Module 1 Lecture 5

The present lecture discusses about structure and function of cytoplasm, nucleus and mitochondria

Structure and function of cytoplasm

Cytoplasm was discovered in 1835 and no single scientist can be credited for discovering cytoplasm the discovery was possible due to contribution of several scientists. It is worth mentioning that the discovery of different organelles in the cytoplasm was attributed to different scientist. The cytoplasm is the part of the cell outside the largest organelle, the nucleus. Cytoplasm appears as thick, gel-like semitransparent fluid that is found in both plant and animal cell. It is bounded by the plasma membrane, and contains many organelles in a eukaryotic cell (cell containing membrane bounded nucleus). The constituent parts of cytoplasm are cytosol, organelles and cytoplasmic inclusions. The cytosol, the aqueous part of the cytoplasm outside all of the organelles, also contains its own distinctive proteins.

Cytosol

Cytosol is the part of the cytoplasm that is not occupied by any organelle. It accounts for almost 70% of the total cell volume. Cytosol (cytoplasmic matrix) like many colloidal systems, shows the property of phase reversal. Under the natural conditions, the phase reversal of the cytosol (cytoplasmic matrix) depends on various physiological, mechanical and biochemical activities of the cell. It is a gelatinous substance consisting mainly of cytoskeleton filaments, organic molecules, salt and water. Chemically, the cytoplasmic matrix is composed of many chemical elements in the form of atoms, ions and molecules. Of the 92 naturally occurring elements, approximately 46 are found in the cytosol (cytoplasmic matrix). Twenty four of these are essential elements, while others are present in cytosol only because they exist in the environment with which the organism interacts. Of the 24 essential elements, six play especially important roles in living systems. These major elements are carbon (C, 20 per cent), hydrogen (H, 10 per cent), nitrogen (N, 3 per cent), oxygen (O, 62 per cent), phosphorus (P, 1.14 per cent) and sulphur (S,0.14 per cent). Most organic molecules are built with these six elements. Another five essential elements found in less abundance in living systems are calcium

(Ca, 2.5 per cent), potassium (K, 0.11 per cent), sodium (Na, 0.10 per cent), chlorine (Cl, 0.16 per cent) and magnesium (Mg, 0.07 per cent).Several other elements, called trace elements, are also found in minute amounts in animal and plant cell cytosol. These are iron (Fe, 0.10 per cent), iodine (I, 0.014 per cent), molybdenum (Mo), manganese (Mn), Cobalt (Co), zinc (Zn), selenium (Se), copper (Cu), chromium (Cr), tin (Sn), vanadium (V), silicon (Si), nickel (Ni), fluorine (F) and boron (B).

The cytoplasmic matrix consists of various kinds of ions. The ions are important in maintaining

osmotic pressure and acid-base balance in the cells. Retention of ions in the matrix produces an increase in osmotic pressure and, thus, the entrance of water in the cell. The concentration of various ions in the intracellular fluid (matrix) differs from that in the interstitial fluid. For example, in the cell K^+ and Mg^{++} can be high, and Na^+ and Cl^-high outside the cell. In muscle and nerve cells a high order of difference exists between intracellular K^+ and extracellular Na^+. Free calcium ions (Ca^{++}) may occur in cells or circulating blood. Silicon ions occur in the epithelium cells of grasses.

Chemical compounds present in cytosol are conventionally divided into two groups: organic and inorganic. Organic compounds form 30 per cent of a cell, rest are the inorganic substances such as water and other substances. The inorganic compounds are those compounds which normally found in the bulk of the physical, non-living universe, such as elements, metals, non-metals, and their compounds such as water, salts and variety of electrolytes and non-electrolytes. In the previous section, we have discussed a lot about the inorganic substances except the water which will be discussed in the following paragraph. The main organic compounds of the matrix are the carbohydrates, lipids, proteins, vitamins, hormones and nucleotides.

Properties of cytoplasmic matrix

The most of the physical properties of the matrix are due to its colloidal nature. The cytosol shows Tyndal effect (light scattering by particle in colloidal solution) and Brownian motion (random moving of particles). Due to the phase reversal property of the cytoplasmic matrix, the intracellular streaming or movement of the matrix takes place and is known as the cyclosis. The cyclosis usually occurs in the sol-phase of the matrix and is effected by the hydrostatic pressure, temperature, pH, viscosity, etc. Cyclosis has been observed in most animal and plant cells. The amoeboid movement depends directly on the cyclosis. The amoeboid movement occurs in the protozoans, leucocytes, epithelia, mesenchymal and other cells. Due to cyclosis matrix moves these pseudopodia and this causes forward motion of the cell. The cytoplasmic matrix being a liquid possesses the property of surface tension. The proteins and lipids of matrix have less surface tension, therefore, occur at the surface and form the membrane, while the chemical substances such as NaCl have high surface tension, therefore, occur in deeper part of the matrix. Besides surface tension and adsorption, the matrix possesses other mechanical properties, *e.g.*, elasticity, contractility, rigidity and viscosity which provide to the matrix many physiological utilities. The colloidal system due to its stable phase gives polarity of the cell matrix which cannot be altered by centrifugation of other mechanical means. The matrix has a definite pH value and it does not tolerate significant variations in its pH. Yet various metabolic activities produce small amount of excess acids or bases which is maintained by certain chemical compounds as carbonate-bicarbonate buffers. The matrix is a living substance and possesses various biological properties as irritability, conductivity, movement, metabolic activity, growth and reproduction.

