

STUDY OF MICROSCOPE

In recent times, many improvements have been made in the microscope, particularly in reference to proper use of various sources of light. Most commonly used microscopes are:

1. Light microscope 2. X-ray microscope 3. Electron microscope

1. Light microscope

In these microscopes, ordinary light is used as a source of illumination. There are series of microscopes that have been developed by using different advance techniques. Generally, in laboratory simple (dissecting) and compound microscope are used.

A. Simple microscope:

In this microscope, convex lens is used for purposes of dissection of material which is not possible to be done with naked human eyes. This microscope consists of basal foot and short limb. The upper end of the limb is fitted with a convex lens which may move horizontally. The foot is fitted with a reflecting mirror for source of light. In between mirror and lens a plan glass stage is situated. For viewing the object, one should bring his eyes close to lens and thereafter, lens is moved over the glass plate so as to adjust it at the desired distance and direction.

Parts of microscope:

1. **Eye piece:** The eyepiece is the top part the microscope; it is the lens you look through to see your specimen.
2. **Arm:** It is the large metal band attaching the base to the lens and eyepiece. When you carry a microscope, use one hand to hold the Arm, and place the other under the base.
3. **Fine Adjustment Knob:** It is the smaller round knob on the side of the microscope used to fine-tune the focus of your specimen after using the coarse adjustment knob.
4. **Course Adjustment Knob:** The Course Adjustment Knob is the largest knob out of the two knobs on the side of microscope. It is used to focus on the specimen; it may move either the stage or the upper part of the microscope (in a relative up and down motion). Always focus with the course adjustment knob first.
5. **Objective lenses:** Most of the microscopes have 2, 3, or more lenses that magnify at different powers. Always start with the lowest power and work your way up to the strongest when examining a specimen. The shortest lens is usually the lowest power.

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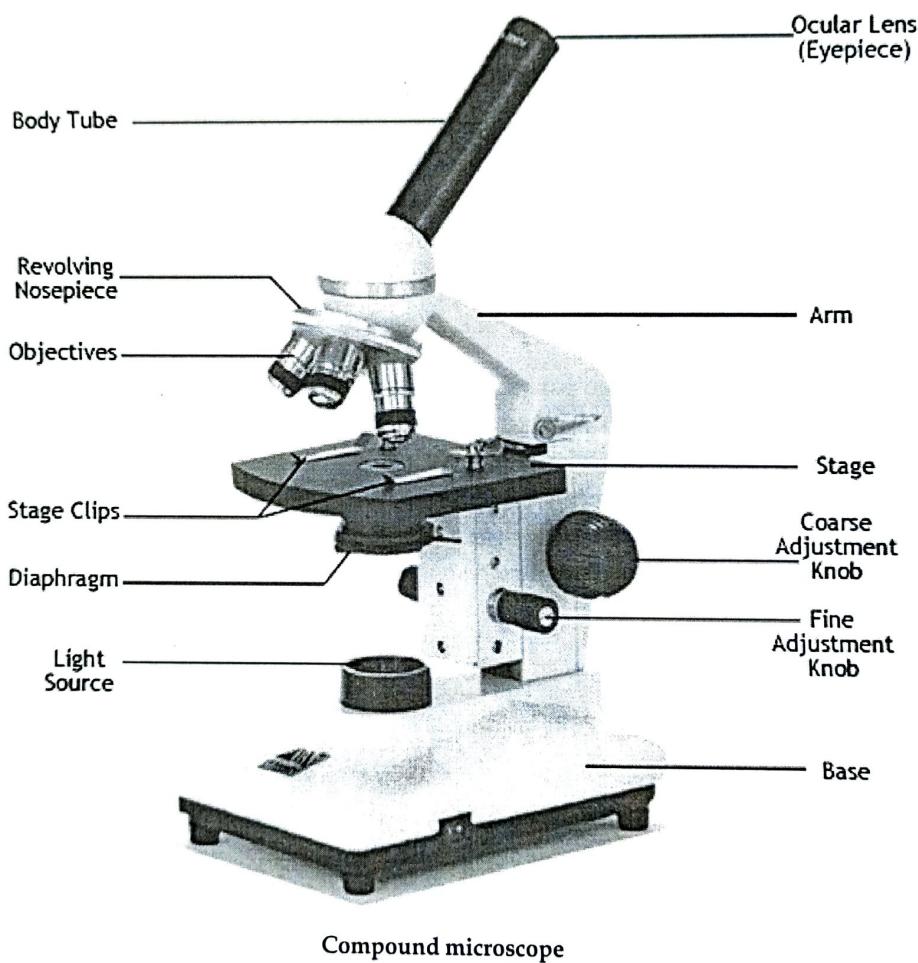
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6. **Stage:** It is where the sample or specimen is placed for examination.
7. **Iris diaphragm:** It is what allows you to control the amount of light on the specimen that comes through the stage (Through the Aperture).
8. **Light Source:** It can be a bulb or a mirror, and is usually found near the base of microscope shining up through the stage.
9. **Aperture:** It is the hole in the stage that allows light through for better viewing of the specimen.

B. Compound microscope:

The light microscope fitted with number of glass lenses is called compound microscope. The material, with a size of less than 50 microns across can be studied under this microscope. A compound microscope along with its component parts is shown in figure. Its main parts are described as follows:



- i. **Body of microscope:** A curved limb with a high joint forming main body of the microscope stand over the horse shoe shaped strong basal foot.
- ii. **Mirror stand:** Mirror with plane and concave sides, which can be moved to any direction for receiving and reflecting suitable light rays, is fixed at the lower most region of the limb.

- iii. **Stage:** A central aperture is attached at the stand, which works as a platform for mounting of the slide. The stage is provided with two clips which firmly hold slides in position. Some advance microscopes are provided with a mechanical stage along with rack and pinion for correct adjustment of the slide containing material on the stage.
- iv. **Condenser:** The condenser is fix below the stage for concentrating light rays which are reflected by the mirror on the object. The condenser is provided with an iris diaphragm in order to control the aperture for allowing the light to pass through the condenser lens. The condenser is also provided with gass filter.
- v. **Body tube:** The body tube is vertically attached with the limb. This tube cab be lowered or lifted by the help of adjustment screw, known as coarse and fine adjustment knobs.
- vi. **Nose piece:** At the lower end of the body tube, a resolving nose piece is attached which carries objectives of different powers of magnification.
- vii. **Objective:** The objective is made up of a system of lenses with short focal lengths for magnifying primary image of the material (object. The objective may be used as dry type or as immersion type.
 - a. **Dry type:** Space between objectives and object is occupied by air. Therefore, high rays passing through the material to the objective lens are refracted as a result of which resolution power of the microscope is affected.
 - b. **Immersion type:** Space between objective and object is filled by an oil of high refractive index. Thus refraction of light rays is completely prevented. The refractive index in immersion objectives range from 1.46 to 1.66 depending upon the kind of immersion medium used, whereas it remains 1 in case of dry objectives.

Magnification:

The total magnification of the microscope is the product of magnification powers of both eye piece and objective. If the eyepiece has 10X and objective, 40X the total magnification of the microscope will be $10x \times 40x = 400x$. In other words, image of the material is enlarge by 400 times of the original size. The magnifying powers of eye pieces and objectives are engraved on their metal bodies.

Resolving power of the microscope:

The resolving power of the microscope is defined as its ability to discriminate between closely adjacent structural components of the object and to reveal them as distinct and wide as possible. It may be determined from the following formula:

$$R = 0.61 \lambda / NA$$

Where,

R is the limit of resolution or minimum distance between any two resolved point.

λ is the wavelength of the light.

NA is the numerical aperture of the objective lens

The value of 0.61 is a constant which represents the minimum difference in detectable contrast.

The resolving power of a microscope is inversely proportional to the wavelength of light which means shorter the wavelength, more would the resolving power of the microscope. The magnification is different from the resolving power. By increasing magnification power, clearly of images decline but by increasing resolving power, clarity is restored. Resolving power depends on numerical aperture (NA) also. Higher the aperture, greater would the resolving power of the objective. The formula for NA is as follows:

$$NA = r \sin \mu$$

Where,

'r' is the refractive index of the medium present between the material and the objective lens and μ is the half of the single of the effective cone of rays entering in to the objective. The maximum value of μ is 90° and the $\sin \mu$ is $\sin 90^\circ$ which is equal to 1. Hence, the maximum NA of the objective lens can be 1X refractive index of the medium. NA of the objective lens using oil immersion medium is 1.4.

Handling of compound light microscope:

The following useful instructions may be kept in mind while using the microscope:

1. Place the microscope under a glass or cloth cover when it is not in use.
2. Knobs and screws of the microscope should be occasionally oiled.
3. Carry the microscope with both the hands in upright position after taking it out from the wooden case. Hold its arms with one hand and put other hand under the base.
4. Handle very carefully with almost no jerk on stiff mechanical stage, fine adjustment or nose piece, otherwise problems like inaccurate and hazy images along with disturbed alignment of lenses would result.
5. If the eyepiece, objective and mirror are dusty or cloudy, wipe than gently with the help of tissue paper unidirectionally.
6. Rotate the nosepiece to the extent that the low power objective comes in line with the body tube, a gentle jerk would signify the objective being set at the correct position.
7. Adjust mirror by turning it towards the source of light. Look through the eyepiece (ocular) and move the first surface of the mirror until it reflects light upward through the condenser and round hole of stage.
8. Adjust iris diaphragm so that the round screen (field of view) is evenly illuminated and does not show any glare.
9. Place the slide with the specimen on the stage of the microscope. Thereafter, focus the specimen under low power objective by moving the body tube downward with the help of coarse adjustment knob. Fine adjustment screw may also be used in order to receive clearer focus of the specimen.
10. In case the specimen is to be studied at higher magnification, raise the body tube slightly upward and then nose piece is rotated to the next desire higher power objectives. The specimen is focused again by using very carefully the fine adjustment screw.
11. For using oil immersion objective, place a drop of immersion oil on the cover slip of the specimen and rotate the nosepiece until oil immersion objective touches the oil drop. Look through the ocular and focus the specimen with fine adjustment screw. There should not be any air bubble left in the oil otherwise the proper image may not be seen.

