The Cell & Molecular Genetics



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THE CELL

The cell is the smallest structural unit of a living organism. So, everything that we are able to do is possible because of the 10 trillion cells present in our body, which were made by Allah. The number is huge and of course, the function performed by them is even bigger.

The introduction to cell began back in year 1655 when a revolutionary observation was made by scientist **Robert Hook**. This observation made by him was so huge that it went on to change the basic biological theory and research forever. So, how was the cell discovered?

Robert Hook was examining a dried section of the cork tree using a crude light microscope. In this analysis, he observed multiple small chambers which he named the cells. Thereafter, over the next 175 years, several kinds of research were made which led to the formation of the cell theory that we know today. The first such theory was proposed by the German botanist Matthias Jacob **Schleiden** and the German physiologist Theodore **Schwann** in 1838. This theory was formalized in the year 1858 by the German researcher Rudolf **Virchow**. Schleiden's contributions on plants were acknowledged by Schwann as the basis for his comparison of animal and plant structure.

Schleiden and Schwann's descriptive statements concerning the cellular basis of biologic structure are straightforward and acceptable to modern thought. They recognized the common features of cells to be the membrane, nucleus, and cell body and described them in comparisons of various animal and plant tissues. A statement by Schleiden pointed toward the future direction of cell studies:

Cell Theory

- The cell is the basic functional and structural unit of life. All the living organisms are composed of cells.
- All cells are formed by the division of the already existing cells which in terms of biology means reproduction. Every cell of our body comprises of genetic material which is passed down during the process.
- All the basic physiological and chemical functions i.e. the growth, repair, movement, communication, immunity and digestions are performed inside the cells.
- All the activities of the cell depend mainly on the activities of the sub cellular structures that lie within the cell.

Types of cell

Broadly, there are two key types of cells i.e. the Prokaryotic Cell and the Eukaryotic Cell. The difference between the two is defined mainly by the presence or the absence of the nuclear membrane. Let's know more about the two types of cells.



The development of microscope

As cells are extremely small, they could not be seen until the development of the microscope. This invention paved the way to a greater understanding of the structure and function of living matter.

The road to the development of the microscope began when the Romans first made glass in the first century BCE. At this early time, it was observed that objects viewed through a droplet of glass were magnified, even though the image was fuzzy it was not until the late sixteenth century that the quality of glass and the technology to finely grind it was developed. These developments allowed the production of spectacles.

Work done in the 1590s by the spectacle maker Zacharias Janssen produced lenses that could magnify nine times. In 1595, he and his father built the first compound microscope. This consisted of two lenses separated in a hollow tube. The image produced, however, was not very clear and tended to split the light like a prism. This affected the microscope's resolution limits - the limit at which tiny objects that are close together stop being visibly separate.

These first microscopes were thought of as toys rather than scientific instruments. Part of the problem was that the lenses were not particularly smooth or of even thickness. Second, the smaller an object is, the less light reflects from it. Seeing anything really tiny requires a very good light source, something that was not available at the time. Nevertheless, using such a microscope, in 1660, the Italian Marcello Malpighi was able to see blood capillaries in the tail of live fish. This showed that blood circulates in the body. Prior to this it was thought that blood was produced in the intestines and moved to other parts of the body where it was consumed.

In 1665 the Englishman Robert Hooke observed the bark of a cork tree under a microscope using reflected light. He found it was composed of minute chambers, which he called cells because they looked like the 'cells' in which monks lived. Although it is now known that there are no cells in dead cork, the modern term 'cell' comes from this label. Hooke used a simple microscope (single lens) for examining specimens using transmitted light (light passing through the specimen) in order to get clearer images.

Dutchman Antonie van Leeuwenhoek further developed the microscope and improved the way it was used. He developed his interest in microscopes during his apprenticeship as a draper in Amsterdam in 1648 where he was able to observe the fibers that comprised different quality materials.

He learned to grind and polish small glass spheres (a biconvex lens) of such quality that they had a magnification of 270. Leeuwenhoek mounted this lens on a flat copper plate and held it very close to his eye The object being studied was placed on the head of a movable pin attached to the plate on the other side of the lens.

Using this simple microscope, Leeuwenhoek was the first to shine a light through living single-celled animals (protozoans) in pond water. He called them animalcules

For about 150 years microscopic work was limited to these two 'primitive' types of microscope. By the late 1800s many of the distortions of the lenses were corrected due to the work by German engineer Carl Zeiss and his employees, Schott and Abbe They developed optical systems that could focus on objects smaller than a wavelength (approximately 0.2 um (micrometres)). Because they used light as an imaging system, they were called light microscopes. Although a powerful and useful tool, light microscopes still could not provide a structure of the While some recent techniques have been employed in improving light microscopes, they are still limited as an understanding of the physical nature of matter, particularly the structure of the MICRO detailed observation of the internal Leeuwenhoek's microscope atom, developed, and new technologies arose. The application of these advances led to the development of microscopes with higher magnification and resolution

In the 1930s, the electron microscope was developed by Ernst Ruska and Max Knoll. Like its predecessors, this changed the possibilities for science. This microscope uses a narrow beam of electrons. The beam width is adjustable, but at it's smallest in below the size of a hydrogen atom. The beam is focused with magnets and the final image is converted to light in a way similar to that on a television screen. A photograph called an electron micrograph is taken of the image. This is necessary because the electron beam prevents the magnified image from being viewed directly. For the same reason, the electron microscope is difficult to use on living material.

In summary, technological advancements in physics allowed magnification and resolution to be massively improved because

the electron microscope uses a very narrow beam of electrons as opposed to the broader beam of light used previously

LIGHT MICROSCOPY. Modern light microscopes are versatile scientific instruments used to study living and prepared specimens as well as specimens with inherent or applied fluorescent properties.

A. Light microscope components

1. A light microscope has a built-in light source and an adjustable sub stage condenser, which project light into the objectives.

2. A mechanically operated stage carries the specimen.

3. Objectives magnify the specimen image, and oculars complete the image formation process.

B. Image formation

1. Modern light microscopes have a resolution limit of 0.2-0.4 µm, or approximately one- twentieth the diameter of a human erythrocyte.

a. Many subcellular organelles exist in this size range. When specimens are carefully prepared, these organelles are visible in the light microscope.

b. Scientists were not aware that organelles smaller than this existed until the electron microscope was developed. The resolving power of a light microscope depends on three variables: objective magnification, 2 objective numerical aperture (N.A.), and the wavelength of light used to illuminate the specimen. a. A typical compound microscope has a low-power (4x) objective, one or two intermediate-power (10x, 40x) objectives, and a high-

power (100x) oil-immersion objective. Oculars provide some magnification (usually 10x) as well, so total magnification ranges from 40x to 1000 x.

b. Every objective has an NA, which is calculated by the formula N.A.= n sine @

where n is the refractive index of the medium between the specimen and the objective lens, and 8 is half the angle of the cone of light gathered by the objective.

(1) A low-power objective has a low N.A.: a high-power objective has a high N. image resolution improves as the N.A. increases.

(2) If air (refractive index of 1 by definition) is the medium between specimen and objective, no lens can gather light from a light cone when $\frac{1}{2}$ angle > 90°. However, if a high refractive index immersion oil is the intervening medium, the objective can gather light from a wider angle light cone, thereby increasing the objective N.A. and improving image resolution. c. The specimen produces a complex diffraction pattern in the back focal plane of the microscope, which is resynthesized into a recognizable magnified image by the oculars. d. Resolution improves as the wavelength of light used to illuminate the specimen shortens

C. Types of light microscopy

1. Brightfield microscopy uses standard lenses and condensers and has a limit of resolution of approximately 0.3 μ m.

2. Phase-contrast microscopy uses modified objective lenses and condensers to permit direct examination of living cells without fixation or staining. a. Phase-contrast microscopes have substage condensers and objectives that convert slight differences in the refractive indices of specimen structures and domains into distinct differ- ences in light intensity. b. Each organelle and domain in a cell has a unique chemical composition or concentration Of chemical constituents, which determines its refractive index. A phase-contrast microscope exaggerates these differences so that cell nuclei, cytoplasm ground substance, mitochondria, and other cytoplasmic structures appear in contrast to one another. c. This type of microscopy does not harm living cells and allows scientists to examine cell behavior under many artificial circumstances.

3. Differential interference contrast microscopy (Nomarski light microscopy) uses special condensers and objective lenses to transform differences in refractive index into an image thatA. Thus, appears to have a three-dimensional character. In this type of microscopy, the nucleus and various particulate cytoplasmic inclusions appear in low relief. 4. Fluorescence light microscopy is used to localize inherently fluorescent substances or substances labeled with fluorescent tags. Fluorescence microscopes have a high intensity light source and two special filters. a. The excitor filter, located between the light source and the specimen, blocks all light wavelengths except those that excite the fluorochrome. b. The barrier filter, located between the specimen and the ocular, blocks all light wavelengths except those emitted by the fluorochrome.

IV. **ELECTRON MICROSCOPY** is used to view fixed and sectioned or metal-coated specimens under magnification high enough to resolve fine details of the specimen.

A. Electron microscope components and image formation 1. In contrast to light microscopes, electron microscopes illuminate specimens with a short wavelength stream of electrons rather than photons, and they form images with magnetic lenses rather than glass lenses. a. The resolving power of modern electron microscopes is high enough to examine details of all types of cellular organelles and some macromolecules. b. Due to the short wavelength of electron radiation, electron microscopes have a high theoretical resolving power. However, the magnetic lens N.A. is so low that the theoretical limit is unattainable.