Organelles

Cytoplasm contains all the organelles like nucleus, mitochondria, endoplasmic reticulum, lysosomes and Golgi apparatus. Besides, it also contains chloroplast in plant cells. Each organelle is bounded by a lipid membrane, and has specific functions.

Cytoplasmic inclusions

Some insoluble suspended substances found in cytosol. They are basically granules of starch and glycogen, and they can store energy. Besides, crystals of some minerals and lipid droplets can also be found in cytoplasm. Lipid droplets act as storage site of fatty acid and steroids.

Functions of Cytoplasm

Cytoplasm is the site of many vital biochemical reactions crucial for maintaining life.

1. It is the place where cell expansion and growth take place.
2. It provides a medium in which the organelles can remain suspended.
3. Besides, cytoskeleton found in cytoplasm gives the shape to the cell, and facilitates its movement.
4. It also assists the movement of different elements found within the cell. The enzymes found in the cytoplasm breaks down the macromolecules into small parts so that it can be easily used by the other organelles like mitochondria. For example, mitochondria cannot use glucose present in the cell, unless it is broken down by the enzymes into pyruvate. They act as catalysts in glycolysis, as well as in the synthesis of fatty acid, sugar and amino acid.
5. Cell reproduction, protein synthesis, anaerobic glycolysis, cytokinesis are some other vital functions that are carried out in cytoplasm. However, the smooth operation of all these functions depend on the existence of cytoplasm, as it provides the medium for carrying out these vital processes.

Cell Organelles

Nucleus

Nucleus means kernel and was the first organelle to be discovered. It was discovered and named by Robert Brown in 1833 in the plant cells and is recognized as a constant feature of all animal and plant cells. Certain eukaryotic cells such as the mature sieve tubes of higher plants and mammalian erythrocytes contain no nucleus. It is the largest cellular organelle in eukaryotes. Prokaryotic cells lack nucleus and is complemented by nucleoid. In mammalian cells, the average diameter of the nucleus is approximately 6 micrometers (μm), occupying about 10% of the total cell volume. The contents of the nucleus are DNA genome, RNA synthetic apparatus, and a fibrous matrix. It is

surrounded by two membranes, each one a phospholipid bilayer containing many different types of proteins. The inner nuclear membrane defines the nucleus itself. In most cells, the outer nuclear membrane is continuous with the rough endoplasmic reticulum, and the space between the inner and outer nuclear membranes is continuous with the lumen of the rough endoplasmic reticulum. The two nuclear membranes appear to fuse at nuclear pores, the ringlike complexes composed of specific membrane proteins through which material moves between the nucleus and the cytosol. It contains cell's genetic material, organized as multiple long linear DNA molecules in complex with histones, to form chromosomes. The genes within these chromosomes are the cell's nuclear genome. The function is to maintain the integrity of the genes that controls the activities of the cell by regulating gene expression. The schematic presentation of nucleus is in Figure 1.

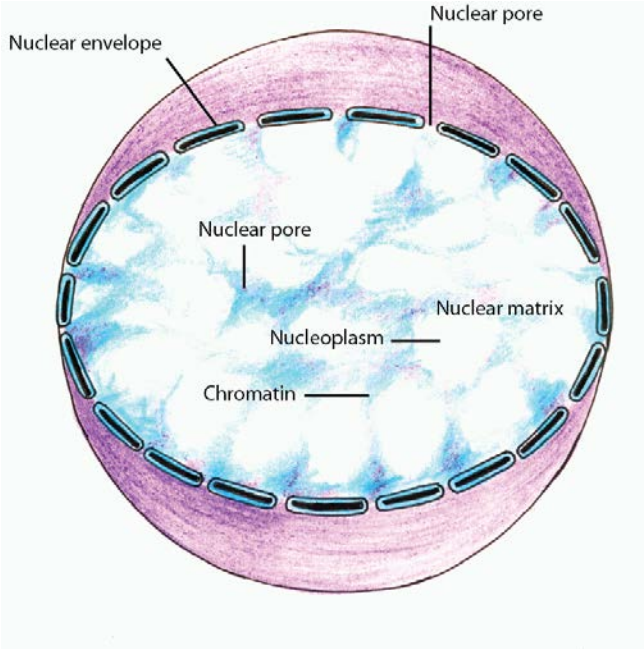

Figure 1: The schematic representation of nucleus.

In a growing or differentiating cell, the nucleus is metabolically active, replicating DNA and synthesizing rRNA, tRNA, and mRNA. Within the nucleus mRNA binds to specific proteins, forming ribonucleoprotein particles. Most of the cell's ribosomal RNA is synthesized in the nucleolus, a subcompartment of the nucleus that is not bounded by a phospholipid membrane. Some ribosomal proteins are added to ribosomal RNAs within the nucleolus as well. The finished or partly finished ribosomal subunits, as well as

tRNAs and mRNA-containing particles, pass through a nuclear pore into the cytosol for use in protein synthesis. In a nucleus that is not dividing, the chromosomes are dispersed and not dense enough to be observed in the light microscope. Only during cell division are individual chromosomes visible by light microscopy. In the electron microscope, the nonnucleolar regions of the nucleus, called the nucleoplasm, can be seen to have dark and light staining areas. The dark areas, which are often closely associated with the nuclear membrane, contain condensed concentrated DNA, called heterochromatin. Fibrous proteins called lamins form a two-dimensional network along the inner surface of the inner membrane, giving it shape and apparently binding DNA to it. The breakdown of this network occurs early in cell division.