Microscope Accessories:

Following microscopic accessories are generally in use:

1. Camera lucida
2. Illuminators
3. Light filters
4. Micrometers
5. Pointer eyepiece

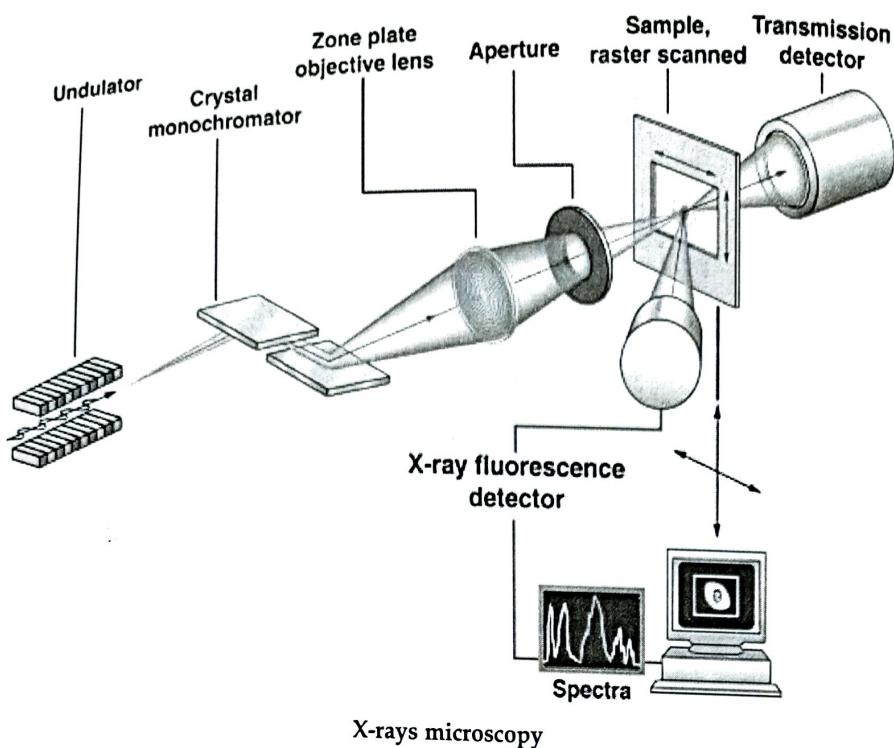
1. **Camera lucida:** It is device which enables a cytologist to sketch the exact outlines of the object seen under a microscope. The camera lucida consists of reflecting prism and mirror. The prism is silvered except in the centre which coincides with hole of eyepiece. Prism deflects the image of object at 90° angle which is further reflected at 90° by the mirror. The image so projected by the mirror may be sketched exactly on a paper sheet with the help pencil.
2. **Illuminators:** The illuminators are usually used as day light alternative during night or under such laboratory conditions where the sun light is not available for the microscope work. The illuminators produce the uniform and dependable light for the purpose
3. **Light filters:** Filters are necessary for good microscopy. They produce specific effects on light, for example, neutral filters (ultraviolet filters) cut down the intensity of illumination. The blue filter facilitated the effects of daylight to artificial light. Green filters are useful in studying the Feulgen, acetocarmine and aceto-orcein preparations.
4. **Micrometers:** A device consisting of ocular and stage micrometers is occasionally used to measure the exact size of the object under the microscope.
5. **Pointer eye piece:** The pointer eyepiece is used for indicating component of the object present under the field of view of microscope.

Precautions:

1. In monocular microscope, both eyes should be used alternatively and both should be kept open all the time as closing of one or the other may cause unnecessary strain and loss of vision.
2. Be careful against spilling water or stain on any part of the microscope specially on the stage, condenser and objective as even a trace of moisture can spoil optical components by growing fungus as well as the chemicals can cause corrosion.
3. The trace of dirt, stain or other chemicals should be washed with xylol.
4. All parts of the microscope should be thoroughly cleaned before and after use.
5. The coarse and fine adjustment screw, condenser, diaphragm, mirror and stage clips should be used carefully.

2. X-rays microscopy

The X-rays microscopy is very efficient cytological tool to analyze three dimensional molecular structure of cell. The X-rays have shorter wavelength with greater penetrating ability than electrons. It is, therefore, an advancement over electron microscopy. It possesses resolution power of about 0.25 microns. The device consists of electromagnetic lenses (made up of curved reflecting mirrors) which focus X-rays beam and as a result of which image is formed on the recording film.



3. Electron Microscope

The modern electron microscopes have a common basic construction pattern with standard components such as:

1. Electron generator (gun)
2. Electromagnetic coils (lenses) - 3 sets
3. Screen for viewing
4. Vacuum pump

An electron microscope is the instrument which helps biologists to study magnified image of ultra structure of various organelles of cells and tissues by means of electrons. It has very high resolution power because stream electrons possesses exceedingly short wavelength with the result that objects varying from 2000 \AA^0 and 3000 \AA^0 in diameter are detected easily. The wavelength of electrons is a function of voltage at which they are generated and can be expressed as:

$$\lambda = 12.2 / \sqrt{v} \text{ \AA}^0$$

where,

λ is the wavelength and v is the voltage at which electrons are generated. For example, at 50000 volts, the wavelength of electrons will be about 0.05 \AA^0 . The resolution of the electron microscope will be one -half of 0.05 \AA^0 is about 100000 times shorter than that of ordinary light (5500 \AA^0).

The electron microscopy has been the invention of scientists like Knoll and Ruska (1932), Marton (1934) and Preus and Miller (1934).

Types of electron microscope:

There are two basic types of electron microscope. There are :

1. Transmission electron microscope (TEM)
2. Scanning electron microscope (SEM)

(1) Transmission Electron Microscope (TEM):

Early electron microscope was, in fact, developed as electron transmitting microscope. The stream of electron produced in highly evacuated cathode tube is deflected towards the specimen by an electromagnetic field which acts as a condenser. After passing through the object, these electrons are again deflected by an electromagnetic coil, which works as an objective lens producing a magnified image of the object. This enlarged image is reviewed by the third electromagnetic lens called as an ocular or projection lens which further magnifies the image of the object for final viewing on the fluorescent screen or photographic plate. The image forms as result of differential scattering of electrons from molecular constituents of the cell.

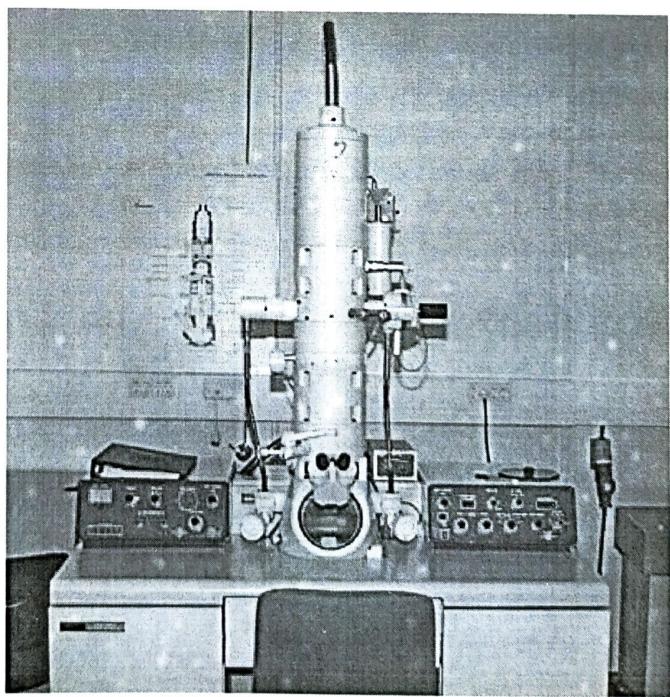
2. Scanning Electron Microscope (SEM):

SEM is primarily used for visualizing the surface architecture of the specimen (pollen grains, hairs, membranes, etc.) rather than the internal details. In a SEM, an electron beam is scanned across the surface and a three dimensional image is produced.

The construction plan and working principles of a SEM is different from that of TEM. In SEM, an accelerated beam of electrons is produced from electron gun and is focussed on the specimen by the condenser lens. The magnetic lenses of a SEM are so constructed to produce extremely thin beam of electrons.

The primary electron beam as it strikes the specimen, forces, out electrons from the surface (of the specimen). These are the secondary electrons and are transmitted to collector. During this process, some of the primary electrons also are reflected and transmitted to the collector, but their (primary electrons) number is far less than the secondary electrons. As a result, the image signal is developed more by the secondary electrons than by primary electrons. The electrons are then transmitted from the collector to a detector which has a substance that emits light when struck by electrons. The light so emitted is converted to an electrical current which is used to control brightness of an image on a CRT (Cathode Ray Tube) screen.

The secondary electrons deflected out of the specimen will be a replica of the refractive index of the surface and thus produce an image on the CRT screen revealing all the topographical details. Image contrast mainly depends on surface topography which determines the number of secondary electrons reaching the detector. The image on the CRT screen will be three dimensional. Magnification of the image of the specimen in SEM is not achieved through the lenses as in a light microscope or TEM. It is dependent upon the ratio of the length of the scan across the specimen surface of the length of the scan of CRT.



Scanning electron microscope

Practical Exercise

1. Sketch the diagram of compound microscope and label it?

2. Write the function of each of the following terms: Resolving power, contrast field of view, depth of field, working distance.

STUDY OF MICROSCOPE

3. Calculate the magnification power of the compound microscope?

4. Know the function of each part of the microscope.

Plant Cell Structure

Plant Cell Definition

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.

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.

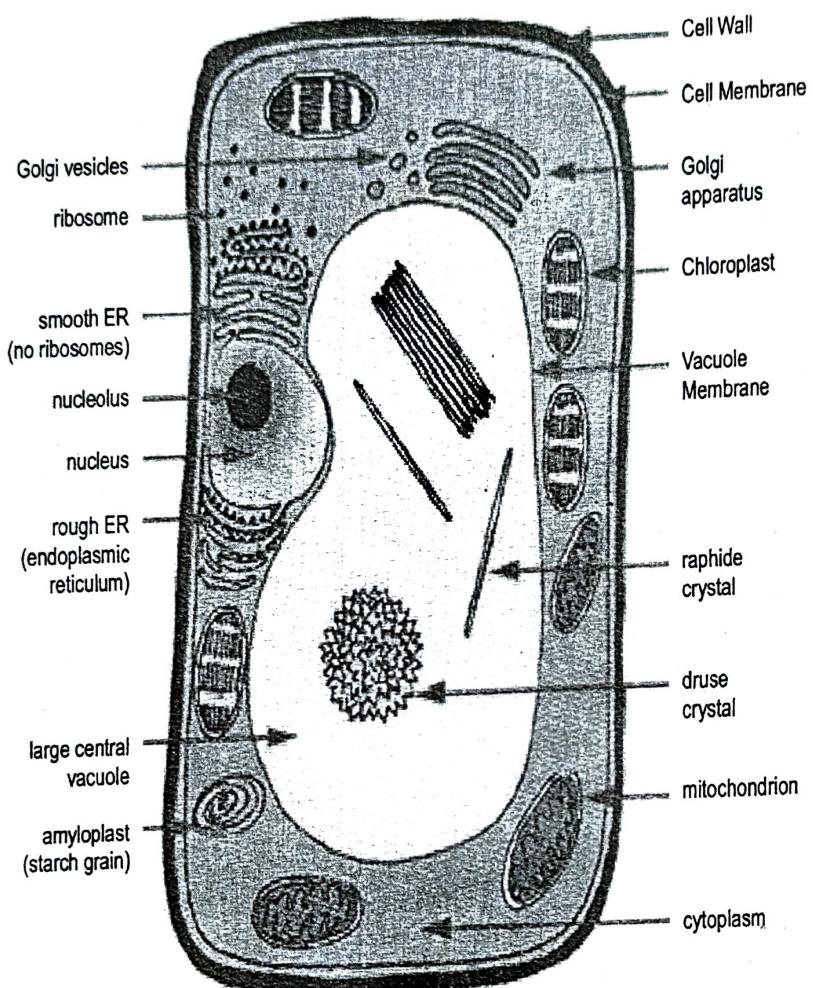
Structure of Plant Cell :

The plant cell has many different parts. Each part of the cell has a specialized function. These structures are called organelles.

PLANT CELL STRUCTURE

This diagram shows the various parts of a plant cell. Specialized structures in plant cells include chloroplasts, a large vacuole, and the cell wall.