2. Specimens are illuminated in an evacuated column within the microscope, because the electron beam would be scattered by air.

3. The magnetic lenses form an image that is displayed on a video screen. Often, the image is photographed to generate a permanent record. These electron micrographs show the fine details of a specimen, often to the molecular level of resolution.

B. Types of electron microscopy. TEM and SEM are distinct types of electron microscopy that employ different methods of specimen preparation, irradiation, and image formation to study the details of cell structures and components.

1. TEM uses thinly sliced, plastic-embedded sections that are stained with heavy metal salts. TEM is used to study the fine details of cell structure, such as the morphology of cell surfaces (in cross section) and the internal elements of cells; it can resolve features as small as 0.5 nm. 2. SEM uses whole specimens that are subjected to critical point drying and then coated with a thin layer of gold and palladium. SEM is used to study three-dimensional features of cell surfaces; it can resolve features as small as 5 nm.

Most microscopes can be divided into another two groups depending on how the imaging beam (light or electrons) interacts with the specimen. In scanning microscopes, the beam reflects off the surface of the specimen to produce a three-dimensional image. Transmission microscopes send the beam through the specimen and a two-dimensional image is formed. For a specimen to be useful in the transmission microscope, the beam has to be able to pass through it and reveal its internal detail. Specimens need to be stained with special chemicals to produce clear detul Coloured dyes are used in light microscopy while heavy metals are used for electron microscopy. The stain produces variations in absorption by different parts of the specimen, Large or thick specimens will not allow the beam through them. They must be sectioned or sliced, then stained. The object is embedded in a material (commonly, wax in light microscopy and plastic for electron microscopy) that will hold it together while being thinly sliced. Sequences of these sections can then be used to translate the twodimensional information to a three-dimensional model.



The **plasma membrane that surrounds and keeps** the cell intact and regulates what enters and exits a cell. The plasma membrane is a phospholipids bilayer that is said to be selective permeable because it allows certain molecules but not others to enter the cell the **nucleus is a large, centrally located structure** that can often be seen with a light microscope. The nucleus contains the chromosomes and is the control center of the cell. It controls the metabolic functioning and structural characteristics of the cell. The **nucleolus is a region inside the nucleus.** The **cytoplasm is** the portion of the cell between the nucleus and the plasma membrane. The matrix of the cytoplasm is a semi fluid medium that contains water and various types of molecules suspended or dissolved in the medium. The presence of proteins accounts for the semi fluid nature of the matrix the cytoplasm contains various organelles. Organelles are small membranous structures that can usually only be seen with an electron microscope. Each type of organelle has a specific function. Cells also have a cytoskeleton, **a network of** interconnected filaments and microtubules that occur in the cytoplasm. The name cytoskeleton is convenient in that it allows us to compare the cytoskeleton to the bones and muscles of an animal. Bones and muscle give an animal structure produce movement. Similarly, the elements of and the cytoskeleton maintain cell shape and allow the cell and its contents to move. Some cells move by using cilia and flagella, which are also made up of microtubules.



Fig. of a typical animal cell

The structure of Plasma Membrane

An animal cell is surrounded by an outer plasma membrane. The plasma membrane marks the boundary between the outside of the cell and the inside of the cell. The plasma membrane is a phospholipids bilayer with attached or embedded proteins. The structure of phospholipids is such that the molecule has a polar head and nonpolar tails. The polar heads, being charged, are hydrophilic (water loving) and face outward, toward the cytoplasm on one side and the tissue fluid on the other side. The nonpolar tails are hydrophobic (not attracted to water) and face inward toward each other, where there is no water. Fluid-mosaic model, a working description of membrane structure, says that the protein molecules have a changing pattern (form a mosaic) within the fluid phospholipid bilayer. Cholesterol lends support to the membrane. Short chains of sugars are attached to the outer surface of some protein and lipid molecules (called glycoprotein and glycolipids, respectively). It is believed that these carbohydrate chains, specific to each cell, help mark it as belonging to a particular individual. They account for why people have different blood types, for example. Other glycoproteins have a special configuration that allows them to act as a receptor for a chemical messenger like a hormone. Some plasma membrane proteins form channels through which certain substances can enter cells; others are carriers involved in the passage of molecules through the membrane



Transport in plasma membrane

How substances move across the Plasma Membrane?

Dye molecules diffuse into water



Dye crystals on bottom

Dye molecules evenly distributed

Substances need to pass through the membrane to enter or leave the cell and they do so in a number of ways. Some of these processes require no energy i.e. they are passive, while

others require energy i.e. they are active. Passive processes include: a) diffusion and b) osmosis, while active processes include: c) active transport, d) phagocytosis, e) pinocytosis and f) exocytosis. These will be described below.

a) Diffusion

Although you may not know it, you are already familiar with the process of diffusion. It is diffusion that causes a smell (expensive perfume or smelly socks) in one part of the room to gradually move through the room so it can be smelt on the other side. Diffusion occurs in the air and in liquids.

The up picture shows what happens when a few crystals of a dark purple dye called potassium permanganate are dropped into a beaker of water. The dye molecules diffuse into the water moving from high to low concentrations so they become evenly distributed throughout the beaker. In the body, diffusion causes molecules that are in a high concentration on one side of the cell membrane to move across the membrane until they are present in equal concentrations on both sides. It takes place because all molecules have an in-built vibration that causes them to move and collide until they are become evenly distributed. It is an absolutely natural process that requires no added energy. Small molecules like oxygen, carbon dioxide, water and ammonia as well as fats, diffuse directly through the double fat layer of the membrane. The small molecules named above as well as a variety of charged particles (ions) also diffuse through the protein-lined channels.

Larger molecules like glucose attach to a carrier molecule that aids their diffusion through the membrane. This is called facilitated diffusion.

In the animal's body diffusion is important for moving oxygen and carbon dioxide between the lungs and the blood, for moving digested food molecules from the gut into the blood and for the removal of waste products from the cell.



b) Osmosis

Although the word may be unfamiliar, you are almost certainly acquainted with the effects of osmosis. It is osmosis that plumps out dried fruit when you soak it before making a fruit cake or makes that wizened old carrot look almost like new when you soak it in water. Osmosis is in fact the diffusion of water across a membrane that allows water across but not larger molecules. This kind of membrane is called a semi-permeable membrane. Take a look at side A of diagram 3.6. It shows a container divided into two parts by an artificial semi-permeable membrane. Water is poured into one part while a solution containing salt is poured into the other part. Water can cross the membrane but the salt

cannot. The water crosses the semi-permeable membrane by diffusion until there is an equal amount of water on both sides of the membrane. The effect of this would be to make the salt solution more diluted and cause the level of the liquid in the right-hand side of the container to rise so it looked like side B of diagram 3.6. This movement of water across the Semi-permeable membrane is called osmosis. It is a completely natural process that requires no outside energy. Although it would be difficult to do in practice, imagine that you could now take a plunger and push down on the fluid in the right-hand side of container B so that it flowed back across the semi-permeable membrane until the level of fluid on both sides was equal again. If you could measure the pressure required doing this, this would be equal to the osmotic pressure of the salt solution. (This is a rather advanced concept at this stage but you will meet this term again when you study fluid balance later in the course).



The plasma membrane of cells acts as a semi-permeable membrane. If red blood cells, for example, are placed in water, the water crosses the membrane to make the amount of water

on both sides of it equal. This means that the water moves into the cell causing it to swell. This can occur to such an extent that the cell actually bursts to release its contents. This bursting of red blood cells is called hemolysis. In a situation such as this when the solution on one side of a semi-permeable membrane has a lower concentration than that on the other side, the first solution is said to be hypotonic to the second.

Now think what would happen if red blood cells were placed in a salt solution that has a higher salt concentration than the solution within the cells. Such a bathing solution is called a hypertonic solution. In this situation the "concentration" of water within the cells would be higher than that outside the cells. Osmosis (diffusion of water) would then occur from the inside of the cells to the outside solution, causing the cells to shrink.



Red cells placed in an isotonic solution, a solution that contains 0.9% salt has the same concentration as body fluids and the

solution within red cells. Cells placed in such a solution would neither swell nor shrink.

This solution is called an isotonic solution. This strength of salt solution is often called normal saline and is used when replacing an animal's body fluids or when cells like red blood cells have to be suspended in fluid. Remember - osmosis is a special kind of diffusion. It is the diffusion of water molecules across a semipermeable membrane. It is a completely passive process and requires no energy. Sometimes it is difficult to remember which way the water molecules move. Although it is not strictly true in a biological sense, many students use the phrase "SALT SUCKS" to help them remember which way water moves across the membrane when there are two solutions of different salt concentrations on either side. As we have seen water moves in and out of the cell by osmosis. All water movement from the intestine into the blood system and between the blood capillaries and the fluid around the cells (tissue or extra cellular fluid) takes place by osmosis. Osmosis is also important in the production of concentrated urine by the kidney.

c) Active transport

When a substance is transported from a low concentration to a high concentration i.e. uphill against the concentration gradient, energy has to be used. This is called active transport. Active transport is important in maintaining different concentrations of the ions sodium and potassium on either side of the nerve cell membrane. It is also important for removing valuable molecules such as glucose, amino acids and sodium ions from the urine.



d) Phagocytosis

Phagocytosis is sometimes called "cell eating". It is a process that requires energy and is used by cells to move solid particles like bacteria across the plasma membrane. Finger-like projections from the plasma membrane surround the bacteria and engulf them as shown in the diagram. Once within the cell, enzymes produced by the lysosomes of the cell (described later) destroy the bacteria. The destruction of bacteria and other foreign substance by white blood cells by the process of phagocytosis is a vital part of the defense mechanisms of the body.

e) Pinocytosis

Pinocytosis or "cell drinking" is a very similar process to phagocytosis but is used by cells to move fluids across the plasma membrane. Most cells carry out pinocytosis (note the pinocytotic vesicle in the up diagram.

f) Exocytosis

Exocytosis is the process by means of which substances formed in the cell are moved through the plasma membrane **into the** fluid outside the cell (or extra-cellular fluid). It occurs in all cells but is most important in secretory cells (e.g. cells that produce digestive enzymes) and nerve cells.