Cell Nucleus: Ultrastructure

The structure of a cell nucleus consists of a nuclear membrane (nuclear envelope), nucleoplasm, nucleolus, and chromosomes. Nucleoplasm, also known as karyoplasm, is the matrix present inside the nucleus. Following section discusses in brief about the several parts of a cell nucleus.

a. Nuclear Membrane

It is a double-membrane structure each 5–10 nm thick . Numerous pores occur in the envelope, allowing RNA and other chemicals to pass, but not the DNA. Because the nuclear membrane is impermeable to most molecules, nuclear pores are required to allow movement of molecules across the envelope. These pores cross both of the membranes, providing a channel that allows free movement of small molecules and ions. The movement of larger molecules such as protein requires active transport regulated by carrier proteins. Figure 2 illustrates the nuclear membrane. The nuclear envelope (or perinuclear cisterna) encloses the DNA and defines the nuclear compartment of interphase and prophase nuclei.

The spherical inner nuclearmembrane contains specific proteins that act as binding sites for the supporting fibrous sheath ofintermediate 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. Like the membrane of the rough ER, the outer surface of outer nuclear membrane is generally

studded with ribosomes engaged in protein synthesis. The proteins made on these ribosomes are transported into space between the inner and outer nuclear membrane, called perinuclear space. The perinuclear space is a 10 to 50 nm wide fluid-filled compartment which is continuous with the ER lumen and may contain fibres, crystalline deposits, lipid droplets or electrondense material. Nuclear pores and nucleocytoplasmic traffic. The nuclear envelope in all eukaryotic forms, from yeasts to

humans, is perforated by nuclear pores which have the following structure and function:

Structure of nuclear pores: Nuclear pores appear circular in surface view and have a diameter between 10nm to 100 nm. Previously it was believed that a diaphragm made of amorphous to fibrillar material extends across each pore limiting free transfer of material. Such a diaphragm called annulus has been observed in animal cells, but lack in plant cells. Recent electron microscopic studies have revealed that a nuclear pore has far more complex structure, so it is called nuclear pore complex with an estimated molecular weight of 50 to 100 million daltons. Negative staining techniques have demonstrated that pore complexes have an eight-fold or octagonal symmetry.

Nuclear Pore density: In nuclei of mammals it has been calculated that nuclear pores account for 5 to 15 per cent of the surface area of the nuclear membrane. In amphibian oocytes, certain plant cells and protozoa, the surface occupied by the nuclear pores may be as high as 20 to 36 per cent.

Arrangement of nuclear pores on nuclear envelope: In somatic cells, the nuclear pores are

evenly or randomly distributed over the surface of nuclear envelope. However, pore arrangement in other cell types is not random but rather range from rows (spores of *Eqisetum*) to Clusters (oocytes of *Xenopus laevis*) to hexagonal (Malpighian tubules of leaf hoppers) packing order.

Nucleo-cytoplasmic traffic: Quite evidently there is considerable trafficking across the nuclear envelope during interphase. Ions, nucleotides and structural, catalytic and regulatory proteins are imported from the cytosol (cytoplasmic matrix); mRNA, tRNA are exported to the cytosol (cytoplasmic matrix). However, one of the main functions of the nuclear envelope is to prevent the entrance of active ribosomes into the nucleus.

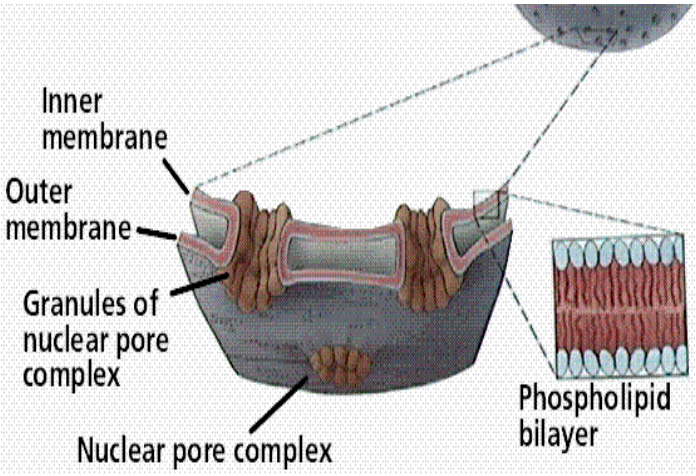

Figure 2: An illustration of the nuclear membrane

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 which is composed mainly of nucleoproteins

but it also contains other inorganic and organic substances, namely nucleic acids, proteins, enzymes and minerals. The most common nucleic acids of the nucleoplasm are the DNA and RNA. The nucleoplasm contains many types of complex proteins