Plant Cell



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 color. Thylakoids are stacked on top of one another in vascular plants in stacks called grana.

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 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.

Cell Wall:

The cell wall is a tough layer found on the outside of the plant cell that gives it strength and also maintains high turgidity. In plants, the cell wall contains mainly cellulose, along with other molecules like hemicellulose, pectin, and lignins. 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.

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**.

Mitochondria are also found in plant cells. They produce ATP through cellular respiration. 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.

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.

Difference between Prokaryotic cell and Eukaryotic cell:

All cells are broadly classified into prokaryotic cells and eukaryotic cells, according to whether their genetic materials are enclosed by a nuclear envelope or not.

Prokaryotic cells (pro-primitive, karyon- nucleus): From the morphological point of view, prokaryotic cells are the most primitive cells. They do not contain a definite nucleus. The chromatin bodies remain scattered inside the cytoplasm. Such a type of nucleus without a nuclear membrane is called a nucleoid. eg. bacteria, cyanobacteria (blue green algae) etc.

Eukaryotic cells: These are believed to have been evolved from the prokaryotes. They contain a definite nucleus. The chromatin bodies are enclosed by a nuclear membrane. Eukaryotic cells are larger than the prokaryotes. They show better structural organization and increased functional efficiency than prokaryotes.

Sr. No.	Prokaryotic cell	Eukaryotic cell
1.	Size is 0.1- 5.0 um	The size is 5-100 um
2.	Cell wall, if present, contains mucopeptide or peptidoglycan.	Cell wall, if present, contains cellulose, peptidoglycan is absent.
3.	A typical nucleus is absent.	A typical nucleus made of nuclear envelope, chromatin, nucleoplasm, nuclear matrix and nucleoli
4.	DNA content is low	DNA lies inside the nucleus, mitochondria and plastids.
5.	DNA is generally circular.	DNA is commonly linear
6.	DNA is naked or without any association with histone proteins.	DNA is associated with histones.
7.	Introns are commonly absent in DNA, RNA, therefore, does not require splicing.	Introns are quite common. RNA, therefore, requires splicing before becoming operational.
8.	Plasmids may occur.	Plasmids are rare.
9.	Cell membrane may have infolding called mesosome.	Mesosome absent
10.	Mitochondria are absent	Mitochondria are often present
11.	Ribosomes are 70 S	Ribosomes are 80 S occur in cytoplasm.
12.	Cytoplasm does not possess endoplasmic reticulum.	Endoplasmic reticulum is usually present.
13.	Golgi apparatus is absent	Golgi apparatus is present
14.	Lysosomes, sphaerosomes and glyoxysomes are absent.	They often present.
15.	Microtubules and microfilaments are rare.	They are usually present.
16.	Centrosome is absent	Centrosome is present except in flowering plants and a few others.
17.	Sexual reproduction is absent.	Sexual reproduction is commonly present.

18.	Cell division does not show distinction of interphase and M phase	A distinction of interphase and mitotic phase occurs during cell cycle.
19.	Endocytosis and exocytosis are absent.	They occur in eukaryotic cells
20.	Flagella are smaller. A distinction of axoneme and sheath is absent in the flagellum.	Flagella are longer. A flagellum shows distinction of axoneme and sheath.
21.	Cyclosis is absent.	Cyclosis or cytoplasmic streaming is common.
22.	It may have pili and fimbriae.	Pili and fimbriae are absent
23.	Transcription occurs in the cytoplasm	Transcription occurs inside the nucleus.

Supplementary Exercise

1. Sketch the plant cell structure and label it?

2. Sketch the diagrams of following cell organelles:
 i. Chloroplast

PLANT CELL STRUCTURE

ii. Vacuole:

iii. Mitochondria

Mitosis and Meiosis Cell Division

Cell is a basic unit of structure and function in all living systems. The process of reproduction of formation of new cells from the pre-existing cells is referred to as cell division. The cell which undergoes division is known as mother cell and the new cells which are formed by the process of cell division are termed as daughter cell.

Functions of cell division:

1. The main function of cell division is to produce daughter cells from a single cell. Repeated cell divisions generate a large number of cells. These cells function in :
 - a. growth and development of somatic tissues of organisms,
 - b. regeneration of damaged tissues,
 - c. production of new organs and tissues,
 - d. replacement of old organs and tissues and
 - e. asexual and sexual reproduction.
2. Cell division keeps the size of cells within a limit range.
3. New cells can not arise from pre-existing cells without cell division. Therefore, cell division is a prerequisite for the continuity of life and the evolution of various life forms.

Types of cell division:

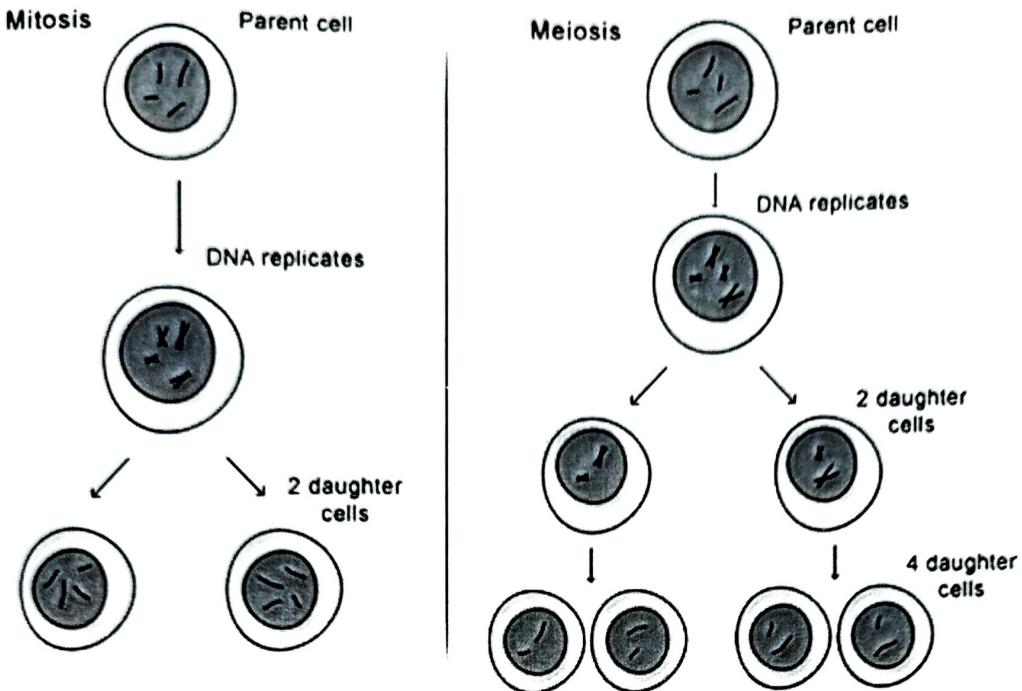
There are mainly two types of cell division: 1. mitosis and 2. meiosis.

1. **Mitosis:** When a cell makes two daughter cells with same chromosome number, it is known as mitosis.
2. **Meiosis:** When the cell is divided into four daughter cells with halved chromosome number, it is called meiosis.

The various events occurring during cell division may be grouped into two categories:

1. Karyokinesis and
2. Cytokinesis.

MITOSIS AND MEIOSIS CELL DIVISION



1. **Karyokinesis:** It is the division of chromosomes of a cell into two groups. These give rise to two daughter nuclei.
2. **Cytokinesis:** It is the division of cytoplasm of a cell into two halves to produce two daughter cells. Each cell ordinarily contains a single daughter nucleus. As a rule, cytokinesis follows karyokinesis.

Meiosis

In meiosis, growing cells has been separated in to the following various stages:

(A) First meiotic division:

- | | | |
|----------------|----------------|---------------|
| 1. Prophase I | (a) Leptonene | (b) Zygotene |
| | (c) Pachytene | (d) Diplotene |
| | (e) Diakinesis | |
| 2. Metaphase I | | 3. Anaphase I |
| 4. Telophase I | | 5. Interphase |

(B) Second meiotic division:

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

Note: In second meiotic division, each of the two daughter nuclei undergo a mitotic division. The distinguishing features of the various stages of second meiotic division are the same as those of the mitosis except few differences which are given below:

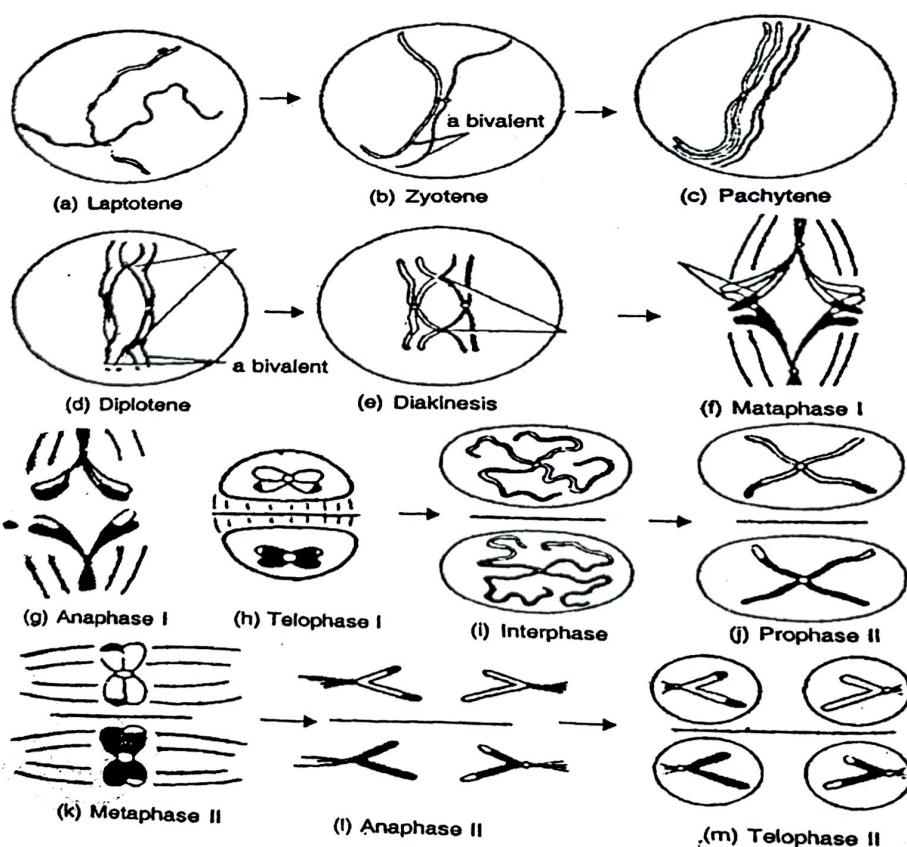
1. Chromosomes are widely separated from each other and exhibit no coiling.
2. Chromosomes are present in haploid number.
3. Both the nuclei may be seen undergoing meiotic division simultaneously.

(A) First meiotic division:

In meiosis, this stage is very long.