The Cytoplasm

Within the plasma membrane is the cytoplasm. It consists of a clear jelly-like fluid called the a) cytosol or intracellular fluid in which b) cell inclusions, c) organelles and d) microfilaments and microtubules are found.

a) **Cytosol**

The cytosol consists mainly of water in which various molecules are dissolved or suspended. These molecules include proteins, fats and carbohydrates as well as sodium, potassium, calcium and chloride ions. Many of the reactions that take place in the cell occur in the cytosol.

b) Cell inclusions

These are large particles of fat, glycogen and melanin that have been produced by the cell. They are often large enough to be seen with the light microscope. For example, the cells of adipose tissue (as in the insulating fat layer under the skin) contain fat that takes up most of the cell.

c) Organelles

Organelles are the "little organs" of the cell - like the heart, kidney and liver are the organs of the body. They are structures with characteristic appearances and specific "jobs" in the cell. Most cannot be seen with the light microscope and so it was only when the electron microscope was developed that they were discovered. The main organelles in the cell are the ribosome, endoplasmic reticulum, mitochondrion, Golgi complex and lysosomes.

Chemical composition of Cytoplasm:

Cytoplasm is the semi-fluid substance of a cell that is present within the cellular membrane and surrounds the nuclear membrane. It is sometimes described as the nonnuclear content of the protoplasm. All the cellular contents in prokaryote organisms are contained within cell's cytoplasm. In eukaryote organisms, the nucleus of the cell is separated from the cytoplasm.

Cytoplasm is a thick and semi-transparent fluid. The cytoplasm was discovered in the year 1835 by Robert Brown and other scientists.

The cytoplasm is made of 70% - 90% water and is colorless usually. Most of the cellular activities occur in the cytoplasm.

Metabolic pathways like glycolysis and cellular processes like cell division take place in the cytoplasm. The outer clear and glassy layer of the cytoplasm is called the ectoplasm or the cell cortex and the inner granular mass is called the endoplasm. In plants cells, a process known as cytoplasmic streaming takes place where there is a movement of the cytoplasm around the vacuoles.

Chemically cytoplasm contains 90% water and 10% include a mixture of organic and inorganic compounds in various proportions.

Inorganic Compounds of Protoplasm:

The inorganic components of the protoplasm mostly occur in the form of water, minerals and salts which are as follows:

(i) **Water:** The most abundant inorganic constituent of the protoplasm is the water. Water constitutes about 65 to 80 per cent of the protoplasm.

In the protoplasm, the water occurs in two forms, viz., free water and bound water. The 95 per cent of the total cellular water is used by the protoplasm as the solvent for various inorganic substances and organic compounds and is known as free water. The remaining 5 per cent of the total cellular water remains loosely linked with protein molecules by hydrogen bonds or other forces and is known as bound water. The water is the best biological solvent for inorganic substances such as mineral ions, salts, etc., and organic compounds such as carbohydrates and proteins. The water is used by the cell as a transporting media for the food, nitrogen wastes and other necessary substances. The water is immiscible with the non-polar liquids as the lipids, so its molecules remain stable and unmixed with the lipid contents of the plasma membrane and other intra-cellular membranes.

(ii) Minerals:

• The minerals are the inorganic chemical substances which occur in the crust of the earth. In the protoplasm the minerals usually occur in the form of salts and in combination with the organic compounds. The mineral salts occur in the form of ions in the protoplasm. An ion is an atom or group of atoms which carries one or more positive or negative charge.

• The positively charged ions are known as cations and the negatively charged ions are known as anions. For example, when sodium chloride (NaCl) is dissolved in water, it is ionized to form a sodium cation (Na+) and a chlorine anion (Cl–). The inorganic

compounds which by dissolving in water become ionized are known as electrolytes but those which do not dissociate in the solvent but remain as such in the molecular stated are e known as non-electrolytes. The, protoplasm contains both electrolytes and non- electrolytes.

a) Electrolytes:

• The electrolytes play a vital role in the maintenance of osmotic pressure and acid base equilibrium in the protoplasm. Certain ions such as the magnesium (Mg+), etc., are essential for many enzymatic activities because these ions act as cofactor. The phosphate (PO4) ion is the important constituent of the adenosine triphosphate (ATP) which is the chief supplier of energy for most of the life processes. Other important ions of the protoplasm are sodium (Na+), potassium (K+), calcium (Ca+), chlorine (Cl–), carbonate (CO3), sulphate (SO2), and amino acids.

(b) Non-electrolytes:

• Some of the minerals occur in protoplasm in non-ionising state. The non- electrolytes of the protoplasm are Na, K, Ca, Mg, Cu, I, Fe, Mn, Fl, Mo, Cl, Zn, Co, Ni, etc. The iron (Fe) occurs in the haemoglobin, ferritin cytochromes and some enzymes as catalase and cytochrome oxidase.

• The calcium (Ca) occurs in the blood, protoplasm and the bones. The copper (Cu), manganese (Mn), molybdenum (Mo) and zinc (Zn) are useful as cofactors for enzymatic actions. The iodine and fluorine are essential for the thyroid and the enamel metabolism respectively.

Organic Compounds of cytoplasm

The chemical substances which contain carbon (C) in combination with one or more other elements as hydrogen (H), nitrogen (N), sulphur (S), etc., are called organic compounds. The organic compounds usually contain large molecules which are formed by the similar or dissimilar unit structures known as the monomers.

• A monomer is a simplest unit of the organic molecule which can exist freely.

Some organic compounds such as carbohydrates occur in the protoplasm as the monomers.

• The monomers usually link with other monomers to form oligomers and polymers .The oligomers contain small numbers of monomers, while the polymers contain large number of monomers. The oligomers and polymers contain large-sized molecules or macromolecules.

• When a polymer contains similar kinds of monomers in its macromolecule, it is known as homo-polymer and when the polymer is composed of different kinds of monomers, it is known as the heteropolymer.

• The main organic compounds of the protoplasm are the carbohydrates, lipids, proteins and nucleotides.

i) Carbohydrates

• The carbohydrates are the compounds containing the carbon, Hydrogen and Oxygen. The carbohydrates form the main source of the energy for all living beings. Only green plants and certain microbes have the power of synthesizing the carbohydrates from the water and CO2 in the presence of sunlight and chlorophyll by the process of photosynthesis.

• All the animals, non-green plants (e.g., fungi, bacteria and viruses) depend on green plants for the supply of carbohydrates.

• Chemically the carbohydrates are the polyhydroxy aldehydes or ketones and they are classified as follows:

Carbohydrate classification

1. Monosaccharide's:

• The monosaccharide's are the simple sugars with empirical formula Cn(H2O)n.

• The monosaccharide's are the monomers and cannot split further (or hydrolysed) into the simpler compounds.

• They are classified and named according to the number of carbon atoms in their molecules as follows: (i) Trioses contain three carbon atoms in their molecules, e.g., Glyceraldehyde and Dihydroxy acetone.

• (ii) Tetroses contain four carbon atoms in their molecules, e.g., Erythrulose.

• (iii) Pentoses contain five carbon atoms in their molecules, e.g., Ribose, Deoxyribose, Arabinose and Xylulose.

• (iv) Hexoses contain six carbon atoms in their molecules, e.g., Glucose, Fructose and Galactose.

• (v) Heptoses contain seven carbon atoms in their molecules, e.g., Sedoheptulose.

• The pentose's and hexoses are the most abundantly found monosaccharide's in the protoplasm. The pentose sugar, ribose is the important compound of ribonucleic acid (RNA) and certain coenzymes as nicotinamide adenine dinucleotide (NAD), NAD phosphate (NADP), adenosine triphosphate (ATP) and coenzyme A (CoA).

• Another pentose sugar, the deoxyribose is the important constituent of the deoxyribonucleic acid (DNA). The ribulose is a pentose which is necessary for photosynthesis mechanism. The glucose, a hexose sugar is the primary source of the energy for the cell. The other important hexose sugars of the protoplasm are the fructose and galactose.

2. Oligosaccharides:

• The oligosaccharides contain 2 to 10 monosaccharide's (monomers) in their molecules. The monomers remain linked with each other by the glycosidic linkages.

• Certain important oligosaccharides are as follows:

Disaccharides contain two monomers, e.g., Sucrose, Maltose, Lactose, etc. The most abundant oligosaccharides are the disaccharides, viz., sucrose, maltose and lactose. The sucrose and maltose occur mainly in the protoplasm of plant cells, while lactose occurs exclusively in the protoplasm of animal cells.