categorized into: (i) Basic proteins. The proteins which take basic stain are known as the basic proteins. The most important basic proteins of the nucleus are nucleoprotamines and the nucleohistones. (ii) Non-histone or Acidic proteins. The acidic proteins either occur in the nucleoplasm or in the chromatin. The most abundant acidic proteins of the

euchromatin (a type of chromatin) are the phosphoproteins. The nucleoplasm contains many enzymes which are

necessary for the synthesis of the DNA and RNA. Most of the nuclear enzymes are composed of non-histone (acidic) proteins. The most important nuclear enzymes are the DNA polymerase, RNA polymerase, NAD synthetase, nucleoside triphosphatase, adenosine diaminase, nucleoside phosphorylase, guanase, aldolase, enolase, 3-phosphoglyceraldehyde dehydrogenase and pyruvate kinase. The nucleoplasm also contains certain cofactors and coenzymes such as ATP and acetyl CoA. The nucleoplasm has small lipid content. The nucleoplasm also contains several inorganic compounds such as phosphorus, potassium, sodium, calcium and magnesium. The chromatin comparatively contains large amount of

these minerals than the nucleoplasm.

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 (*chrome*=colour) substance or chromatin fibres. Chromosome will be discussed in detail in the next module.

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 nucleolus is mainly involved in the assembly of ribosomes. After being produced in the nucleolus, ribosomes are exported to the cytoplasm where they translate mRNA. Some of the eukaryotic organisms have nucleus that contains up to four nucleoli. The nucleolus plays an indirect role in protein synthesis by producing ribosomes. Nucleolus disappears when a cell undergoes division and is reformed after the completion of cell-division. 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, 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. A nucleolus is often associated with the nucleolar organizer (NO) which represents the secondary constriction of the nucleolar organizing chromosomes, and are 10 in number in human beings. Nucleolar organizer consists of the genes for 18S, 5.8S and 28S rRNAs. The genes for fourth type of r RNA, *i.e.,* 5S rRNA occur outside the nucleolar organizer. Nucleolus is not bounded by any limiting membrane; calcium ions are supposed to maintain its intact organization. Nucleolus also contains some enzymes such as acid phosphatase, nucleoside phosphorylase and NAD^+ synthesizing enzymes for the synthesis of some coenzymes, nucleotides and ribosomal RNA. RNA methylase enzyme which transfers methyl groups to the nitrogen bases occurs in the nucleolus of some cells. Functionally nucleolus is the site where biogenesis of ribosomal subunits (40S and 60S) takes place. In it three types of rRNAs, namely 18S, 5.8S and 28S rRNAs, are transcribed as parts of a much longer precursor molecule (45S transcript) which undergoes processing (RNA splicing) by the help of two types of proteins such as nucleolin and U3 sn RNP (U3 is a 250 nucleotide containing RNA, sn RNP represents small nuclear ribonucleoprotein). The 5S r RNA is transcribed on the chromosome existing outside the nucleolus and the 70S types of ribosomal proteins are synthesized in the cytoplasm. All of these components of the ribosomes migrate to the nucleolus, where they are assembled into two types of ribosomal subunits which are transported back to the cytoplasm. The smaller (40S) ribosomal subunits are formed and migrate to the cytoplasm much earlier than larger (60S) ribosomal subunits; therefore, nucleolus contains many more incomplete 60S ribosomal subunits than the 40S ribosomal subunits. Such a time lag in the migration of 60S and 40S ribosomal subunits, prevents functional ribosomes from gaining access to the incompletely processed heterogeneous RNA (hn RNA; the precursor of m RNA) molecule inside the nucleus.

Functions of the nucleus

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

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

As the nucleus regulates the integrity of genes and gene expression, it is also referred to as the control center of a cell. Overall, the cell nucleus stores all the chromosomal DNA of an organism.

Mitochondria

Structure and Function

The mitochondria were first observed by Kolliker in 1850 as granular structures in the striated muscles. Mitochondria are called the 'powerhouse of the cell'. They are intracellular organelles found in almost all eukaryotic cells having bilayered membranes. Most eukaryotic cells contain many mitochondria, which occupy up to 25 percent of the volume of the cytoplasm. These crucial organelles, the main sites of ATP production during aerobic metabolism, are generally exceeded in size only by the nucleus, vacuoles, and chloroplasts. They are responsible for aerobic metabolism through oxidative phosphorylation, which leads to energy production in the form of adenosine triphosphate (ATP). Mitochondria contain a number of enzymes and proteins that help in processing carbohydrates and fats obtained from food we eat to release energy. Each human cell contains on average hundreds to thousands of mitochondria. The exception is mature red blood cells, which rely exclusively on anaerobic metabolism and contain no mitochondria. Figure 3 gives the schematic representation of a typical mitochondria.

