are essentially passive in their response to pathogenic invasion, i.e., they do not react actively in their own defence. Similarly, pathogens invading storage organs are poorly specialized since they enter storage organs through wounds or natural openings like tentacles as if they penetrate directly though they are not known to do so. Some varieties with thick skin or small lenticels may escape disease while those with thin skin and wide, open lenticels may not. Thus escape is not a fine resistance mechanism. Hence breeding for resistance in storage organs of plant lacking fine resistance mechanism is a real limitation
- **Difficult Transfer of Alien Genes:** Though sophisticated lab techniques of cell and tissue culture are now known till they are not standardized with all crop species, the transfer of resistance genes from wild relatives to agronomically adapted cultivars of some crops pose a difficulty. Nelson stated that although disease resistance is identified in wild species of *Arachis* interspecific transfer of such genes to cultivated species has not been possible.



- **Undesirable Character Association:** Resistance genes are often linked with such traits as undesirable plant height, vigour, quality and yield. Sometimes a gene conferring resistance to one disease may be linked with susceptibility to other diseases e.g. genes controlling resistance to crown rust in oat are linked with susceptibility to Victoria blight.
- **Difficult Assembly of Polygenes:** VR genes can be selected for easily, but HR genes (polygenes) pose a rather formidable task to accumulate them in agronomically acceptable background. The reason is that genes conditioning HR are most often located on several different chromosomes. Absence of gene flow restricts the process of gene accumulation in selfers. However with appropriate techniques good success can be achieved.
- **Dangers of Cytoplasm Conditioning Susceptibility:** The dangers of genetic uniformity either due to the use of same T cytoplasm in maize (which caused the comleaf blight epidemic in 1970 in USA) and the Tifton 23A cytoplasm in pearl millet (which caused downy mildew assuming an epidemic proportion during 1970-71 in India. Non availability of diverse sources employing the same or a few cytoplasmic or genetic factors may jeopardize the resistance breeding programme. Diversification of the cytoplasmic factors and genetic base might offset such danger.
- **Incredible Physiological Specialization by Pathogens:** Many pathogens are known to produce endless number of new and highly virulent races. A matching response by host genotypes is not possible at that scale. This implies that resistance breeding may be a highly tenuous proposition, since the availability of new resistance genes is not so far. In addition, there is at least one apparent weakness in current plant breeding procedure for disease resistance, mainly in self-pollinated crops.

## NOTES

### **Pest Resistance in Crop Plants: The Concept and Mechanisms**

Insects pose problems mainly in tropical regions rather than in temperate and arid zones, since high temperature tends to enhance insect activity and the reproductive potential. The resistance to pests is at variance with that to pathogens in following aspects:

The rate of multiplication is higher in plants pathogens than in plant pests. Therefore, the spread of disease is faster than that of pest attack. A host plant may support millions of pathogens systemically, while the number of pests per plant can be counted in hundreds, rarely in thousands

The resistance of host plants to pests, not promptly responsive, is attributed to the existing resistance mechanisms. In contrast, the host plant immediately responds hypersensitively to a pathogen invasion. In view of these factors, breeding for pest resistance has not been so frequent as for disease resistance. Plant breeders lack an understanding of the dynamics of insects with regard to their hosts and they regard the insect population as a fixed environmental parameter with all implications. The yield loss due to pests are less grave than due to diseases.

## NOTES

### Mechanism of Pest Resistance

Pest resistance is the collective heritable characters that tend to reduce the probability of a plant to act as a host to the insect or pest. The main resistance mechanisms are non-preference, antibiosis and tolerance and pest avoidance.

**Non preference:** Any inherent feature of the host plant which discourages the feeding, colonization, oviposition or shelter of insect/ pest makes it non preferred by the pest. The non-preference of insect is expressed either in having no contact at all or having limited contact with the resistant host e.g. some biochemical compounds are repellent to pests, some essential oil in tomato against mites, saponins in roots of some varieties or high tannin content in grains of sorghum against birds. Many plant compounds like sugars, amino acids and vitamins act as feeding stimulants to pests, their low concentration might be repellent to pests.