(ii) Lipids (Fats)

• The lipids (Gr., lipos = fat) are the organic compounds which are insoluble in water but soluble in ether, chloroform, benzene, hot alcohol and petroleum ether. They contain long chains of aliphatic hydrocarbons or benzene ring in their molecules. The lipids are nonpolar and hydrophobic. They are important constituents of the cellular membranes, hormones and vitamins of the cells and are the source of energy for the cells. • The lipids contain carbon (C), hydrogen (H) and oxygen (O) and are classified into following types:

Simple lipids \longrightarrow (true fats) ex. Wax & Natural fats

Compound lipids \longrightarrow ex. Phospholipids, Glycolipids, Lipoprotein

Steroids (same chemical properties of lipids) cholesterol and sex hormones.

(iii) Proteins

• The proteins are the most important constituents of the protoplasm. All proteins are composed of carbon (C), hydrogen (H), oxygen (O), nitrogen (N) and some of them in addition contain sulphur (S) and phosphorus (P). The protoplasm is dependent almost entirely upon proteins for its supply of nitrogen, sulphur and phosphorus.

• The proteins are the polymers of the amino acids. An organic compound containing one or more amino groups (-NH2) and one or more carboxyl groups (-COOH) is known as amino acid. The amino acids occur freely in the protoplasm and constitute the so called amino acid pool. The amino acids are derived from the organic acids in which the hydrogen in alpha position is replaced by the amino groups.

Formation of proteins:

• Because molecule of the amino acid contains both amino (NH2) and acidic or carboxyl (-COOH) group, it can behave as an acid and base at a time. The molecules of such organic compounds which contain both acidic and basic properties are known as

amphoteric molecules. Due to amphoteric molecules, the amino acids unite with one another to form complex and large protein molecules.

• When two molecules of amino acids are combined, then the basic group (-NH2) of one amino acid molecule combines with the carboxylic (-COOH) group of other amino acid and the loss of a water molecule takes place. This sort of condensation of two amino acid molecules by -NH-CO linkage or bond is known peptide linkage or peptide bond.

• A combination of two amino acids by the peptide bond is known as dipeptide, when three amino acids are united by two peptide bonds they form tripeptide.

• Likewise, by condensation of few or many amino acids by the peptide bonds, the oligopeptides and polypeptides are formed respectively.

Various molecules of polypeptides unite to form the peptones, proteases and proteins. Thus, protein macromolecules are the polymers of many amino acid monomers. The macromolecules of proteins comparatively have high molecular weights.

Enzymes

• The protoplasm and many cellular organelles contain very important organic compounds known as the enzymes (Gr., en = in+zyme =leaven). The enzymes are the specialized proteins having the capacity to act as catalysts in chemical reactions.

• Like other catalysts of the chemical world, the enzymes are the catalysts of the biological world and they influence the rate of a chemical reaction, while they remain quite unchanged at the end

of the reaction. The substance on which the enzymes act is known as substrate.

• The enzymes play a vital role in various metabolic and biosynthetic activities of the cell such as synthesis of DNA, RNA and protein molecules and metabolism of carbohydrates, lipids, fats and other chemical substances.

Nucleic Acids

• The nucleic acids are the complex macromolecular compounds of immense biological importance. They control the important biosynthetic activities of the cell and carry hereditary information's from generation to generation.

• There occur two types of nucleic acids in living organisms, viz., ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Both types of nucleic acids are the polymers of nucleotides. A nucleotide is composed of nucleoside and phosphoric acid.

• Even the nucleoside is composed of the pentose sugar (ribose or deoxyribose) and nitrogen bases (purines and pyrimidine's). The purines are adenine and guanine and pyrimidine's are cytosine, thymine and uracil.

Vitamins

• The vitamins are complex organic compounds of diverse chemical nature which are required in minute amounts for normal growth, functioning and reproduction of cells. The vitamins play an important role in the cellular metabolism and they act as the enzymes or other biological catalysts in the various chemical activities of the cell.

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• The cell cannot synthesise the vitamins from the standard food and so they are taken along with the food. Their deficiency in the cell causes metabolic disorders and leads to various diseases.

Ribosomes

Ribosomes are composed of two subunits, one large and one small. Each subunit has its own mix of proteins and rRNA. Protein synthesis occurs at the ribosomes. Ribosomes are found free within the cytoplasm either singly or in groups called often polyribosomes. Ribosomes are attached the to endoplasmic reticulum, a membranous system of saccules and channels. Proteins synthesized by cytoplasmic ribosomes are used inside the cell for various purposes. Those produced by ribosomes attached to endoplasmic reticulum may eventually be secreted from the cell.

Membranous Canals and Vesicles

The endomembrane system consists of the nuclear envelope, the vesicles (tiny membranous sacs). This system compartmentalizes the cell so that particular enzymatic reactions are restricted to specific regions.

The Endoplasmic Reticulum the endoplasmic reticulum (ER):

The endoplasmic reticulum is the main component of the endomembrane system, also called the cytoplasmic vacuolar system or cytocavity network. This system comprises following structures 1. The nuclear envelope, consisting of two nonidentical membranes, one opposed to the nuclear chromatin and other separated from the first membrane by a perinuclear space (both forming a cisternae), the two membranes being in contact at the nuclear pores; 2. The endoplasmic reticulum; and 3. The Golgi apparatus, which is mainly related to some of the terminal processes of cell secretion. GERL (or Golgi, ER and lysosome) refers to a special region of endomembrane system, which is more related to the Golgi apparatus and is involved in the formation of lysosomes.

The entire endomembrane system represents a barrier separating cytoplasmic compartments. The membrane of each component of this system has two faces: (i) The cytoplasmic or protoplasmic face and (ii) The luminal face .The luminal face borders the perinuclear cisternae, the cavities of ER and SER, and the Golgi elements. It also corresponds to the interior of the secretory granules, the lysosomes and peroxisomes and also to faces of mitochondrial membranes confronting to outer mitochondrial chamber.

TYPES OF ENDOPLASMIC RETICULUM

Two types of endoplasmic reticulum have been observed in same or different types of cells which are as follows:

1. Agranular or Smooth Endoplasmic Reticulum This type of endoplasmic reticulum possesses smooth walls because the ribosomes are not attached with its membranes. The smooth type of endoplasmic reticulum occurs mostly in those cells, which are involved in the metabolism of lipids (including steroids) and glycogen. The smooth endoplasmic reticulum is generally found in adipose cells, interstitial cells, and glycogen

storing cells of the liver, conduction fibers of heart, spermatocytes and leucocytes. The muscle cells are also rich in smooth type of endoplasmic reticulum and here it is known as sarcoplasmic reticulum. In the pigmented retinal cells it exists in the form of tightly packed vesicles and tubes known as myeloid bodies.

2. Granular or Rough Endoplasmic Reticulum

The granular or rough type of endoplasmic reticulum possesses rough walls because the ribosomes remain attached with its membranes. Ribosomes play a vital role in the process of protein synthesis. The granular or rough type of endoplasmic reticulum is found abundantly in those cells which are active in protein synthesis such as pancreatic cells, plasma cells, goblet cells, and liver cells. The granular type of endoplasmic reticulum takes basiophilic stain due to its RNA contents of ribosomes. The region of the matrix containing granular type of endoplasmic reticulum takes basiophilic stain and is named as ergastoplasm, basiophilic bodies, chromophilic substances or Nissl bodies by early cytologists.




Golgi Apparatus

For the performance of certain important cellular functions such as biosynthesis of polysaccharides, packaging (compartmentalizing) of cellular synthetic products (proteins), production of exocytone (secretory) vesicles and differentiation of cellular membranes, there occurs a complex organelle called Golgi complex or Golgi apparatus in the cytoplasm of animal and plant cells. The Golgi apparatus, like the endoplasmic reticulum, is a canalicular system with sacs, but unlike the endoplasmic reticulum it has parallely arranged, flattened, membranebounded vesicles which lack ribosomes and stainable by osmium tetraoxide and silver salts.

HISTORICAL

An Italian neurologist (ie, physician) Camillo Golgi in 1873 discovered and developed the silver chromate method (termed la reazione nera) for studying histological details of nerve cells,He, thus, opened a new field of scientific inquiry, called neuromorphology. In 1898, Golgi found that Purkinje cells (ie, nerve cells of cerebral cortex of brain) of barn owl contained an internal reticular network which stains black with the silver stain. He called this structure apparato reticolare Interno (internal reticular apparatus).

Due to their presumed high lipid contents Golgi apparatuses were called lipochondria (Baker1951, 1953) Since originally these were known to be networks, they were also called "dictyosomes" (Gr. dicrver-net) Currently, the term Golgi apparatus is more prevalent one, than many other names such as Golgi complex, Golgiosome, Golgi bodies, Golgi material, Golgi membrane, etc. The Golgi apparatus of the cells of plants and lower invertebrates is usually referred to as Golgi body or dictyosome

OCCURRENCE

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Occurrence

*The Golgi apparatus occurs in all cells except the prokaryotic cells (viz., mycoplasmas, bacteria and blue green algae) and eukaryotic cells of certain fungi, sperm cells of bryophytes and pteridiophytes, cells of mature sieve tubes of plants and mature sperm and red blood cells of animals. Their number per plant cell can vary from several hundred as in tissues of corn root and algal rhizoids (ie., more than 25,000 in algal rhizoids), to a single organelle in some algae. Certain algal cells such as Pinularia and Microsterias, contain largest and most complicated Golgi apparatuses. In higher plants, Golgi apparatuses are particularly common in secretory cells and in young rapidly growing cells.