Prophase I: This stage can be identified into the following sub-stages:

- (a) **Leptotene:** In this sub-stage, chromosomes become visible and appear as single threads and longer. Generally chromosomes are distributed throughout the nucleus in a haphazard fashion.
- (b) **Zygotene:** Generally in this sub-stage, homologous chromosomes begin to pair and become shorter and thicker.
- (c) **Pachytene:** This sub-stage can be easily identified because chromosomes look as bivalents. Due to synapsis, chromosomes (bivalents) shorten and become thicker. Nucleolus is present and attached to one chromosome.
- (d) **Diplotene:** Paired chromosomes start separating longitudinally from each other. Each bivalent consist of four chromatids. Bivalents take an appearance of a cross at each point of contact which is known as chiasma. Chromatids can be seen crossing over. Chromosomes becomes shorter and their coiled nature is apparent. Nucleolus decreases in size.
- (e) **Diakinesis:** Separation of chromatids is complete. Chromosomes continue to shorten. Nucleolus starts disappearing and bivalents are found to be evenly distributed through the nucleus.



Metaphase I: Bivalents chromosomes arrange themselves on equatorial plate of the spindle fibres (equatorial plate) keeping their respective centromere to lie in two lines parallel to equatorial plate. Therefore, the centromeres are starts moving towards opposite poles.

Anaphase I: In this stage, paired homologous chromosomes now moves towards the opposite poles. Centromere moves ahead of the rest of the chromosome.

Telophase I: The chromosomes reach the poles and become uncoiled. The nuclear membrane reappear enclosing each group of chromosomes separately. Thus, two nuclei, each containing half number of chromosomes are formed.

(B) Second meiotic division:

This meiotic division is the same as that of mitosis except few differences which are given below:

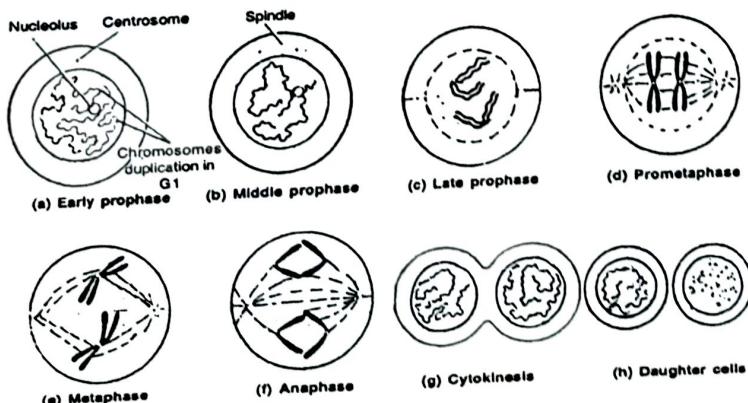
1. Chromosomes number is half
2. Chromosomes are widely separated and move towards the opposite poles.
3. Genetic constitutions of each chromosome is changed.

Mitosis

The different stages of mitosis that can be seen with the help of microscope. These are the following:

- | | |
|---------------|-------------|
| 1. Interphase | 2. Prophase |
| 3. Metaphase | 4. Anaphase |
| 5. Telophase | |

1. **Interphase:** This was originally called the resting phase of the cell and nucleus between two cell divisions. Cells grow in size; nucleus and heterochromatin's (pro-chromosomes) are seen in dark staining bodies. The nucleus in a fixed condition takes very little stain. Chromosomes are extremely thin showing a faintly network.
2. **Prophase:** In this stage can be clearly identified into the following sub-stages:
 - a. **Early prophase:** In this sub-stage, chromosomes are seen thin and long.
 - b. **Middle prophase:** In this sub-stage, chromosomes become shorter and thicker.
 - c. **Late prophase:** Comparatively former two sub-stages chromosomes become thicker and shorter. Each chromosome is longitudinally divided into two chromatids. In this sub-stage nucleolus and nuclear membrane are present.



3. **Metaphase:** In this stage, chromosomes become shorter and thicker. Nuclear membrane and nucleolus disappear. Chromosomes lie on equatorial plate. Each chromosome consists of two chromatids which are arranged towards the opposite poles.
4. **Anaphase:** In this stage, centromere of chromosome is divided into two parts. Daughter chromatids begin to move towards the opposite poles.
5. **Telophase:** In this stage, daughter nuclei form distinctly visible two groups of chromatids which reach towards respective poles. In this stage, two daughter nuclei are formed from one nucleus.

Significance of mitosis:

1. Mitosis is an extremely regular process. It maintains the qualitative and quantitative distribution of hereditary materials to daughter cells.
2. It maintains the constancy of the diploid number of chromosome of species.
3. It maintains the constancy and balance in the DNA and RNA content in cell.
4. It maintains the nuclear and cytoplasmic balance in the cell.
5. Mitosis results in the growth and development of the organism.
6. Repair and replacement of dead cells in ensure by mitosis.
7. It plays an important role in the asexual reproduction and in the formation of gametes in sexually reproducing organisms.

General distinction between mitotic and meiotic cell division

Mitotic division	Meiotic division
1. Shape of the cells in rectangular	1. Shape of the cells in round.
2. Generally the cells are attached to each other.	2. Generally the cells are scattered individually.
3. Chromosomes are seen to be comparatively longer.	3. Chromosomes are seen to be comparatively shorter.
4. Found in somatic cells	4. Found in the reproductive cells.
5. This type of cell division, each time the DNA duplicates the cell divide in two parts.	5. In meiotic division, the DNA duplicate only once but cell divides itself in to two parts.

Exercise 1: Preparation of temporary and permanent slides for cytological study

selection of material: Cytological study can be undertaken either through mitosis or meiosis. For mitotic study the fast developing regions like root tips or shoot tips are selected. The root tips of onion (*Allium cepa*) are widely used for mitotic study.

For meiotic study, the immature flower buds are selected. The selection of flower bus for meiotic study requires critical knowledge and experience of floral biology. The meiotic activities are greatly influenced by temperature. Hence the time of bud selection is an important aspect.

Exercise 2: Identification of various stages of mitosis and preparation of slides.

Preparation of slide for mitotic studies:

Plant Material:

Root tips of onion (*Allium cepa* L., $2n=16$)

The growing point of root tips of *Allium cepa* should be selected for mitotic studies.

Preparation of slide:

1. Excise young root tips either from plant or from germinating onion seeds.
2. Transfer the root tips to vials containing one of the pre-fixatives and keep at 10 to 14 °C for t_{W_0} to four hours.
3. Fix the root tips, after washing in water, in Carnoy's Fluid-I for 24 hours. The highest rate of mitotic cell division in onion is in between 1.00 to 3.00 pm.
4. Root tips are transferred to 70% ethyl alcohol and keep it at 4 to 10 °C for further use.
5. Place the pre-fixed or fixed root tips (not more than two at a time) in a watch glass containing 10 drops of 1% Aceto-orecin and one drop of 1 N HCL.
6. Heat the watch glass with materials, without boiling, over a flame for 10-15 seconds and allow it to cool.
7. Transfer a root tip to a clean slide and add one or two drops of 1% Aceto-orecin.
8. The material is squashed by tapping firmly with a flat end of metal rod or glass rod or pencil to separate the cells.
9. Put a cover slip and press the slide in folds of a blotting paper with the help of thumb to spread the cells and to remove excess stain.
10. Observe the prepared slide under compound microscope on 10 X and then shift it to 45 X to identify the various stages of mitosis.

Viewing Mitosis in Onion Root tips:

Why use onion roots for viewing mitosis?

- The roots are easy to grow in large numbers.
- The cell at the tip of the roots are actively dividing, and thus many cells will be in stage of mitosis.
- The tips can be prepared in a way that allows them to be flattened on microscopic slide so that the chromosomes of individual cells can be observed.
- The chromosomes can be stained to make more easily observable.

Supplementary exercise:

Q.1 A cell of following genotype of an organism undergoes mitosis. What will be the genotypes of the daughter cell?

1. AA
2. Aa
3. aa

Q.2 What is cell cycle? Draw a suitable labeled diagram of it.

Q.3 Write the significance of mitosis.

Exercise 3: Identification of various stages of meiosis and preparation of slides

Preparation of slide:

Plant Material: Young flower buds of *Tradescassia* ($2n=12$)

- Flower bud of *Tradescassia*. Select proper buds.
- Discard the flower which are already opened.
- Discard the mature buds having red colour.
- Select immature white buds with a slight pink or red in colour.
- Arrange selected buds on a moist blotting paper according to their size.

Procedure

- *Tradescassia* have five anthers.
- Fix the flower buds in Carnoy's Fluid-II solution for 24 hours. Transfer the material in to 70% alcohol and stored it at 10 °C for future use.
- Open a bud with the help of pointer.
- Take only one anther and put it on a clean slide with one drop of distilled water. Put a drop of 2% aceto-orcein.
- Crush the anther tissue with pointer and spread it on slide.
- Remove the anther tissues and make thin smear of pollen mother cell.
- Put a cover slip on the material, heat the slide gently and again tap gently to separate the cells.
- Put a blotting paper on it and press it with your thumb to remove the excess stain.
- Observe under the microscope 10X and 45 X magnifications.

Supplementary exercise:

Q.1 In garden pea, the somatic cells are diploid with $2n=14$. How many chromosomes are present in each cell at the following stages of meiosis?

- Prophase I :
- Metaphase I :
- Cytokinesis :
- Prophase II :
- Metaphase II :
- Cytokinesis :

Q.2 Differentiate between mitosis and meiosis.

-
-
-
-
-
-

Q.3 Write the significance of meiosis.

-
-
-
-
-
-

Q.4 Prepare cytological temporary slides. Draw its suitable diagram and write identification of different stages.

Q.5 Prepare acetocarmine or orcein.

	1/4 YY	1/2 Yy	1/4 yy
1/4 RR	1/16 RRYY	1/8 RRYy	1/16 RRyy
1/2 Rr	1/8 RrYY	1/4 RrYy	1/8 Rryy
1/4 rr	1/16 rrYY	1/8 rrYy	1/16 rryy

Summary :

Genotype	RRYY								
Genotypic ratio	1/16	2/16	2/16	4/16	1/16	2/16	1/16	2/16	1/16

(c) Phenotypic or Zygotic Checkerboard:

In this method, segregation for one character is place on left side and the segregation for another character on the upper side of the checkerboard. If genes for both characters show dominance then $Rr \times Rr$ will produce $3/4 R$ and $1/4 r$. Similarly $Yy \times Yy$ will produce $3/4 Y$ and $1/4 y$; multiply them to get phenotypic ratios:

	3/4 Y -	1/4 y y
3/4 R -	9/16 R- / Y -	3/16 R- / y y
1/4 r r	3/16 r r / Y -	1/16 r r / y y

Summary:

Genotype	Phenotypic ratio
R- / Y-	9/16
R- / yy	3/16
rr / Y-	3/16
r r / yy	1/16

Branching or Forked line method:

Another way of knowing segregation for more than two genes is branching or forked line method. When more than two genes are considered, the branching method is more suitable than checker board method which is time consuming, difficulties encountered in classifying and chances of error are more. This method is short and simple than checkerboard and can be used to find out all possible genotypic or phenotypic combinations.

Exercise 1: Determination of genotypes and phenotypes

The heritable features of an organism are called characters or traits. Characters are of two types viz., dominant which are expressed in F_1 and recessive which are suppressed in F_1 . The dominant character is also called wild character and recessive character is also known as mutant character. The dominant character is represented by capital letter and recessive character by small letter. For example, red flower is dominant over white hence, red flower is represented by R and white by r. These red and white colours are known as contrasting characters.