- **Antibiosis:** This relates to the production by host plants of chemical factors injurious to pests, when invaded by the latter. Such antibiotic compound diminishes the pest population, manifested by decreased size, shortened life span, decreased reproduction and increased mortality of the pest. High concentration of chemical compounds like gossypol, a polyphenolic compound released by cotton against many insects/pests.
- **Tolerance:** It enables the host plant to produce yield regardless of insect attack. It does not in any way restrict the pest invasion, nor does it disturb the reproduction of the pest. But it tends to minimize the damage to the host. Thus a tolerant variety will grow more normally and produce higher carry home yield than a sensitive variety under the same regime of pest infestation.
- **Cross Resistance:** Under host pest relationship, some factors cause resistance in a host to one insect may protect it from another.
- **Pest Avoidance:** When the pest population is at its peak, and the host plant is at its susceptible age, pest incidence is maximum. The susceptibility accrues from a coincidence of the life cycle, in whole or part of both the pest and plant with respect to their environmental requirements. Pest avoidance can be achieved successfully by breeding plants to grow outside the insect's environmental optima range.

#### Check Your Progress

3. What is the rate of multiplication in plants as compared to plant pests?
4. What kind of traits are resistance genes are often linked with?

### 14.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Inbreeding depression is the reduced biological fitness in a given population as a result of inbreeding.

2. Hybrid vigour is the tendency of a cross bred individual to show qualities superior to those of both parents.
3. The rate of multiplication is higher in plants pathogens than in plant pests.
4. Resistance genes are often linked with such traits as undesirable plant height, vigour, quality and yield.

*Inbreeding Depression  
Theories, Mutation Breeding  
and Breeding for Disease  
Resistance and Stress  
Tolerance*

## NOTES

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### 14.5 SUMMARY

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- Inbreeding depression is the reduced biological fitness in a given population as a result of inbreeding, or breeding of related individuals.
- Mutation breeding, in the case of self-pollinated crops that are disseminated by seed, is based on the self-fertilization – or selfing – of mutants until the induced desired character is stably expressed in advanced mutant generations.
- Mutation breeding is built on mutation induction and mutation detection. It has many comparative advantages: it is cost effective, quick, proven and robust.
- Hybrids are usually very vigorous in comparison with their parents.
- Repeated selfing renders the majority of species lines relatively less vigorous, when measured as height, weight or reproductive capacity.
- Vigour in inbred lines appear to be inherited and selection of vigorous inbred lines in successive generations may yield lines that are more vigorous than the most outbred.
- Inbreeding depression may express itself at different stages in the life cycle., In *spergula arvensis*, a cornfield weed in which outcrossing is estimated as below 3%, heterozygote for seed coat character germinate faster than either selfed homozygotes.
- Selfers with high levels of homozygosity may be able to maintain vigour by retaining heterozygosity at few loci with marked heterozygote advantage, in a selfing condition heterozygosity at these loci will be strongly selected for the tendency to homozygosity at these loci.
- The loss of vigour on inbreeding in allogamous crops is essentially the consequence of mendelian segregation of heterozygous loci present in the original population, but not of the process of inbreeding itself.
- Dominant alleles at major loci tend to act in the positive direction by favouring increase in size, number and viability of traits of an individual. Recessive alleles act in the opposite direction.
- Inbreeding depression may occur due to physiological limit imposed by negative correlation between unit characters and the complex character. Such association tends to restrict the expression of the full potential of complex traits because of metabolic limits beyond which plant cease to develop normally.