In animal cells, there usually occurs a single Golgi apparatus, however, its number may vary from animal to animal and from cell to cell. Thus, Paramoeba species has two Golgi apparatuses and nerve cells, liver cells and chordate oocytes have multiple Golgi apparatuses, there being about 50 of them in the liver cells.

The GERL Region

Golgi apparatus is a differentiated portion of the endomembrane system found in both animal and plant cells. This membranous component is spatially and temporally related to the endoplasmic reticulum (ER) on one side and by way of secretory vesicles, may fuse with specific portions of the plasma membrane. To the trans face of Golgi is associated the trans-reticular Golgi, TGN (trans or GERL (-Golgi + smooth ER+ lysosomal), in which acid phosphatase enzyme (a characteristic lysosomal enzyme) makes its first appearance. GERL is found to be involved in Golginetwork) the origin of primary lysosomes and of melanin granules; in the processing, condensing and packaging of secretory material in endocrine and exocrine cells; and in lipid metabolism. GERL is also a region of sorting of cellular secretory proteins.

Lysosomes

Lysosomes, vesicles produced by the Golgi apparatus, contain hydrolytic digestive enzymes. Sometimes macromolecules are brought into a cell by vesicle formation at the plasma membrane When a lysosome fuses with such a vesicle, its contents are digested by lysosomal enzymes into simpler subunits that then enter the cytoplasm Even parts of a cell are digested by its own lysosomes (called autodigestion).



The lysosomes (Gr. lyso-digestive+ soma body) are tiny membrane-bound vesicles involved in intracellular digestion. They contain a variety of hydrolytic enzymes that remain active under acidic conditions. The lysosomal lumen is maintained at an acidic pH (around 5) by an ATP-driven proton pump in the membrane. Thus, these remarkable organelles are primarily meant for the digestion of a variety of biological materials and secondarily cause aging and death of animal cells and also a variety of human diseases such as cancer, gout, Pompe's disease, silicosis and I-cell disease.

Lysosomes were observed and named by Christian de Duve in 1955 as cytoplasmic rounded dense bodies. They were termed "suicide bags" by de Duve in 1959, Lysosomes were investigated according to following two schools: 1. C. de Duve and his coworkers (1963, 1964, 1974) worked in Belgium and their approach was biochemical one. 2. Alex Novikoff and his research group (1962, 1964) worked in United States and their approach was morphological and cytochemical For the discovery of lysosomes and a brilliant series of experiments on them, de Duve shared the 1974 Nobel Prize.

Occurrence

The lysosomes occur in most animal and few plant cells. They are absent in bacteria and mature mammalian erythrocytes. Few lysosomes occur in muscle cure or in acinar cells of the pancreas Leucocytes especially granulocytes are a particularly rich source of lysosomesTheir lysosomes areso large-sized that they can be observed under the light microscope. Lysosomes are also numerous in epithelial cells of absorptive, secretory and excretory organs (e.g., intestine, liver, kidney, etc.). They occur in abundance in the epithelial cells of lungs and uterus. Lastly, phagocytic cells and cells of reticuloendothelial system (e.g., bone marrow, spleen and liver) are also rich in lysosomes

Structure

The lysosomes are round vacuolar structures which remain filled with dense material and are bounded by single unit membrane. Their shape and density vary greatly. Lysosomes are 0.2 to 0.5 um in size. Since, size and shape of lysosomes vary from cell to cell and time to time (ie., they are polymorphic), their identification becomes difficult.

FUNCTIONS OF LYSOSOMES

The important functions of lysosomes are as follows:

1. Digestion of large extracellular particles. The lysosomes digest the food contents of the phagosomes or pinosomes. The lysosomes of leucocytes enable the latter to devour the foreign proteins, bacteria and viruses.

2. Digestion of intracellular substances. During the starvation, the lysosomes digest the stored food contents, viz., proteins, lipids and carbohydrates (glycogen) of the cytoplasm and supply to the cell necessary amount of energy.

3. Autolysis. In certain pathological conditions the lysosomes start to digest the various organelles of the cells and this process is known as autolysis or cellular autophagy. When a cell dies, the lysosome membrane ruptures and enzymes are liberated. These enzymes digest the dead cells. In the process of metamorphosis of amphibians and tunicates many embryonic tissues, eg, gills, fins, tail, etc., are digested by the lysosomes and utilized by the other cells. 4. Extracellular digestion. The lysosomes of certain cells such as sperms discharge their enzymes outside the cell during the process of fertilization. The lysosomal enzymes digest the limiting membranes of the ovum and form penetration path in ovum for the sperms. Acid hydrolases are released from osteoclasts and break down bone for the reabsorption; these cells also secrete lactic acid which makes the local pH enough for optimal enzyme activity. Likewise, preceding ossification (bone formation), fibroblasts release cathepsin D enzyme to break down the connective tissue.

<u>Mitochondria</u>

Most mitochondria (sing., mitochondrion) are between 0.5 μ m and 1.0 μ m in diameter and about 7 μ m in length, although the size and the shape can vary. Mitochondria are bounded by a double membrane.



The inner membrane is folded to form little shelves called cristae, which project into the matrix, an inner space filled with a gel like fluid Mitochondria are the site of ATP (adenosine triphosphate) production involving complex metabolic pathways. ATP molecules are the common carrier of energy in cells. The mitochondria convert the chemical energy of glucose products into the chemical energy of ATP molecules. In the process mitochondria use up oxygen and give off carbon dioxide and water mitochondria carry on cellular respiration. The matrix of a mitochondrion contains enzymes for breaking down glucose products, ATP production then occurs. Every cell uses a certain amount of ATP energy to synthesize molecules, but many cells use ATP to carry out their specialized function. For example, muscle cells use ATP for muscle contraction, which produces movement, and nerve cells use it for the conduction of nerve impulses, which make us aware of our environment.

The Cytoskeleton

Several types of filamentous protein structures form а cytoskeleton that helps maintain the cell's shape and either anchors the organelles or assists their movement as appropriate. The cytoskeleton includes microtubules and actin filaments; Microtubules are shaped like thin cylinders and are several times larger than actin filaments. Each cylinder contains 13 rows of tubule, a globular protein, arranged in a helical fashion. Remarkably, microtubules can assemble and disassemble. In many cells, the regulation of microtubule assembly (helping to maintain the shape of the cell and acting as tracks along which organelles move. It is well known that during cell division, microtubules form spindle fibers, which assist the movement of chromosomes actin filaments are long, extremely thin fibers that usually occur in bundles or other groupings. Actin filaments have been isolated from various types of cells, especially those in

which movement occurs. Microvilli, which project from certain cells and can shorten and extend, contain actin filaments. Actin filaments, like microtubules, can assemble and disassemble *Centrioles*



There are nine outer microtubule triplets and no center microtubules there is always one pair of centrioles lying at right angles to one another near the nucleus. Before a cell divides, the centrioles duplicate, and the members of the new pair are also at right angles to one another. During cell division, the pairs of centrioles separate so that each daughter cell gets one pair of centrioles. Centrioles are part of a microtubule organizing center that also includes other proteins and substances. Microtubules begin to assemble in the center, and then they grow outward, extending through the entire cytoplasm. In addition, centrioles may be involved in other cellular processes that use microtubules, such as movement of material throughout the cell or formation of the spindle, a structure that distributes the chromosomes to daughter cells during cell division. Their exact role in these processes is uncertain, however. Centrioles also give rise to basal bodies that direct the formation of cilia and flagella.

Functions of the Cytoskeleton:

- Intermediate filaments determine cell shape.
- Microtubules and microfilaments assemble and disassemble, causing cell movement.
- Microtubules and microfilaments move organelles within the cell.
- Microfilaments form centrioles, the spindle that apportions the genetic material during cell division.

The Nucleus

Nuclei were first discovered and named by Robert Brown in 1833 in the plant cells and were quickly recognized as a constant feature of all animal and plant cells, Nucleoli were described by **M.J. Schleiden in 1838**, although first noted by Fontana (1781). The term nucleolus was coined by **Bowman in 1840**. In 1879, W. Flemming coined the term chromatin for chromosomal meshwork.



The nucleus is found in all the eukaryotic cells of the plants and animals. However, certain eukaryotic cells such as the mature

sieve tubes of higher plants and mammalian erythrocytes contain no nucleus. In such cells nuclei are present during the early stages of development. Since mature mammalian red blood cells are without any nuclei, they are called red blood "corpuscles" rather than cells (L. corpus body, especially dead body or corpse).

Usually the nucleus remains located in the center. But its position may change from time to time according to the metabolic states of the cell.

The nucleus is composed of the following structures:

1. Nuclear Envelope

The nuclear envelope (or perinuclear cisterna) encloses the DNA and defines the nuclear compartment of interphase and prophase nuclei. It is formed from two concentric unit membranes, each 5-10 nm thick. The spherical inner nuclear membrane contains specific proteins that act as binding sites for the supporting fibrous sheath of intermediate filaments (IF), called nuclear lamina Nuclear lamina has contact with the chromatin (or chromosomes) and nuclear RNAs. The inner nuclear membrane is surrounded by the outer nuclear membrane, which closely resembles the membrane of the endoplasmic reticulum, that is continuous with it. It is also surrounded by less organized intermediate filaments.