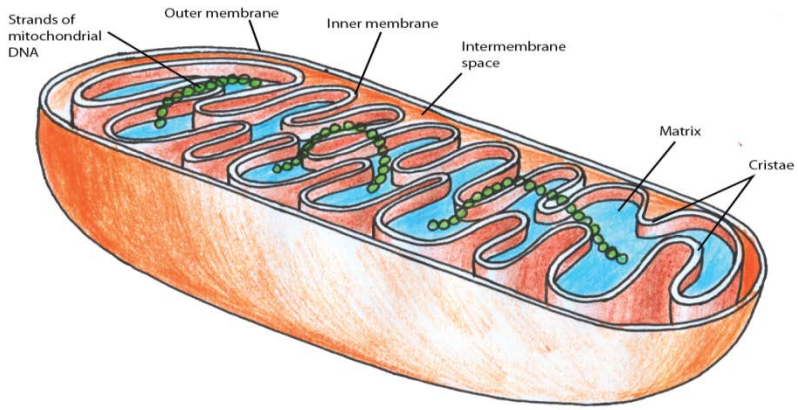

Figure 3: Schematic representation of mitochondria

Localisation:

Mitochondria are present in all eukaryotic cells. They move autonomously in the cytoplasm, so they generally have uniform distribution in the cytoplasm, but in many cells their distribution is restricted. The distribution and number of mitochondria can be correlated with type of function the cell performs. Typically mitochondria with many cristae are associated with mechanical and osmotic work situations, where there are sustained demands for ATP *e.g*., between muscle fibres, in the basal infolding of kidney tubule cells, and in a portion of inner segment of rod and cone cells of retina. Myocardial muscle cells have numerous large mitochondria called sarcosomes that reflect the great amount of work done by these cells. Often mitochondria occur in greater concentrations at work sites, for example, in the oocyte of *Thyone briaeus*, rows of mitochondria are closely associated with RER membranes, where ATP is required for protein biosynthesis. Mitochondria are particularly numerous in regions where ATP-driven osmotic work occurs, *e.g.,* brush border of kidney proximal tubules, the infolding of the plasma membrane of dogfish salt glands and Malpighian tubules of insects, the contractile vacuoles of some protozoans as Paramecium. Non-myelinated axons contain many mitochondria that are poor ATP factories, since each has only single cristae. In this case, there is a great requirement for monoamine oxidase, an enzyme present in outer mitochondrial membrane that oxidatively deaminates monoamines including neurotransmitters (acetylcholine).

Orientation:

The mitochondria have definite orientation. For example, in cylindrical cells the mitochondria

usually remain orientated in basal apical direction and lie parallel to the main axis. In leucocytes, the mitochondria remain arranged radially with respect to the centrioles. As they move about in the mitochondria form long moving filaments or chains, while in others they remain fixed in one position where they provide ATP directly to a site of high ATP utilization, *e.g.,* they are packed between adjacent myofibrils in a cardiac muscle cell or wrapped tightly around the flagellum of sperm.

Morphology:

Number: The number of mitochondria in a cell depends on the type and functional state of the

cell. It varies from cell to cell and from species to species. Certain cells contain exceptionally large number of the mitochondria, for example the *Amoeba*, *Chaos chaos* contain 50,000; eggs of sea urchin contain 140,000 to 150,000 and oocytes of amphibians contain 300,000 mitochondria. Liver cells of rat contain only 500 to 1600 mitochondria. The cells of green plants contain less number of mitochondria in comparison to animal cells. Some algal cells may contain only one mitochondrion.

Shape: The mitochondria may be filamentous or granular in shape and may change from one form to another depending upon the physiological conditions of the cells. Thus, they may be of club, racket, vesicular, ring or round-shape. Mitochondria are granular in primary spermatocyte or rat, or club-shaped in liver cells. Time-lapse picturisation of living cells shows that mitochondria are remarkably mobile and plastic organelles, constantly changing their shape. They sometimes fuse with one another and then separate again. For example, in certain euglenoid cells, the mitochondria fuse into a reticulate structure during the day and dissociate during darkness. Similar changes have been reported in yeast species, apparently in response to culture conditions.

Size: Normally mitochondria vary in size from 0.5 μm to 2.0 μm and, therefore, are not distinctly visible under the light microscope. Sometimes their length may reach up to 7 μm.

Structure: Each mitochondrion is bound by two highly specialized membranes that play a crucial role in its activities. Each of the mitochondrial membrane is 6 nm in thickness and fluidmosaic in ultrastructure. The membranes are made up of phospholipids and proteins. The space in between the two membranes is called the inter-membrane space which has the same composition as the cytoplasm of the cell. Inner and the outer membrane is separated by a 6–8 nm wide space.

Outer Membrane

The two membranes that bound a mitochondrion differ in composition and function. The outer membrane, composed of about half lipid and half protein, contains porins that render the membrane permeable to molecules having molecular weights as high as 10,000 dalton. In this respect, the outer membrane of mitochondria is similar to the outer membrane of gram-negative bacteria. The outer membrane is smooth unlike the inner membrane and has almost the same amount of phospholipids as proteins. It has a large number of special proteins called porins that allow molecules of 5000 daltons or less in weight to pass through it. It is completely permeable to nutrient molecules, ions, ATP and ADP molecules.