Alternative forms of a gene are called allele. Homozygous individuals have similar alleles (AA or aa) and breed true on selfing. Heterozygous individuals have dissimilar alleles (Aa) and segregate on selfing. Phenotypes refers to external appearance of an individual such as red flower and white flower. Genotype refers to genetic constitution such as RR for red flower and rr for white flower.

In monohybrid cross one pair of gene is involved. In dihybrid cross two pairs of genes are involved, each affecting a different character. Trihybrid cross involves three gene pairs. A cross between two individuals is called as direct cross, and cross in which order of male and female is reversed is called reciprocal cross. Cross of F_1 with one of its parents is called back cross and cross of F_1 to the homozygous recessive parent is called test cross.

- Test cross of monohybrid gives 1:1 ratio and dihybrid gives 1:1:1:1 ratio.
- Monohybrid with complete dominance gives 3:1 phenotypic ratio in F_2 generation.
- Monohybrid with incomplete dominance gives 1:2:1 phenotypic ratio in F_2 generation.
- A dihybrid with complete dominance gives 9:3:3:1 phenotypic ratio in F_2 generation.
- The number of gametes formed in F_1 is equal to 2^n , where n is the number of segregating gene pairs.
- The number of homozygous individuals obtained by selfing of F_1 = 2^n .
- The number of genotypes obtained in F_2 = 3^n .
- The number of phenotypes (complete dominance) obtained in F_2 = 2^n .
- The number of heterozygous genotypes in F_2 = $3^n - 2^n$.

Mendel gave the formulae for determining the number of genotypes and phenotypes in F_2 generation:

Mendel gave the following formulae for determining the different kinds of gametes produced by F_1 , heterozygote, different kinds of genotypes in F_2 , different kinds of F_2 genotypes that are homozygous, different kinds of F_2 genotypes that are heterozygous, total number of phenotypes in F_2 in case of complete dominance and perfect population size in F_2 .

When the number of gene pair differences is more than three, the number of possible combinations between them is greatly increased.

Q.2 In four o'clock plant, white flower colour is governed by a recessive allele 'r' and red colour by a allele 'R' which has incomplete dominance over 'r'. Determine the genotypic and phenotypic ratios expected from the following crosses:

(1) RR x rr

(2) RR x Rr

(3) Rr x rr

(4) Rr x Rr

Q.3 In garden pea, tallness is dominant over dwarfness and round seeded character is dominant over wrinkled seed. Determine the genotypic and phenotypic ratios expected in F_2 from a cross between tall round seeded strain (TTRR) and dwarf wrinkled seeded strain (ttrr).

Exercise 2: Study of monohybrid ratio and its modifications

What is Monohybrid?

A cross between two genetically dissimilar homozygous parents involving one gene pair controlling a single character is termed as monohybrid cross and their phenotypic F_1 ratio 3:1 (in case of complete dominance) is known as monohybrid ratio.

Complete Dominance:

When two parents differing in one independently inherited character governed by one pair of gene are crossed, the resultant F_1 is known as monohybrid. The parents involved in a cross are homozygous for dominant and recessive. The F_1 of a cross is heterozygous with dominant effect. This is according

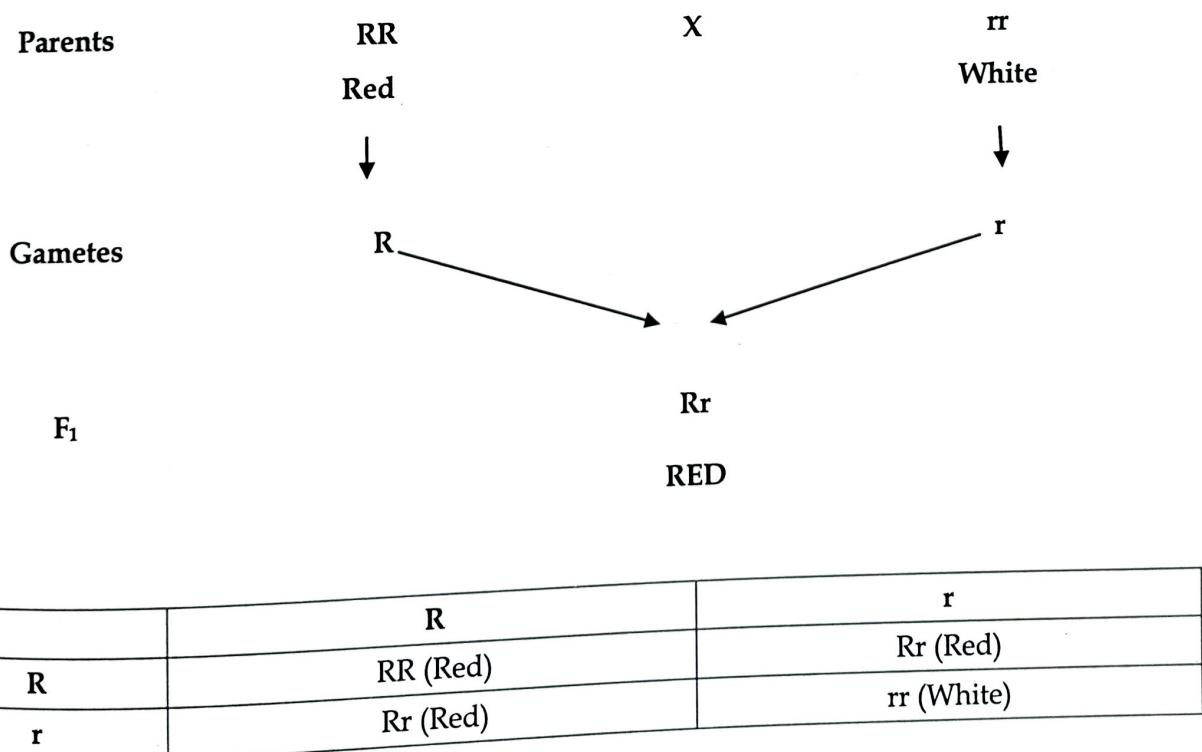
to Mendel's law of dominance in which the dominant gene suppresses the expression of recessive gene.

When F_1 is subjected to self-pollination to obtain F_2 generation, Mendel's second law of segregation of gene will produce one or more phenotypic class. Genes will be separated and enter into different gametes. In the F_2 population $\frac{3}{4}$ (75%) will be dominant and $\frac{1}{4}$ (25%) will be the recessive.

1. Only one allele of each gene pair is expressed in F_1 generation is known as dominant allele. The other allele which is not expressed in F_1 is referred as recessive allele.
2. Only one allele of a gene is present in gametes.
3. Two copies of a gene do not mix each other but stay together in the same cell of F_1 .
4. There is a separation of two alleles of each gene and there transmission into separate gametes of F_1 .
5. There is a production of two types of gametes by F_1 in equal frequencies.
6. A random union between the male and female gametes.
7. Characters of both the parents i.e. dominant as well as recessive characters appear in a definite proportion of 3:1 ratio in F_2 generation.

Presentation:

Mendel had conducted his experiment to find out the inheritance patterns of a gene character governed by a single gene pair. He had crossed red flowered pea with white coloured pea variety. He obtained F_1 with all red flowered plants. This was possible because red flower character is dominant over white flower. He obtained F_2 by self-pollinating F_1 in which he obtained red flowered plants and white flowered plant in ratio of 3 : 1 (i.e., $\frac{3}{4}$ were red flowered and $\frac{1}{4}$ were white coloured plants).



Frequency Distribution Table:

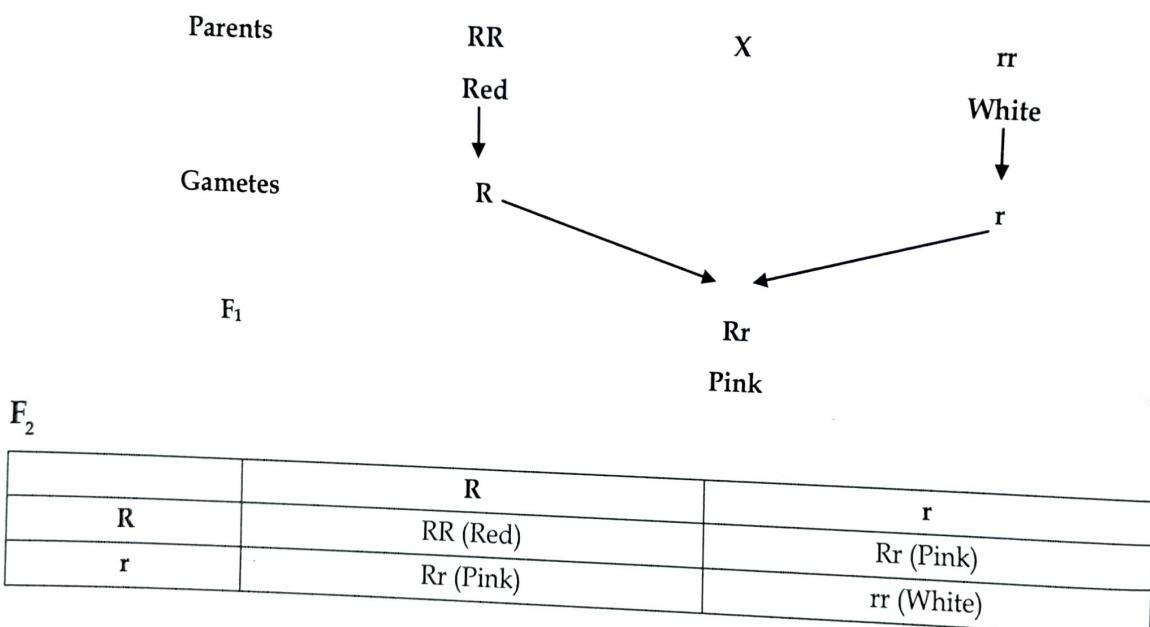
Genotype	Genotypic Frequency	Phenotype	Phenotypic Frequency
RR	1	Red	3
Rr	2	Red	
rr	1	White	1

Modification of Monohybrid:

There are two kinds of modifications in case of monohybrid. They are as under:

(A) Incomplete Dominance:

The red flowered plants of four O'clock or snapdragon are crossed with white flowered plant. The red flower colour is not completely dominant over the white flower. The red flower colour will be obtained when there is presence of two dominant genes, while both the recessive genes will produce white flower colour. In F_1 due to heterozygous condition both the parents fails to produce characters because neither red nor white is dominant over each other. Because of incomplete dominance, F_1 is intermediate type and all will be with pink coloured flower. When this F_1 is subjected to self-pollination, in F_2 red, pink and white flowered plants are obtained in the ratio of 1:2:1 respectively.



Frequency Distribution Table:

Genotype	Genotypic Frequency	Phenotype	Phenotypic Frequency
RR	1	Red	1
Rr	2	Pink	2
rr	1	White	1

(B) Co-dominance:

Allele which lack dominant and recessive relationship is called co-dominant alleles or intermediate alleles. In case of co-dominance, both allele express their phenotypes in heterozygous condition. For example, in case of ABO blood group in human. The people have blood type AB are heterozygous exhibiting phenotypes for both the I^A and I^B alleles.

The main difference between co-dominance and incomplete dominance lies in the way which genes acts. In case of co-dominance, both the alleles are active and expressed in F_1 , while in case of incomplete dominance, only one allele (dominant allele) is active and expressed in F_1 .