2. Nucleoplasm

The space between the nuclear envelope and the nucleolus is filled by a transparent, semi- solid, granular and slightly acidophilic ground substance or the matrix known as the nuclear sap or nucleoplasm or karyolymph. The nuclear components such as the chromatin threads and the nucleolus remain suspended in the nucleoplasm.

The nucleoplasm has at complex chemical composition. It is composed of mainly the nucleoproteins but it also contains other inorganic and organic substances, viz., nucleic acids, proteins, enzymes and minerals.

1. Nucleic acids. The most common nucleic acids of the nucleoplasm are the DNA and RNA. Both may occur in the macromolecular state or in the form of their monomer nucleotides.

2. Proteins. The nucleo-plasm contains many types of complex proteins.

<u>3- Chromatin Fibres</u>

The nucleoplasm contains many thread=like, coiled and much elongated structures which take readily the basic stains such as the basic fuchsin. These thread-like structures are known as the chromatin (Gr., chrome colour) substance or chromatin fibers. Such chromatin fibers are observed only in the interphase nucleus. During the cell division (mitosis and meiosis) chromatin fibers become thick ribbon-like structures which are known as the chromosomes.

Chemically, chromatin consists of DNA and proteins, Small quantities of RNA may also be present but the RNA rarely accounts for more than about 5 per cent of the total Chromatin present.

The fibers of the chromatin are twisted, finely anastomosed and uniformly distributed in the nucleoplasm. Two types of Chromatin material have been recognized, heterochromatin (contains small amount of DNA and large amounts of RNA) and euchromatin (contains comparatively large amounts of DNA)

4. Nucleolus.

Most cells contain in their nuclei one or more prominent spherical colloidal acidophilic bodies, called nucleoli. However, cells of bacteria and yeast lack nucleolus. The size of the nucleolus is found to be related with the synthetic activity of the cell. Therefore, the cells with little or no synthetic activities, e.g., sperm cells, blastomeres, muscle cell, etc., are found to contain smaller or no nucleoli while the oocytes, neurons and secretory cells which synthesize the proteins or other substances contain comparatively large-sized nucleoli. The number of the nucleoli in the nucleus depends on the species and the number of the chromosomes. The number of the nucleoli in the cells may be one, two or four. The position of the nucleolus in the nucleus is eccentric.

Functions of Nucleus

Nucleus is the controlling Centre of the cell.

1- It controls all the metabolic activities of the cell by controlling the synthesis of enzyma required there in.

- 2- The nucleus controls the inheritance of characters from parents to offspring.
- 3- It is responsible for the development of characters. Our phenotype is determined by the genes we have inherited from our parents. These genes are present in the chromosomes of nucleus.

What is the differences between Nucleus and Nucleolus?

CELL CYCLE & CELL DIVISION

Cell life cycle

Cells have two major periods

Interphase

(Cell grows, Cell carries on metabolic processes)

• Cell division (Cell replicates itself, to produce more cells for growth and repair processes)

DNA Replication

• Genetic material duplicated and readies a cell for division into two cells

- Occurs toward the end of interphase
- DNA uncoils and each side serves as a template

MULTIPLICATION OF CELL

New cells are produced from the preexisting cells by division. Ordinarily cells divide by two means, Mitosis and Meiosis. The former is meant for multiplication of cells and is found in somatic cells, while the latter produces gametes, and is naturally restricted to germ cells. A cell division consists of karyokinesis or nuclear division followed by cytokinesis or cytoplasmic division.

MITOSIS

It is a type of cell division in which a constant chromosome number is maintained in the parent (dividing) and daughter (divided) cells. Mitosis affects a qualitative and quantitative distribution of the essential particles of heredity, the genes to daughter cells. It is a dynamic mechanical process, a number of push and pull, stress and strain.

Karyokinesis

The whole cycle of karyokinesis is a continuous one and its division to prophase, prometaphase, metaphase, anaphase and telophase is only conventional.

Prophase. The prophase is initiated with the division of the centrosome into two and the appearance of the chromosomes. During this stage, two halves of the centrosome travels in opposite directions around the nucleus until they are 180° apart and aster is formed around each daughter centrosome.

Each chromosome is composed of two closely parallel chromatids. The division of a chromosome into two chromatids takes place before the beginning of mitosis and the cell division is really concerned not with the multiplication of the chromosomes but only with the distribution of the products.

Each chromosome has a non-staining gap, the centromere, which consists mainly or entirely of protein, with little or no DNA or RNA, and plays an important role in attaching the chromosome to the spindle. Throughout the prophase stage the chromatids are slightly irregular, woolly or hairy in appearance and by the end of the prophase they become smooth in outline. The nuclear membrane usually disappears at the end of this stage.

Prometaphase* . The spindle is formed. It is a relatively solid gelatinous body, apparently composed entirely of protein, with a very small amount of RNA. Centrosomes possibly act as organizers of the spindle and are placed at the two poles. The chromosomes arrange themselves with their centromeres on the equatorial plane of the spindle.



Metaphase. The chromosomes reach maximal condensation and the chromatids are united only at the centromere, which is still undivided. Metacentric chromosomes usually appear V-shaped at metaphase, while acrocentrics appear as straight or slightly curved rods.

Anaphase. The centromere divides into two daughter centromeres and separate from one another. At this time the equatorial region of the spindle between the daughter centrosomes elongates and the poles of the spindle become further apart in the late anaphase. The equatorial part of the spindle narrows and appears as a thin bundle of thread-like structure, the stem body, between the two separating groups of daughter chromosomes.

Telophase. The two groups of chromosomes do not actually reach the poles of the spindle but are at some distance from the equator. The polar caps of the spindle disappear; the chromosomes lose their smooth outline, undergo decondensation and form a tangled mass. A nuclear membrane appears around the chromosomes, the stem body disappears, and two daughter nuclei are formed.

Cytokinesis

The cytoplasmic division starts in the telophase or even in the anaphase in some cases. A furrow appears around the cytoplasm which gradually moves inward and finally cleaves the cytoplasm into two halves, each receiving a daughter nucleus, and two daughter cells are formed.

Significance of Mitosis.

1. The exact longitudinal division of the chromosomes ensures equal distribution of genetic constitution in the daughter cells.

2. Lineal heredity is established whether it be from cell to cell or organism to organism.

3. It ensures the maintenance of constancy of a species which is essential in the perpetuation of the race since its inception.

MEIOSIS

Meiosis is the antithesis of fertilization. It occurs mainly during the formation of gametes. In diploid organisms it results in the haploid number of chromosomes. In meiosis the nucleus divides twice but chromosomes divide only once. A gap of interphase of uneven duration is present between the successive divisions Each division is broadly separated into five stages

Prophase, Prometaphase, Metaphase, Anaphase and Telophase.

FIRST MEIOSIS

Prophase

It is a very long stage and may be divided into the following subdivisions.

A. Leptotene

1. The chromosomes are not longitudinally divided and consist of a single chromat

2. The threads are composed of a series of granules (chromomeres) connected by non-staining gaps.

3. Leptotene chromosomes preserve the same structure and number as in the somatic tissue.

B. Zygotene

1. The homologous chromosomes come to pair which begin at one or more points, usually at centromere.

2. Pairing is not merely between homologous chromosomes but strictly bet homologous regions, which is revealed from various sorts of loops, and it seems that the force of attraction is a mutual one between homologous chromomeres (or genes) resulting in a delay in the splitting of the chromosomes.

C. Pachytene

1. As a result of pairing the number of the chromosomes is reduced to half.

2. The paired chromosomes are called bivalents, each of which now splits longitudinally. The bivalents have two distinct centromeres.

3. In pachytene, the threads are strictly parallel in the beginning but in some they are coiled and on splitting it seems that the two threads are wound around each other.

D. Diplotene

1. With the splitting of the pachytene chromosomes the force of attraction between the paternal and maternal chromosomes is replaced by a force of attraction between the chromatids.

2. The moment the two homologous chromosomes begin to separate, marks the transition from pachytene to diplotene.

3. They do not actually separate completely but remain held together at some points called chiasmata. There is usually at least one chiasma in each bivalent.

4. Chiasma in a bivalent is accompanied by three changes.

(i) Shortening and thickening of the threads.

(ii) Rotation of 180° is most marked in bivalents with a single chiasma.

(iii) Actual moving of the chiasma towards the end or terminalization.

E. Diakinesis

1. The bivalents become much thicker and shorter.

2. The split in some cases becomes invisible.

3- Terminalization may still continue.



Prometaphase. It is the period between the disappearance of the nuclear membrane and the moment when the spindle is fully

formed. Here the diakinesis bivalents have contracted still further and begin to be associated with the developing spindle.

Metaphase

1. Each bivalent possesses two centromeres, one maternal and the other paternal.

2 They are generally far apart and they arrange themselves at equal distances above and below the equatorial plane.

3 The centromeres have not divided nor they do so until the second meiotic division.

Anaphase

- 1. As the centromeres separate, they drag after them the chromatids which are attached to them.
- 2. The unterminalised chiasmata move to the end, away from the centromere and slip off at the ends.