Inner Membrane

The inner membrane is much less permeable, than the outer membrane. It has about 20 percent lipid and 80 percent protein. The surface area of the inner membrane is greatly increased by a large number of infoldings, or finger like projections called cristae, that protrude into the matrix, or central space, increasing the surface area for the complexes. It contains the complexes of the electron transport chain and the ATP synthetase complex, they also serve to separate the matrix from the space that will contain the hydrogen ions, allowing the gradient needed to drive the pump. It is permeable only to oxygen, carbon dioxide and water and is made up of a large number of proteins that play an important role in producing ATP, and also helps in regulating transfer of metabolites across the membrane. In general, the cristae of plant mitochondria are tubular, while those of animal mitochondria are lamellar or plate-like. Some mitochondria, particularly those from heart, kidney and skeletal muscles have more extensive cristae arrangements than liver mitochondria. In comparison to these, other mitochondria (from fibroblasts, nerve axons and most plant tissues) have relatively few cristae.

Attached to matrix face of inner mitochondrial membrane are repeated units of stalked particles, called elementary particles, inner membrane subunits or oxysomes. They are also identified as F1 particles or F0-F1 particles and are meant for ATP synthesis (phosphorylation)

and also for ATP oxidation (acting as ATP synthetase and ATPase). F0-F1 particles are regularly spaced at intervals of 10 nm on the inner surface of inner mitochondrial membrane. According to some estimates, there are 104 to 105 elementary particles per mitochondrion. When the mitochondrial cristae are disrupted by sonic vibrations or by detergent action, they produce submitochondrial vesicles of inverted orientation. In these vesicles, F0-F1 particles are seen attached on their outer surface. These submitochondrial vesicles are able to per- form respiratory chain phosphorylation. However, in the absence of F0-F1 particles, these vesicles lose their capacity of phosphorylation as shown by resolution (removal by urea or trypsin treatment) and reconstitution of these particles.

Matrix

The matrix is a complex mixture of enzymes that are important for the synthesis of ATP molecules, special mitochondrial ribosomes, tRNAs and the mitochondrial DNA. Besides these, it has oxygen, carbon dioxide and other recyclable intermediates.

Chemical composition

Mitochondria are found to contain 65 to 70 per cent proteins, 25 to 30 per cent lipids, 0.5 per cent RNA and small amount of the DNA. The lipid contents of the mitochondria is around 90 per cent phospholipids (lecithin and cephalin), 5 per cent or less cholesterol and 5 per cent free fatty acids and triglycerides. The inner membrane is rich in one type of phospholipid, called cardiolipin which makes this membrane impermeable to a variety of ions and small molecules (Na^+, K^+, Cl^-, NAD^+,AMP, GTP, CoA and so on). The outer mitochondrial membrane has typical ratio of 50 per cent proteins and 50 per cent phospholipids of ‘unit membrane’. However, it contains more unsaturated fatty acids and less cholesterol. It has been estimated that in the mitochondria of liver 67 per cent of the total mitochondrial protein is located in the matrix, 21 per cent is located in the inner membrane, 6 per cent is situated in the outer membrane and 6 per cent is found in the outer chamber. Each of these four mitochondrial regions contains a special set of proteins that mediate distinct functions. Besides Porin, enzymes of outer membrane consists of, other proteins involved in mitochondrial lipid synthesis and those enzymes that convert lipid substrates into forms that are subsequently metabolized in the matrix. Certain

important enzymes of this membrane are monoamine oxidase, rotenone-insensitive NADH-cytochrome-C-reductase, kynurenine hydroxyalase, and fatty acid CoA ligase. Enzymes of intermembrane space contains several enzymes that use the ATP molecules passing out of the matrix to phosphorylate other nucleotides. The main enzymes of this part are adenylate kinase and nucleoside diphosphokinase. Enzymes of inner membrane contains proteins with three types of functions: 1. Those that carry out the oxidation reactions of the respiratory chain; 2. an enzyme complex, called ATP synthetase that makes ATP in matrix ; and 3. specific transport proteins The significant enzymes of inner membrane are enzymes of electron transport pathways, namely nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), diphosphopyridine nucleotide (DPN) dehydrogenase, four cytochromes (Cyt. b, Cyt. c, Cyt.c1, Cyt. a and Cyt. a3), ubiquinone or coenzyme Q10, non-heme copper and iron, ATP synthetase, succinate dehydrogenase; β-hydroxybutyrate dehydrogenase; carnitive fatty acid acyl transferase. Enzymes of mitochondrial matrix contains various enzymes, including those required for the oxidation of pyruvate and fatty acids and for the citric acid cycle. The matrix also contains several identical copies of the mitochondrial DNA, special 55S mitochondrial ribosomes, tRNAs and various enzymes required for the expression of mitochondrial genes. Thus, the mitochondrial matrix contains malate dehydrogenase, isocitrate dehydrogenase, fumarase, aconitase, citrate synthetase, ⟨-keto acid dehydrogenase, β-oxidation enzymes.

Viewing mitochondria

Mitochondria can be isolated by cell fractionation brought about by differential centrifugation. Homogeneous fractions of mitochondria can be obtained from liver, skeletal muscle, heart, and some other tissues. They can be observed easily in cells cultured *in vitro*, particularly under darkfield illumination and phase contrast microscope. Janus green stains living mitochondria greenish blue due to its action with cytochrome oxidase system present in the mitochondria. This system maintains the vital dye in its oxidized state. In the surrounding cytoplasm the stain is reduced to a colourless base. Fluorescent dyes (rhodamine 123), which are more sensitive, have been used in isolated mitochondria and intact cultured cells. Such stains are more suitable for *in situ* metabolic

studies of mitochondria. Different parts of mitochondria have distinct marker enzymes for histochemical markings, such as cytochrome oxidase for inner membrane, monoamine oxidase for outer membrane, malate dehydrogenase for matrix and adenylate kinase for outer chamber.