## NOTES

- The rapid increase in homozygosity on inbreeding promotes genetic differentiation. As a result, numerous genetically different inbred lines are evolved.
- Variation within inbred lines is purely of environmental nature, since a high degree of homozygosity would freeze genetic variation. Therefore, average phenotypic variance between highly inbred lines and their hybrids provide the best measure of environmental variance.
- Inbreeding tends to increase the genetic correlation between relatives and in turn brings about an increase in the prepotency.
- Vertical resistance is likely to be more valuable when legislative control is possible, (by compelling or forbidding the planting of a particular vertical host population etc).
- Vertical resistance is unlikely to be valuable in perennial crops or in crop that is difficult to breed, because replacement of variety is difficult with such crops.
- The impulse of progress in crop improvement through plant breeding was propelled by exploitation of heterosis, the gain invigour on crossing two inbreds.
- Vertical resistance is likely to be more valuable if there is a closed season (winter or long tropical dry season unfavourable for pathogen).
- Vertical resistance is likely to be more valuable against a simple interest disease than against a compound interest disease because the infection rate is slower in the former than in the latter.
- Inbreeding tends to increase the genetic correlation between relatives and in turn brings about an increase in the prepotency.
- The impulse of progress in crop improvement through plant breeding was propelled by exploitation of heterosis, the gain invigour on crossing two inbreds.
- The heterosis resulting from hybridization between two races or species tends to cause an excessive increase in size, weight and growth rate in interspecific or interracial hybrids.
- Three approaches have been traded to control pests and diseases i.e avoidance through quarantine, good husbandry, crop rotation, hygiene or sanitation of seeds and soil, control of vectors and preventive chemicals etc., direct control measures through various pesticides and fungicides and biological control through hyperparasites or predators and resistant crop varieties.
- Vertical resistance will breakdown more quickly if the protection it confers is incomplete.
- Crop and plant patterns of vertical resistance in space are valuable against compound interest disease. The crop pattern tend to contain the infection rate hence is useful in controlling the compound interest disease.
- Vertical resistance is unlikely to be valuable against a pathogen with a higher vertical mortality since frequent mutability of the pathogen jeopardizes the variety with vertical resistance.

- Breeding for disease resistance is precisely a purposeful manipulation of interorganism genetics.
- Increased fitness associated with dominance and heterozygosity at single locus is called heterozygote advantage and this may be expressed through increased vigour in comparison with homozygotes.
- The loss of vigour on inbreeding in allogamous crops is essentially the consequence of mendelian segregation of heterozygous loci present in the original population, but not of the process of inbreeding itself.
- Inbreeding tends to increase the genetic correlation between relatives and in turn brings about an increase in the prepotency. The latter determines precisely the success of pure line breeding for improvement of the self-pollinated crops.
- Resistant cultivars evolved through directed plant breeding practices have contributed to the welfare of mankind by restricting or eliminating the pathogenic invasion on crop plants.
- Near immune (vertical resistance) varieties promote a great directional selection pressure favouring resistance breaking variants of parasites.
- Development of partially resistant (horizontal resistance) varieties which minimize the effects of parasitism to an acceptable level by and large more sensible than to develop near immune cultivars.

## NOTES

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### 14.6 KEY WORDS

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- **Inbreeding:** The production of offspring from the mating or breeding of individuals or organisms that are closely related genetically
- **Hybrid vigour:** The improved or increased function of any biological quality in a hybrid offspring. An offspring is heterotic if its traits are enhanced as a result of mixing the genetic contributions of its parents
- **Breeding:** The mating and production of offspring by plants or animals to produce desirable characters
- **Resistance:** Hybridization is done when resistant genes are available either in the germplasm or in wild species of crop plants.

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### 14.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

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#### Short Answer Questions

1. What do you mean by hybrid vigour?
2. What is inbreeding? How is it different from hybrid vigour?
3. What is the impact of inbreeding?

## NOTES

4. List a few crops intolerant to inbreeding.
5. What do you mean by genetic basis of inbreeding in allogamous crops?
6. What are inbreds and variation within inbreds?
7. List the categories of heterosis.

### Long Answer Questions

1. "Repeated selfing renders the majority of species lines relatively less vigorous, when measured as height, weight or reproductive capacity." Discuss.
2. "There is relationship between selfing, homozygosis and inbreeding depression is beyond dispute." Discuss this relationship.
3. Write a detailed functional note on inbreeding.
4. "Characters that form an important component of fitness show a reduction on inbreeding, whereas characters that contribute little to fitness show little or no change in vigour." Explain.
5. "The impulse of progress in crop improvement through plant breeding was propelled by exploitation of heterosis." From your learning of the text discuss this in detail.

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## 14.8 FURTHER READINGS

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## NOTES

## NOTES