3. Each centromere, as it moves to the pole, drags after it two chromatids.

4. The result of this stage is often said to be a separation of whole chromosomes.

5. The chromosomes which separate at the first anaphase are not the same as the maternal and paternal chromosomes which came together at zygotene but these have interchanged parts of their length by crossing over, so that the actual chromosomes which separate at first division are new combinations of segments of the maternal and paternal chromosomes. **6.** There is no correlation between the mode of orientation of one bivalent and another in the same cell.

7. Alike mitosis, here also the spindle elongates to form the stem body and completes the anaphase separation of two groups of chromosomes.

Telophase

- **1.** The telophase nuclei are formed.
- 2. They pass into a more or less complete resting stage.

SECOND MEIOSIS

Prophase

If the chromosomes have not gone into a resting stage during interkinesis, there will naturally be no prophase to the second meiosis. In this case, the telophase nuclei of the first meiosis will pass directly into prometaphase of the second meiosis. Prophase . if present, is too short.

Prometaphase

It differs from the ordinary mitotic division in the following.

1. The number of chromosome is half the somatic number.

2. The chromatids diverge widely being only held together at the centromere and not approximated throughout their length.

Metaphase

- 1. The chromatids tend however to come in closer contact.
- 2. The chromosomes have only one spiral.

Anaphase

- 1. Centromeres divide.
- 2. Two sister chromatids move to opposite poles.

Telophase

1. Four daughter nuclei are formed with haploid number of chromosomes.

2. The nuclear division is followed by cytokinesis and four cells are formed at the end of meiosis.

Significance of meiosis

At fertilization two gametes unite but their chromosomes remain separate. In meiosis, the chromosome number is reduced to half (haploid) and in this way the original number (diploid) is restored in the zygote.

THE STRUCTURE OF A CHROMOSOME

1. In 1888. Waldeyar introduced the term chromosome, because of its affinity towards dyes.

2. The chromosomes are darkly stained, individualised, protoplasmic bodies located in the nucleus.

3. In an interphase nucleus, these form an interwoven network of fine chromatin.

4. They appear as well defined structures during cell division and are most distinct in metaphase only.

5. The chromosomes are sell duplicating structures and maintain their morphological and physiological characteristics all along. Microscopic structure

A chromosome has three distinct zones - pellicle, matrix and chromonemata.

1. Pellicle. The very thin outer achromatic envelope.

2. Matrix. The ground substance.

3. Chromonemata. Two identical, spirally coiled thread-like structures embedded in the matrix. They are tightly coiled together and appear as a single thread of about 800 A thicknesses.(a) A chromonemata consists of about 8 micro fibrils, each of which is formed of double helix of DNA.

(b) Chromomeres. These are small, dense masses at regular intervals on the chromo T nemata, and found only in leptotene and zygotene stages of meiosis.

(c) Interchromomeres. The highly stained parts of the chromonemata in between the chromomeres.

(d) Centromere or Kinetochore. A constricted zone where two chromonemata are joined together. This is also called primary constriction.

(i) The chromosome attaches itself to the spindle fibre with the centromere during cell division.

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(ii) The centromere helps in the orientation of the chromosomes on the spindle. It divides during cell division and play an important role in the segregation of chromosomes to the poles.

(iii) The centromere is usually one(monocentric); the number may be two

(dicentric), many (polycentric) or none (acentric). The acentric chromosomes do not survive.

(iv)The centromere may be located in different sites of a chromosome. Telocentric occupying terminal position; acrocentric, subterminal position; metacentric, middle position. In metaphase the telocentric chromosome assumes rod- shape, acrocentric, rod-shaped with one small anterior arm and metacentric,V-shaped.

4. The part of the chromosome on either side of centromere represents arms.

5. Nucleolus organiz organizer region or secondary constriction. This is sometimes present in one or both the arms of a chromosome. This area is associated with the nucleolus and participates in its formation.

6. Satellite. Sometimes the secondary constriction is subterminal and the small part at the terminal end beyond the constriction appears as a knob. The shape and size of the satellite are constant for a particular chromosome.

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DNA, RNA and Genes

How did DNA discovered?

DNA as an acidic substance present in nucleus was first identified by
Friedrich Meischer in 1868.
He named it as 'Nuclein'.



• In 1953, James Watson and Francis Crick,

described a very simple but famous Double Helix model for the Structure of DNA. The scientific framework for their breakthrough was provided by other scientists including



- □ Linus Pauling
- **Rosalind Franklin and Maurice Wilkins**
- □ Erwin Chargaff



Rosalind Franklin

She worked in same laboratory as Maurice Wilkins.
She study X-ray diffraction to study wet fibers of DNA.
She made marked advances in X-ray diffraction techniques with DNA



□ The diffraction pattern she obtained suggested several

structural features of DNA

□ Helical

- $\hfill\square$ More than one strand
- □ 10 base pairs per complete turn

DNA Structure

- DNA structure is often divided into four different levels primary, secondary, tertiary and quaternary.
- > DNA has three main components

1. Deoxyribose (a pentose sugar **2**. Base (there are four different ones)



3. Phosphate

The Nitrogenous Bases



THEY ARE DIVIDED INTO TWO GROUPS

- > Pyrimidines and purines
- > PYRIMIDINES (MADE OF ONE 6 MEMBER RING)

Thymine T

Cytosine C



- PURINES (MADE OF A 6 MEMBER RING, FUSED TO A 5 MEMBER RING)
- ➤ Adenine A
- ➢ Guanine G



□ THE RINGS ARE NOT ONLY MADE OF CARBON



Nucleotide Structure

- Nucleotides are formed by the condensation of a sugar, phosphate and one of the 4 bases
- > The following illustration represents one nucleotide



Base + sugar → nucleoside

□ Example

- \Box Adenine + ribose = Adenosine
- □ Adenine + deoxyribose = Deoxyadenosine
- □ Base + sugar + phosphate(s) → nucleotide

□ Example

- □ Deoxyadenosine monophosphate (dAMP)
- □ Deoxyadenosine diphosphate (dADP)
- **Deoxyadenosine triphosphate (dATP)**





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DNA Double Helix & Hydrogen bonding

Salient features of the Double-helix structure of DNA:

□ It is made of two polynucleotide chains, where the backbone

is constituted by sugar-phosphate, and the bases project inside.

□ The two chains have anti- parallel polarity. It means, if one chain has the polarity $5' \rightarrow 3'$, the other has $3' \rightarrow 5'$.





The bases in two strands are paired through hydrogen bond (H-bonds) forming base pairs (bp). Adenine forms two hydrogen bonds with Thymine from opposite strand and vice-versa. Similarly, Guanine is bonded with Cytosine with three H-bonds.

□ Based on the observation of Erwin Chargaff that for a double stranded

DNA, the ratios between Adenine and Thymine; and Guanine and Cytosine are constant and equals one.

□ Hydrogen bond:-A chemical bond consisting of a hydrogen atom between two electronegative atoms (e.g., oxygen or nitrogen) with one side be a covalent bond and the other being an ionic bond.



The two strands are coiled in a right-handed fashion (Clockwise).

The pitch of the helix is 3.4 nm (a nanometer is one billionth of a meter, that is 10-9 m) and there are roughly 10 bp in each turn. Consequently, the distance between a bp in a helix is approximately equal to 0.34 nm.

□ The plane of one base pair stacks over the other in double helix. This, in addition to H-bonds, confers stability of the helical structure.



There are two asymmetrical grooves on the outside of the helix:

- a) Major groove
- b) Minor groove

Groove:-any furrow (slight depression in the smoothness of a surface) or channel on a bodily structure or part.

□ Certain proteins can bind within these groove

□ They can thus interact with a particular sequence of bases.



Structure of Double-helix

□ Three major forms:

B-DNA A-DNA Z-DNA

• B-DNA

is biologically the most common

 $\hfill\square$ It is a -helix meaning that it has a Right handed, or clockwise, spiral.

- □ Complementary base pairing
- A-T
- G-C
- □ Ideal B-DNA has 10 base pair per turn(3600 rotation of helix)
- \Box So each base is twisted 360 relative to adjacent bases.

□ Base pair are 0.34 nm apart.

□ So complete rotation of molecule is 3.4 nm.

□ Axis passes through middle of each basepairs.

B-DNA

□ Minor Groove is Narrow, Shallow.

□ Major Groove is Wide, Deep.

□ This structure exists when plenty of water

surrounds molecule and

there is no unusual base sequence in DNA-

Condition that are likely to

be present in the cells.

□ B-DNA structure is most stable



configuration for a random sequence of nucleotides under physiological condition.

<u>A-DNA</u>

- ✓ Right-handed helix
- ✓ Wider and flatter than B-DNA
- ✓ 11 bp per turn
- ✓ Its bases are tilted away from main axis of molecule
- ✓ Narrow Deep major Groove and Broad, Shallow minor Groove.
- ✓ Observed when less water is present.
 i.e.Dehydrating condition.
- ✓ A-DNA has been observed in two context:

• Active site of DNA polymerase (~3bp)



• Gram (+) bacteria undergoing sporulation

<u>Z-DNA</u>

- ✓ A left-handed helix
- ✓ Seen in Condition of High salt concentration.
- ✓ In this form sugar-phosphate backbones zigzag back and forth, giving rise to the name Z-DNA(for zigzag).
- ✓ 12 base pairs per turn.
- ✓ A deep Minor Groove.
- ✓ No Discernible Major Groove.
- ✓ Part of some active genes form Z-DNA, suggesting that Z-DNA may play a role in regulating gene transcription.