Function of mitochondria

1. The most important function of the mitochondria is to produce energy. The food that we eat is broken into simpler molecules like carbohydrates, fats, etc., in our bodies. These are sent to the mitochondrion where they are further processed to produce charged molecules that combine with oxygen and produce ATP molecules. This entire process is known as oxidative phosphorylation.
2. It is important to maintain proper concentration of calcium ions within the various compartments of the cell. Mitochondria help the cells to achieve this goal by serving as storage tanks of calcium ions.
3. Mitochondria help in the building of certain parts of the blood, and hormones like testosterone and estrogen.
4. Mitochondria in the liver cells have enzymes that detoxify ammonia.

Although most of the genetic material of a cell is contained within the nucleus, the mitochondria have their own DNA. They have their own machinery for protein synthesis and reproduce by the process of fission like bacteria do. Due to their independence from the nuclear DNA and similarities with bacteria, it is believed that mitochondria have originated from bacteria by endosymbiosis.

Lecture 6

In previous lecture we had discussion about few cell organelles like mitochondria, nucleous etc. During current lecture, we will have discussion about few other cell organelles. The present lecture discusses about ribosome, endoplasmic reticulum, golgi bodies and lysosomes.

Ribosomes

Ribosomes are the protein synthesis units of a cell described by G.E. Palade in 1952. They are complex of ribosomal RNA and various proteins. Their presence in both free and endoplasmis reticulum membrane attached form (rough endoplasmic reticulum) was confirmed by Palade and Siekevitz by the electron microscopy. We will have discussion about endoplasmic reticulum in this lecture after discussion about ribosome. Ribosomes are small, dense, rounded and granular particles of the ribonucleoprotein. As mentioned, they occur either freely in the matrix of mitochondria, chloroplast and cytoplasm or remain attached with the membranes of the endoplasmic reticulum. They occur in most prokaryotic and eukaryotic cells and provide a scaffold for the ordered interaction of all the molecules involved in protein synthesis. They are the most abundant RNA-protein complex in the cell, which directs elongation of a polypeptide at a rate of three to five amino acids added per second. Small proteins of 100–200 amino acids are therefore made in a minute or less. On the other hand, it takes 2–3 hours to make the largest known protein, titin, which is found in muscle and contains about 30,000 amino acid residues.

Occurrence and distribution:

The ribosomes occur in both prokaryotic and eukaryotic cells. In prokaryotic cells the ribosomes often occur freely in the cytoplasm or sometimes as polyribosome. In eukaryotic cells the ribosomes either occur freely in the cytoplasm or remain attached to the outer surface of the membrane of endoplasmic reticulum. The yeast cells, reticulocytes or lymphocytes, meristamatic plant tissues, embryonic nerve cells and cancerous cells contain large number of ribosomes which often occur freely in the cytoplasmic matrix. Cells like the erythroblasts, developing muscle cells, skin and hair which synthesize specific proteins for the intracellular utilization and storage contain also contain large number of free ribosomes. In cells with active protein synthesis, the ribosomes remain attached with the membranes of the endoplasmic reticulum. Examples

are the pancreatic cells, plasma cells, hepatic parenchymal cells, Nissls bodies, osteoblasts, serous cells, or the submaxillary gland, thyroid cells and mammary gland cells.

Types of ribosomes:

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

70S Ribosomes: Prokaryotes have 70S ribosemes. The 70S ribosomes are comparatively smaller in size and have sedimentation coefficient 70S with molecular weight 2.7×10^6 daltons. Electron microscopy measures the dimension of the 70S ribosomes as$170 \times 170 \times 200$ A^o. They occur in the prokaryotic cells of the blue green algae and bacteria and also in mitochondria and chloroplasts of eukaryotic cells.

80S Ribosomes: Eukaryotes have 80S ribosomes. The 80S ribosomes have sedimentation coefficient of 80S and molecular weight 40×10^6 daltons. The 80S ribosomes occur in eukaryotic cells of the plants and animals. The ribosomes of mitochondria and chloroplasts are always smaller than 80S cytoplasmic ribosomes and are comparable to prokaryotic ribosomes in both size and sensitivity to antibiotics. However their sedimentation values vary in different phyla, 77S in mitochondria of fungi, 60S in mitochondria of mammals and 60S in mitochondria of animals.

Number of ribosomes:

An *E. coli* cell contains 10,000 ribosomes, forming 25 per cent of the total mass of the bacterial

cell. Whereas, mammalian cultured cells contain 10 million ribosomes per cell.

Chemical composition:

The ribosomes are chemically composed of RNA and proteins as their major constituents; both occurring approximately in equal proportions in smaller as well as larger subunit. The 70S ribosomes contain more RNA (60 to 40%) than the proteins (36 to 37%). The ribosomes of *E. coli* contain 63% rRNA and 37% protein. While the 80S ribosomes contain less RNA (40 to 44%) than the proteins (60 to 56%), yeast ribosomes have 40 to 44% RNA and 60 to 56% proteins; ribosomes of pea seedling contain 40% RNA and 60% proteins. There is no lipid content in ribosomes.