Supplementary Exercise:

Q.1 In foxes, silver black coat governed by a recessive allele "b" and red colour by its dominant allele "B". Determine the genotypic and phenotypic ratios expected from the following mating:

- (a) Pure red X Silver Black
- (b) Carrier red X Silver black

Q.2 In cowpea, white flower colour is governed by a recessive allele "w" and purple by its dominant allele "W". Determine the genotypic and phenotypic ratio expected from the following mating:

- (a) Pure purple X Carrier purple
- (b) Pure purple X white
- (c) Carrier purple X White

Q.3 Differentiate between followings:

- (1) Incomplete dominance and co-dominance
- (2) Backcross and Testcross

CALCULATION OF RATIOS

Q.4 A tall homozygous (TT) is crossed with a heterozygous plant (Tt). What are the genotypes and phenotypes of the offspring? Draw a Punnett square.

Q.5 In four O'clock flowers, red flower colour 'R' is incompletely dominant over white 'r', the heterozygous being pink flowered. In the following crosses, in which the genotypes of the parents are given, what are the gametes produced by each parent and what will be the flower colour of the offspring from each cross: Rr x RR, rr x Rr, RR x rr, Rr x Rr?

CALCULATION OF RATIOS

Q.6 Black wool sheep is due to a recessive allele 'b' and white wool to its dominant allele 'B'. A white buck (male) is crossed to a white ewe (female), both animals carrying the allele for black. They produce a white buck lamb which is then backcrossed to the female parent. What will be the resulting offspring from the cross?

Exercise 3: Study of dihybrid ratio

What is Dihybrid?

When two parents differing in two independently inherited characters governed by two pairs of genes are crossed, the resulting F_1 is known as di-hybrid. The ratio observed by self pollination the F_1 is known as di-hybrid ratio.

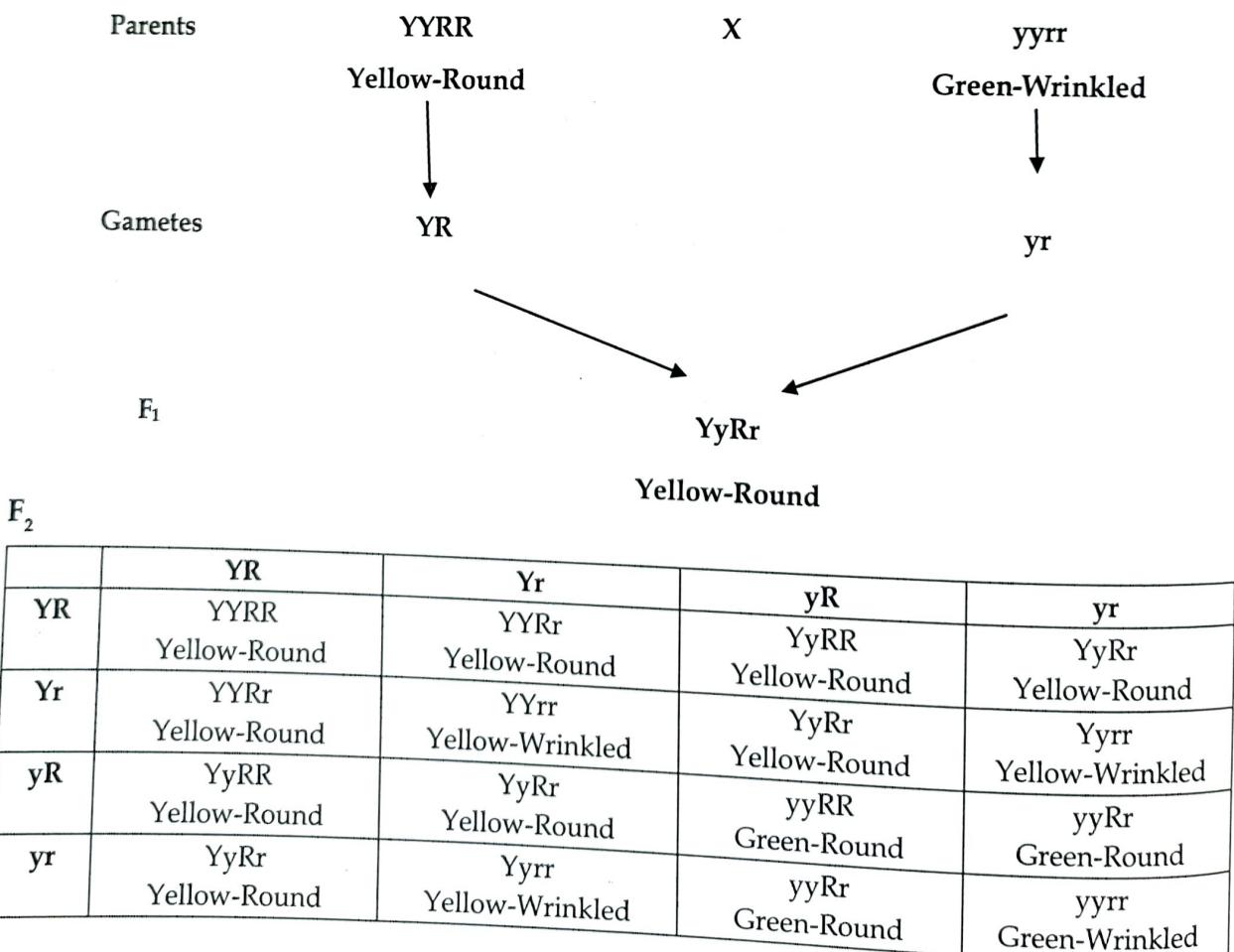
The parents in a cross are homozygous for dominant and recessive genes. The F_1 of across is heterozygous with dominant effect.

F_1 is subjected to self pollination to obtain F_2 generation. Hence characters segregate independently of each other.

Explanation:

Mendel made a cross between two pea plants which differ in two characters. He crossed plants having round yellow cotyledon colour with wrinkled green cotyledon colour. Yellow seed colour is dominant over green colour seed. Round shape of the seed is dominant over wrinkled shape seed. The F_1 population showed both the dominant characters.

All the plants were yellow-round, on self-fertilizing the F_1 generations, F_2 with four different phenotypes were observed in the ratio of 9:3:3:1. Two of the phenotypes resembled the parents and two of them were of new type-the recombinants.



CALCULATION OF RATIOS

Frequency Distribution Table:

Genotype	Genotypic Frequency	Phenotype	Phenotypic Frequency
YYRR	1	Yellow-Round	9
YYRr	2		
YyRR	2		
YyRr	4		
YYrr	1	Yellow-Wrinkled	3
Yyrr	2		
yyRR	1	Green-Round	3
yyRr	2		
Yyrr	1	Green-Wrinkled	1

Supplementary Exercise:

Q.1 In the garden pea, Mendel found that yellow cotyledon colour was dominant to green ($Y > y$) and round seed shape was dominant to shrunken ($R > r$).

- (a) What phenotypic ratio would be expected in the F_2 from a cross of a pure yellow, round X green shrunken:
- (b) What is the F_2 ratio of yellow:green and round:shrunken?

LABORATORY MANUAL ON FUNDAMENTAL OF GENETICS

Q.2 The position of flower on the stem of garden pea is governed by a pair of alleles. Flowers growing in the axils are produced by the action of a dominant allele "T", those governing only at the tip of stem by its recessive allele "t". The coloured flower are produced by a dominant allele "C" and white flowers by its recessive allele "c". A dihybrid plant with pure coloured flowers in the leaf axils is crossed to a pure strain of the white flowers at the tip of the stem. What genotypic and phenotypic ratios are expected in the F_1 and F_2 generations?

Exercise 4: Study of trihybrid ratio

What is trihybrid ratio?

When two parents differing in three independently inherited characters governed by three pairs of genes are crossed, the resulting F_1 is known as tri-hybrid. The ratio obtained by self-fertilization of F_1 is known as trihybrid ratio.

Explanation:

An individual heterozygous for three pairs of alleles, the parents involved in a cross are homozygous for dominant and recessive. The F_1 of a cross is heterozygous with dominant effect. When three pairs of characters are brought together in a cross, they segregate independently of each other.

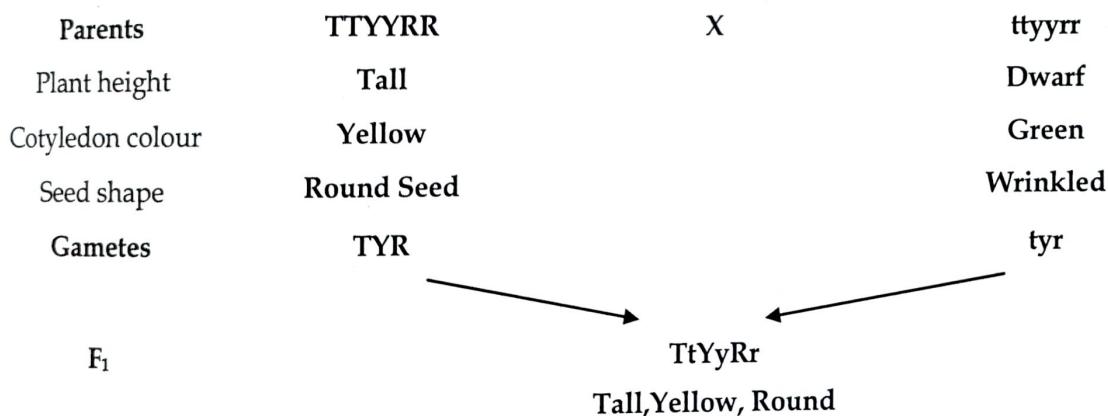
Example:

The following three pairs of characters are considered in garden pea.

1. Tall plant vs. Dwarf plant, i.e. TT vs. tt.
2. Yellow cotyledon Colour vs. Green cotyledon Colour, i.e., YY vs. yy
3. Round Seed vs. Wrinkled Seed, i.e., RR vs. rr.

The parents homozygous for dominance is having "TTYYRR" and parents homozygous for recessive is having "ttyyrr" are crossed. F_1 hybrid should have all the three dominant characters. On self-fertilizing the F_1 generation the F_2 generation with eight phenotypes in a ration of 27:9:9:3:3:3:1.

TRIHYBRID RATIO:



Gametes: G_2 Fork line method

Gene for first character	Gene for second character	Gene for third character	Gametes formation
T	Y	R	TYR
		r	Tyr
	y	R	TyR
		r	Tyr
t	Y	R	tYR
		r	tYr
	y	R	tyR
		r	tyr

Frequency Distribution Table:**27 Genotypes will give 8 different phenotypes:**

Genotype	Genotypic Frequency	Phenotype	Phenotypic Frequency
TTYYRR	1	Tall-Yellow-Round	27
TTYYRr	2		
TTYyRR	2		
TTYyRr	2		
TtYYRR	4		
TtYYRr	4		
TtYyRR	4		
TtYyRr	8		
TTyyRR	1	Tall-Green-Round	9
TTyyRr	2		
TtyyRR	2		
TtyyRr	4		
TTYYrr	1	Tall-Yellow-Wrinkled	9
TTYyrr	2		
TtYyrr	2		
TtYYrr	4		
ttYYRR	1	Dwarf-Yellow-Round	9
ttYyRR	2		
ttYYRr	2		
ttYyRr	4		
TTyyrr	1	Tall-Green-Wrinkled	3
Ttyyrr	2		
ttYYrr	1	Dwarf-Yellow-Wrinkled	3
ttYyrr	2		
ttyyRR	1	Dwarf-Green-Round	3
ttyyRr	2		
ttyyrr	1	Dwarf-Green-Wrinkled	1

CALCULATION OF RATIOS

Supplementary Exercise:

Q.1 In pea, tall vine (D) is dominant over dwarf (d); green pods (G) over yellow (g) and round seed (W) over wrinkled (w). A homozygous dwarf, green, wrinkled pea plant is crossed with a homozygous tall, yellow, round. Utilizing forked line method, give the genotypes and phenotypes of parents, F_1 and F_2 progenies.