Property	<u>B-DNA</u>	<u>A-DNA</u>	<u>Z-DNA</u>
Strand	antiparallel	antiparallel	antiparallel
Type of Helix	Right-handed	Right- handed	Left- handed
Overall shape	Long &	Short & wide	Elongated &
	narrow		narrow
Base pair per turn	10	11	12
Distance between	0.34nm	0.23nm	0.38nm
adjacent bases			
Pitch/ turn of Helix	3.40nm	2.82nm	4.56nm
Helical diameter	2.0nm	2.3nm	1.8nm



Tilt/inclination of bp to axis	10	200	90
<u>Major Groove</u>	Wide& Deep	Narrow& Deep	No discrenible
Minor Groove	Narrow, Shallow	Broad, Shallow	Narrow, Deep

DNA Supercoiling

- DNA supercoiling refers to the over or under-winding of strands.
- DNA supercoiling is important for DNA packaging within all cells. Because the length of DNA can be of thousands of times that of a cells, packaging this material into the cell or nucleus (in Eukaryotes) is a difficult feat.
- Supercoiling of DNA reduces the space and allows for much more DNA to be packaged.



Nucleosome Structure

- Nucleosomes are the basic unit of the chromatin organization.
- In Eukaryotes DNA associated with Proteins. (In prokaryotes DNA is naked)
- Nucleosomes= basic bead like unit of DNA packing
 - ✓ Made of segment of DNA wound around a protein core that is composed of 2 copies of each 4 types of Histones.
- > Nucleosomes have:
 - ✓ 8 Histones in the core
 - ✓ DNA wrapped twice around the core
 - ✓ One Histone holding the Nucleosome together
 - ✓ A DNA 'linker' continues towards the next nucleosome.
- The DNA has a negatively charged backbone(because of PO4 3- group)
- > The Protein(Histones) are positively charged.
- The DNA and Protein are Electromagnetically attracted to each other to form chromatin.



RNA Ribonucleic Acid

- RNA is a polymer of ribonucleotides linked together by phosphodiester linkage.
- > RNA was first genetic material.
- In 1967 Carl Woese found the catalytic properties of RNA and speculated that the earliest forms of life relied on RNA both to carry genetic information and to catalyse biochemical reactions.
- Their theories were not validated until the work of Nobel Prize laureate Thomas R. Cech. In the 1970s, Cech was studying the splicing of RNA in a single-celled organism, Tetrahymena thermophila, when he discovered that an unprocessed RNA molecule could splice itself. He announced his discovery in 1982 and became the first to show that RNA has catalytic functions.
- Usually single stranded and helical in structure.
- But double stranded also present in some viruses
- RNA exists in several different single-stranded structures, most of which are directly or indirectly involved in protein synthesis or its regulation.
- > It also acts as the genetic material in some viruses.
- It function as messenger (mRNA), adapter(tRNA), structural (rRNA) and in some cases as a catalytic molecule(Ribozyme).

RNA strands are typically several hundred to several thousand nucleotides in length.



RNA structure

- □ There are also three main component
- a) Phosphate Group
- b) Sugar (Ribose)
- c) And Nitrogenous base
- □ The Nitrogenous Bases



□ They are divided into two groups:

i. Purine

- ii. Pyrimidine
- □ Purines (made of a 6 member ring, fused to a
- 5 member ring)
 - > Adenine
 - > Guanine
- □ Pyrimidine (made of a 6 member ring)
 - > Cytosine
 - > Uracil

RNA Nucleotide

- Nucleotides are formed by the condensation of a sugar, phosphate and one of the 4 bases
- The following illustration represents one nucleotide



Base + sugar \rightarrow nucleoside

- □ Example
- \Box Adenine + ribose = Adenosine
- \Box Base + sugar + phosphate(s) \rightarrow nucleotide
- □ Example
- □ Adenosine monophosphate (AMP)

□ Adenosine diphosphate (ADP)

□ Adenosine triphosphate (ATP)



* Covalent bonding B/W Nucleotides

Nucleotides are linked together by <u>covalent bonds</u> called <u>phosphodiester linkage</u>.





Hydrogen bonding

- Usually RNA is single stranded, But in some viruses RNA present in double stranded form.
- The bases in two strands are paired through hydrogen bond (Hbonds) forming base pairs (bp). Adenine forms two hydrogen bonds with Uracil from opposite strand and vice-versa. Similarly, Guanine is bonded with Cytosine with three H-bonds.

dsRNA Structure

- > There are double-stranded RNA structures
- ✓ RNA can fold back on itself
- ✓ Depends on base sequence
- ✓ Gives stem (double-strand) and loop (single-strand structures)
- **b** ds RNA has an A-like conformation
- ✓ Steric clashes between 2'-OH groups prevent the B-like conformation.



Types of RNA

- In all prokaryotic and eukaryotic organisms, three main classes of RNA molecules exist1) Messenger RNA(m RNA)
 - 2) Transfer RNA (t RNA)
 - 3) Ribosomal RNA (r RNA)
- ➤ The other are –
- ✓ small nuclear RNA (SnRNA),
- ✓ micro RNA(mi RNA) and
- ✓ small interfering RNA(Si RNA) and
- ✓ Heterogeneous nuclear RNA (hnRNA).

Messenger RNA (m-RNA)

□ All members of the class function as messengers carrying the information in a gene to the protein synthesizing machinery



Structure

- The 5' terminal end is capped by 7- methyl guanosine triphosphate cap.
- The cap is involved in the recognition of mRNA by the translating machinery.
- > It stabilizes m RNA by protecting it from 5' exonuclease.
- > The 3'end of most m-RNAs have a polymer of Adenylate

Residues (20-250).

5'

- > The tail prevents the attack by 3' exonucleases.
- On both 5' and 3' end there are non-coding sequences which are not translated (NCS)
- The intervening region between non coding sequences present between 5' and 3' end is called coding region.

This region encodes for the synthesis of a protein.

 The structure of a typical human protein coding mRNA including the untranslated regions (UTRs)

 Cap
 5' UTR
 Coding sequence (CDS)
 3' UTR
 Poly-A tail

3

Eukaryotic mRNA molecule



Heterogeneous nuclear RNA (hnRNA) [Precursor mRNA]

□ In mammalian nuclei , hnRNA is the immediate product of gene transcription

□ The nuclear product is heterogeneous in size

(Variable) and is very large.

□ 75 % of hnRNA is degraded in the nucleus, only 25% is processed to mature m RNA.

□ Mature m –RNA is formed from primary transcript by capping, tailing, splicing and base modification. Transfer RNA (t-RNA)

□ Transfer RNA are the smallest of three major species of RNA molecules

□ They have 74-95 nucleotide residues

□ They transfer the amino acids from cytoplasm to the protein synthesizing machinery, hence the name tRNA.

□ They are also called Adapter molecules, since they act as adapters for the translation of the sequence of nucleotides of the m RNA in to specific amino acids

□ There are at least 20 species of tRNA one corresponding to each of the 20 amino acids required for protein synthesis.

□ tRNA is the only RNA species that contains the nucleoside thymidine. Structure

1) Primary structure- The nucleotide

sequence of all the t RNA molecules

allows extensive intrastand

complementarity that generates a

secondary structure.

2) Secondary structure- Each single t- RNA shows extensive internal base pairing and acquires a clover leaf like structure. The structure is stabilized by hydrogen bonding between the bases and is a consistent feature. Secondary structure (Clover leaf structure)

All t-RNA contain 5 main arms or loops which are as followsa) Acceptor arm

b) Anticodon arm

- c) D HU arm
- d) TΨ C arm
- e) Extra arm
- (DihydroUracil)

Thymidine Pseudouridine Cytosine



Secondary structure of tRNA. CCA tail in yellow, Acceptor stem in purple, Variable loop in orange, D arm in red, Anticodon arm in blue with Anticodon in black, T arm in green.

3) Tertiary structure of t-RNA

The L shaped tertiary structure is formed by further folding of the clover leaf due to hydrogen bonds between T and D arms.
 The base paired double helical stems get arranged in to two double helical columns, continuous and perpendicular to one another.



Ribosomal RNA (rRNA)

□ Ribosomal ribonucleic acid (rRNA) is the RNA component of the ribosome, and is essential for protein synthesis in all living organisms.

□ The functions of the ribosomal RNA molecules in the ribosomal particle are not fully understood, but they are necessary for ribosomal assembly and seem to play key roles in the binding of mRNA to ribosomes and its translation

□ Recent studies suggest that an rRNA component performs the peptidyl transferase activity and thus is an enzyme (a ribozyme).

□ It constitutes the predominant material within the ribosome, which is approximately 60% rRNA and 40% protein by weight.

□ Ribosomes contain two major rRNAs and 50 or more proteins.

□ The ribosomal RNAs form two subunits, the large subunit Major types of small RNA molecules:

> Small nuclear RNA (snRNA) - involved in mRNA splicing.

> Small nucleolar RNA (snoRNA) - directs the modification of ribosomal RNAs.

(LSU) and

small subunit (SSU). The LSU rRNA acts as a ribozyme, catalysing peptide bond formation.

Small RNA molecules

□ Micro RNA (miRNA) and short interfering RNA (siRNA) -regulate gene expression

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