Probability

Probability is a concept which numerically measures the degree of certainty of the occurrence of events.

A probability is a number between 0 and 1 that predicts the frequency of a random event; the sum of the probabilities of all possible outcomes is 1. During meiosis in a heterozygote A^1A^2 , a gamete is equally likely to receive A^1 or A^2 , and the probability of each is 1/2. In general:

If an event E happens in m of n equally likely events, then the probability of event E is $P [E] = m/n$.
Ex.1. In a cross between two heterozygotes, $A^1A^2 \times A^1A^2$, what is probability of a heterozygous offspring? Because alleles segregate in 1:1 ratio i.e. half the eggs and sperm are A^1 and half are A^2 type. Fertilization is random with respect to the gametes, haplotypes, so there are four equally likely outcomes for each progeny: A^1A^1 , A^1A^2 , A^2A^1 or A^2A^2 . There are two ways to get heterozygous offspring (egg A^1 and sperm A^2 or vice versa) and $m/n = 2/4$. Thus, the probability that one of the progeny will be heterozygous is 1/2. This can be visualized as under:

Sperm/Eggs	$1/2A^1$	$1/2A^2$
$1/2A^1$	$1/4 A^1A^1$	$1/4 A^1A^2$
$1/2A^2$	$1/4 A^1A^2$	$1/4 A^2A^2$

Ex.2. Two coins are tossed simultaneously. What is the probability of getting at least one head?

Solution: When two coins are tossed simultaneously, all possible outcomes are HH, HT, TH, TT.

Total number of possible outcomes = 4

Let E be the event of getting at least one head.

Then, the favorable outcomes = 3

Therefore, $P (\text{getting at least one head}) = P (E) = 3/4$

Ex.3. Three coins are tossed simultaneously. What is the possibility of getting at least one head and one tail?

Solution: When three coins are tossed simultaneously, all possible combinations are as follows:

PROBABILITY

S.N.	Coin A	Coin B	Coin C	Probability	
1	H	H	H	$\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$	= 1/8
2	H	H	T	$\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$	= 1/8
3	H	T	H	$\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$	= 1/8
4	H	T	T	$\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$	= 1/8
5	T	H	H	$\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$	= 1/8
6	T	H	T	$\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$	= 1/8
7	T	T	H	$\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$	= 1/8
8	T	T	T	$\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$	= 1/8

Multiplication Rule:

The probability that two independent events X and Y will both happen is the product of their probabilities.

$$P(X \text{ and } Y) = P(X) P(Y)$$

P = Probability.

X and Y independent events.

Ex.4. A couple plan to have three children. They want to know the probability that all three will be boys.

$$P[3 \text{ boys}] = \{P[\text{boys}]\}^3 = (1/2)^3 = 1/8.$$

Addition Rule:

If X and Y are independent events, the probability that X or Y will happen is the probability of X plus the probability of Y minus. The probability of their joint occurrence "X or Y" includes the co-occurrence of X and Y. If X and Y are mutually exclusive, then $P[X \text{ and } Y] = 0$ and the addition rule still applies.

$$P(X \text{ or } Y) = P(X) + P(Y) - P[X \text{ and } Y]$$

Ex.5. Two genes A and B are genetically unlinked. A woman and man, both with genotype $A^1 A^0 B^1 B^0$, plan to have children. They want to know the probability of having a child with genotype $A^0 A^0$ or $B^0 B^0$, including $A^0 A^0 B^0 B^0$.

$$\begin{aligned} P[A^0 A^0 \text{ or } B^0 B^0] &= P[A^0 A^0] + P[B^0 B^0] - P[A^0 A^0 \text{ and } B^0 B^0] \\ &= 1/4 + 1/4 - 1/4 \times 1/4 \\ &= 1/4 + 1/4 - 1/16 \\ &= 7/16. \end{aligned}$$

Ex.6. What is the probability that a family of six children will contain three boys and three girls?
 $P[3 \text{ Boys and 3 Girls}] = [6! / 3! 3!] (1/2)^3 (1/2)^3$

$$\frac{6 \times 5 \times 4 \times 3 \times 2 \times 1}{3 \times 2 \times 1 \times 3 \times 2 \times 1} \times 1/2 \times 1/2 \times 1/2 \times 1/2 \times 1/2 \times 1/2$$

LABORATORY MANUAL ON FUNDAMENTAL OF GENETICS

$$= 20 \times 1/8 \times 1/8$$

$$= 20/64 = 5/16 = 0.31$$

Determination of the probability:

Probability of a combination of two independent events is determined according to the following formula:

$$P(A \text{ occurs } k \text{ times}) = n! / k! (n-k)! p^k q^{n-k}$$

Where,

P	=	Probability of a combination of independent events
n	=	Total number of independent events
k	=	Number of one of the two mutually exclusive events
n-k	=	Number of the other mutually exclusive events
p	=	Probability of the first of the two mutually exclusive events
q	=	Probability of the other mutually exclusive events
!	=	Factorial
n!	=	1x2x3.....x n
k!	=	1x2x3..... x k
n -k)!	=	(1x2xn ---(nk))

Supplementary Exercise

- Both parents of a family have genotype A/A⁰. What is the probability that exactly three of six of their children will have genotype A⁰/A⁰?

PROBABILITY

2. A coin tossed 6 times what is the probability of the following combinations of heads (H) and tails (T)
- a) 3H2T
 - b) 4H2T
 - c) atleast 3H
 - d) more than 4 H
3. A coin is tossed 5 times, what is the probability of 3H and 2T?

DNA and RNA Model

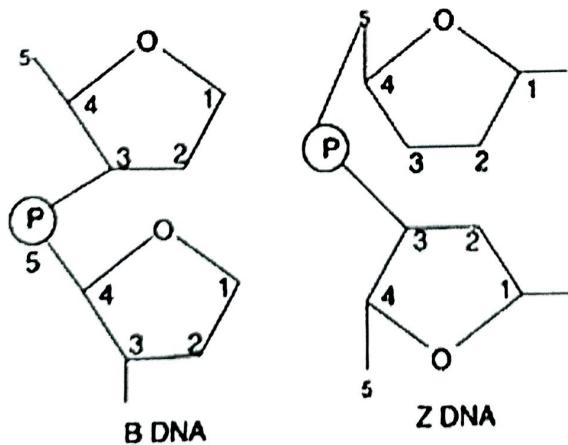
In 1953, Wilkins and Franklin got very fine X-ray photographs of DNA. The photographs showed that DNA was a helix with a width of 2.0 nm. One turn of the helix was 3.4 nm with 10 layers of bases stacked in it. Watson and Crick (1953) worked out the first correct double helix model from the X-ray photo-graphs of Wilkins and Franklin. Wilkins, Watson and Crick were awarded Nobel Prize for the same in 1962.

Types of DNA:

DNA duplex model proposed by Watson and Crick is right handed spiral and is called B-DNA (Balanced DNA). In the model the base pairs lie at nearly right angles to the axis of helix. Another right handed duplex model is A-DNA (Alternate DNA). Here, a single turn of helix has 11 base pairs.

The base pairs lie 20° away from perpendicular to the axis. C-DNA (complementary DNA) has 9 base pairs per turn of spiral while in D-DNA the number is only 8 base pairs. Both are right handed. Z-DNA (Zigzag DNA) is left-handed double helix with zigzag back-bone, alternate purine and pyrimidine bases, single turn of 45 Å length with 12 base pairs and a single groove.

B-DNA is more hydrated and most frequently found DNA in living cells. It is physiologically and biologically active form. However, it can get changed into other forms. Right handed DNA is known to change temporarily into the left handed form at least for a short distance. Such changes may cause changes in gene expression.



Orientation of adjacent sugar molecules in B and Z DNA.

Circular and Linear DNA:

In many prokaryotes the two ends of a DNA duplex are covalently linked to form circular DNA. Circular DNA is naked, that is, without association with histone proteins, though polyamines do occur. In linear DNA the two ends are free. It is found in eukaryotic nuclei where it is associated with histone proteins.

Chargaff's Rules:

Chargaff (1950) made observations on the bases and other components of DNA. These observations or generalizations are called Chargaff's base equivalence rule.

- (i) Purine and pyrimidine base pairs are in equal amount, that is, adenine + guanine = thymine + cytosine. $[A + G] = [T + C]$, i.e., $[A+G] / [T+C] = 1$
- (ii) Molar amount of adenine is always equal to the molar amount of thymine. Similarly, molar concentration of guanine is equalled by molar concentration of cytosine.
 $[A] = [T]$, i.e., $[A] / [T] = 1$; $[G] = [C]$, i.e., $[G] / [C] = 1$
- (iii) Sugar deoxyribose and phosphate occur in equimolar proportions.
- (iv) A-T base pairs are rarely equal to C-G base pairs.
- (v) The ratio of $[A+T] / [G+C]$ is variable but constant for a species. It can be used to identify the source of DNA. The ratio is low in primitive organisms and higher in advanced ones.

Structure of DNA:

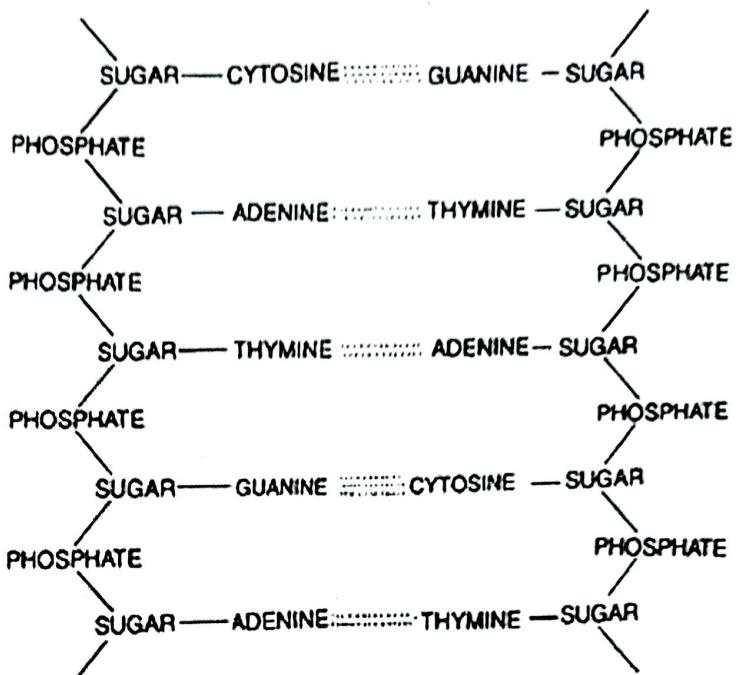
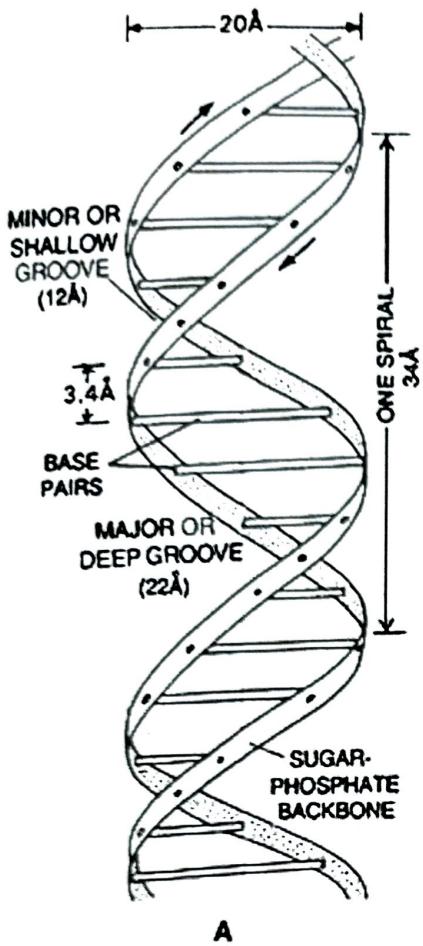
DNA or deoxyribonucleic acid is a helically twisted double chain polydeoxyribonucleotide macromolecule which constitutes the genetic material of all organisms with the exception of rhinoviruses. In prokaryotes it occurs in nucleoid and plasmids. This DNA is usually circular. In eukaryotes, most of the DNA is found in chromatin of nucleus.

It is linear. Smaller quantities of circular, double stranded DNA are found in mitochondria and plastids (organelle DNA). Small sized DNAs occur in viruses, $\phi \times 174$ bacteriophage has 5386 nucleotides. Bacteriophage lambda (Phage X) possesses 48502 base pairs (bp) while number of base pairs in Escherichia coli is 4.6×10^6 . A single genome (haploid set of 23 chromosomes) has about 3.3×10^9 bp in human beings. Single-stranded DNA occurs as a genetic material in some viruses (e.g., phage $\phi \times 174$, coliphage fd, M₁₃). DNA is the largest macromolecule with a diameter of 2 nm (20\AA or $2 \times 10^{-9}\text{m}$) and often having 3 length in millimetres.

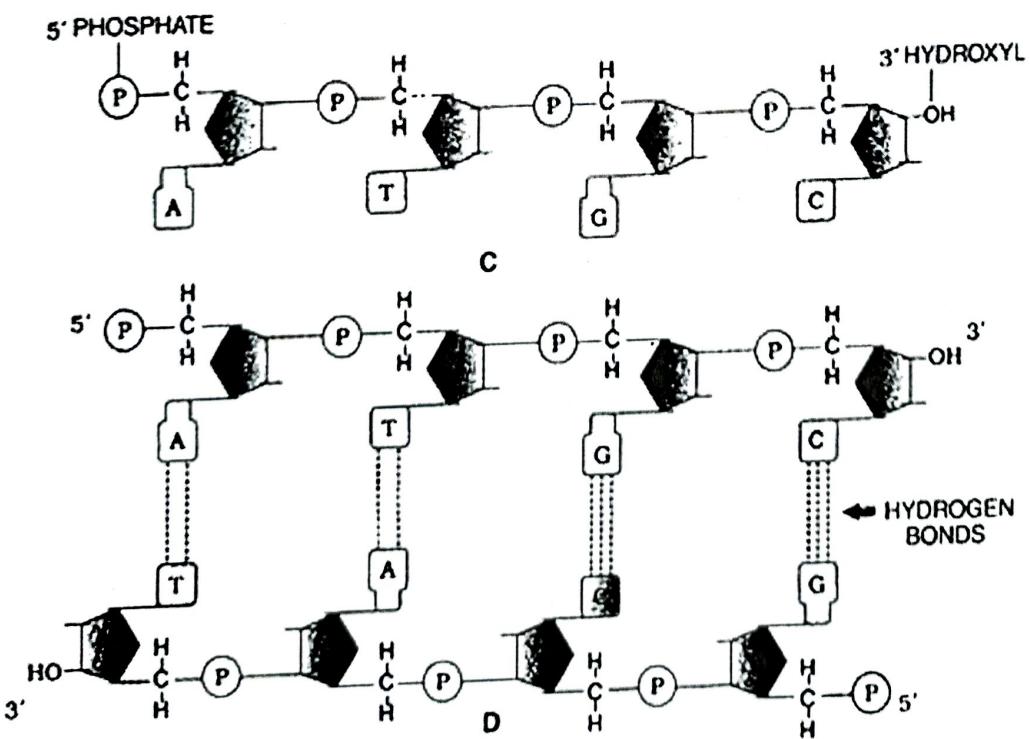
Salient Features of B model of DNA of Watson and Crick:

1. DNA is the largest biomolecule in the cell.
2. DNA is negatively charged and dextrorotatory.
3. Molecular configuration of DNA is 3D.
4. DNA has two polynucleotide chains.
5. The two chains of DNA have antiparallel polarity, $5' \rightarrow 3'$ in one and $3' \rightarrow 5'$ in other.
6. Backbone of each polynucleotide chain is made of alternate sugar-phosphate groups. The nitrogen bases project inwardly.

DNA AND RNA MODEL



B



7. Nitrogen bases of two polynucleotide chains form complementary pairs, A opposite T and N opposite G.
8. A large sized purine always comes opposite a small sized pyrimidine. This generates uniform distance between two strands of helix.
9. Adenine (A) of one polynucleotide chain is held to thymine (T) of opposite chain by two hydrogen bonds. Cytosine (C) of one chain is similarly held to guanine of the other chain by three hydrogen bonds.
10. The double chain is coiled in a helical fashion. The coiling is right handed. This coiling produces minor and major grooves alternately.
11. The pitch of helix is 3.4 nm (34 Å) with roughly 10 base pairs in each turn. The average distance between two adjacent base pairs comes to about 0.34 nm (0.34×10^{-9} m or 3.4 Å).
12. Planes of adjacent base pairs are stacked over one another. Alongwith hydrogen bonding, the stacking confers stability to the helical structure.
13. DNA is acidic. For its compaction, it requires basic (histone) proteins. The histone proteins are +vely charged and occupy the major grooves of DNA at an angle of 30° to helix axis.

Sense and Antisense Strands:

Both the strands of DNA do not take part in controlling heredity and metabolism. Only one of them does so. The DNA strand which functions as template for RNA synthesis is known as template strand, minus (-) strand or antisense strand.

Its complementary strand is named nontemplate strand, plus (+) strand, sense and coding strand. The latter name is given because by convention DNA genetic code is written according to its sequence.
(5') GCATTCGGCTACTAAC (3')

DNA Nontemplate, Sense (+) or Coding Strand

(32') CGTAAGCCGATCATTG (5')

DNA Template, Antisense, or Noncoding or (-) Strand

(52') GCAUUCGGCUAGUAAC (3')

RNA Transcript

RNA is transcribed on $3' \rightarrow 5'$ (-) strand (template/antistrand) of DNA in $5' \rightarrow 3'$ direction. The term antisense is also used in wider prospective for any sequence or strand of DNA (or RNA) which is complementary to mRNA.

RNA:

Structurally speaking, ribonucleic acid (RNA), is quite similar to DNA. However, whereas DNA molecules are typically long and double stranded, RNA molecules are much shorter and are typically single stranded. RNA molecules perform a variety of roles in the cell but are mainly involved in the process of protein synthesis (translation) and its regulation.

RNA Structure

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

Functions of RNA in Protein Synthesis:

Cells access the information stored in DNA by creating RNA to direct the synthesis of proteins through the process of **translation**. Proteins within a cell have many functions, including building cellular structures and serving as enzyme catalysts for cellular chemical reactions that give cells their specific characteristics. The three main types of RNA directly involved in protein synthesis are **messenger RNA (mRNA)**, **ribosomal RNA (rRNA)**, and **transfer RNA (tRNA)**.

In 1961, French scientists François Jacob and Jacques Monod hypothesized the existence of an intermediary between DNA and its protein products, which they called messenger RNA.¹¹ Evidence supporting their hypothesis was gathered soon afterwards showing that information from DNA is transmitted to the ribosome for protein synthesis using mRNA. If DNA serves as the complete library of cellular information, mRNA serves as a photocopy of specific information needed at a particular point in time that serves as the instructions to make a protein.

The mRNA carries the message from the DNA, which controls all of the cellular activities in a cell. If a cell requires a certain protein to be synthesized, the gene for this product is “turned on” and the mRNA is synthesized through the process of **transcription**. The mRNA then interacts with **ribosomes** and other cellular machinery (Figure 3) to direct the synthesis of the protein it encodes during the process of **translation**. mRNA is relatively unstable and short-lived in the cell, especially in prokaryotic cells, ensuring that proteins are only made when needed.

rRNA and tRNA are stable types of RNA. In prokaryotes and eukaryotes, tRNA and rRNA are encoded in the DNA, then copied into long RNA molecules that are cut to release smaller fragments containing the individual mature RNA species. In eukaryotes, synthesis, cutting, and assembly of rRNA into ribosomes takes place in the nucleolus region of the nucleus, but these activities occur in the cytoplasm of prokaryotes. Neither of these types of RNA carries instructions to direct the synthesis of a polypeptide, but they play other important roles in protein synthesis.

Ribosomes are composed of rRNA and protein. As its name suggests, rRNA is a major constituent of **ribosomes**, composing up to about 60% of the ribosome by mass and providing the location where the mRNA binds. The rRNA ensures the proper alignment of the mRNA, tRNA, and the ribosomes; the rRNA of the ribosome also has an enzymatic activity (**peptidyl transferase**) and catalyzes the formation of the peptide bonds between two aligned amino acids during protein synthesis. Although rRNA had long been thought to serve primarily a structural role, its catalytic role within the ribosome was proven in 2000. Scientists in the laboratories of Thomas Steitz (1940) and Peter Moore (1939) at

Yale University were able to crystallize the ribosome structure from *Haloarcula marismortui*, a halophilic archaeon isolated from the Dead Sea. Because of the importance of this work, Steitz shared the 2009 Nobel Prize in Chemistry with other scientists who made significant contributions to the understanding of ribosome structure.

Transfer RNA is the third main type of RNA and one of the smallest, usually only 70–90 nucleotides long. It carries the correct amino acid to the site of protein synthesis in the ribosome. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain being synthesized. Any mutations in the tRNA or rRNA can result in global problems for the cell because both are necessary for proper protein synthesis.

Supplementary Exercise

1. What units make up nucleic acids? What are the chemical components that make up those units?
2. What are pentoses? To what organic group do pentoses belong? Are nucleoside formed of only one type of pentose?
3. Into which two groups can the nitrogenous bases that form DNA and RNA be classified? What is the criterion used in that classification?

DNA AND RNA MODEL

4. What is the differences between DNA and RNA from the point of view of the nitrogenous bases that are present in their nucleotide?
5. What parts of nucleotide bind to form nucleic acids? What is meant by the 5' and 3' extremities of nucleic acids?
6. Who were James Watson, Francis Crick and Maurice Wilkins?