Ribosomal RNAs:

RNA constitutes about 60 percent of the mass of a ribosome. The 70S ribosomes contain three types of rRNA, *viz*., 23S rRNA, 16S rRNA, 5S rRNA. The 23S and 5S rRNA occur in the larger 50S ribosomal subunit, while the 16S rRNA occurs in the smaller 30S ribosomal subunit. Assuming an average molecular weight for one nucleotide to be 330 daltons, one can calculate the total number of each type of rRNA. Thus, the 23S rRNA consists of 3300 nucleotides, 16S rRNA contains 1650 nucleotides and 5S rRNA includes 120 nucleotides in it. The 80S ribosomes contain four types of rRNA, 28S rRNA (or 25-26 rRNA in plants, fungi and protozoa), 18S rRNA, 5S rRNA and 5.8S rRNA. The 28S, 5S and 5.8S rRNAs occur in the larger 60S ribosomal subnit, while the 18S rRNA occurs in the smaller 40S ribosomal subunit. About 60 per cent of the rRNA is helical (*i.e*., double stranded) and contains paired bases. These double stranded regions are due to hairpin loops between complimentary regions of the linear molecule.

The 28S rRNA has the molecular weight 1.6×10^6 daltons and its molecule is double stranded

and having nitrogen bases in pairs. The 18S rRNA has the molecular weight 0.6x106 daltons and

consists of 2100 nucleotides. The 18S and 28S ribosomal RNA contain a characteristic number of methyl groups, mostly as 2'-O-methyl ribose. The molecule of 5S rRNA has a clover leaf shape and a length equal to 120 nucleotides. The 5.8S rRNA is intimately associated with the 28S rRNA molecule and has, therefore, been referred to as 28S-associated ribosomal RNA (28S-A rRNA). The 55S ribosomes of mammalian mitochondria lack 5S rRNA but contain 21S and 12S rRNAs. The 21S rRNA occurs in larger or 35S ribosomal subunits, while 12S rRNA occur in smaller or 25S ribosomal subunit. It is thought that each ribosomal subunit contains a highly folded ribonucleic acid filament to which the various proteins adhere. But as the ribosomes easily bind the basic dyes so it is concluded that RNA is exposed at the surface of the ribosomal subunits, and the protein is assumed to be in the interior in relation to non-helical part of the RNA.

Ribosomal Proteins:

A ribosome is composed of three (in bacteria) or four (in eukaryotes) different rRNA molecules and as many as 83 proteins, organized into a large subunit and a small subunit. The primary structure of several of these proteins has been elucidated. Most of the recent knowledge about the structure of ribosomal proteins has been achieved by dissociation of ribosomal subunits into their component rRNA and protein molecules. When both 50S and 30S ribosomal subunits are dissociated by centrifuging both of them in a gradient of 5 M cesium chloride, then there are two inactive core particles (40S and 23S, respectively) which contain the RNA and some proteins called core proteins (CP) at the same time several other proteins—the so-called split proteins (SP) are released from each particle (Fig. 14.3). There are SP50 and SP30 proteins which may reconstitute the functional ribosomal subunit when added to their corresponding core. Some of the split proteins are apparently specific for each ribosomal subunit. The split proteins have been further fractionated and divided into acidic (A) and basic (B) proteins. According to Nomura (1968, 1973) and Garett and Wittmann (1973) each 70S ribosome of *E. coli* is composed of about 55 ribosomal proteins. Out of these 55 proteins, about 21 different molecules have been isolated from the 30S ribosomal subunit, and some 32 to 34 proteins from the 50S ribosomal subunit. Similar organization of ribosomal proteins and RNA is found in 80S Ribosomes. Different rRNA molecules evidently play a central role in the catalytic activities of ribosomes in the process of protein synthesis.

Metallic Ions:

The most important low molecular weight components of ribosomes are the divalent metallic ions such as Mg++, Ca++ and Mn++.

Structure

The ribosomes are oblate spheroid structures of 150 to 250A^{o} in diameter. Each ribosome is porous, hydrated and composed of two subunits. One ribosomal subunit is large in size and has a domelike shape, while the other ribosomal subunit is smaller in size, occurring above the larger subunit and forming a cap-like structure. The small ribosomal subunit contains a single rRNA molecule, referred to as small *rRNA.* The large subunit contains a molecule of large *rRNA* and one molecule of 5S rRNA, plus an additional molecule of 5.8S rRNA in vertebrates. The lengths of the rRNA molecules, the quantity of proteins in

each subunit, and consequently the sizes of the subunits differ in bacterial and eukaryotic cells. The assembled ribosome is 70S in bacteria and 80S in vertebrates. There are great structural and functional similarities between ribosomes from all species which is another reflection of the common evolutionary origin of the most basic constituents of living cells.

The 70S ribosome consists of two subunits, 50S and 30S. The 50S ribosomal subunit is larger in size and has the size of 160 A^o to 180 A^o. The 30S ribosomal subunit is smaller in size and occurs above the 50S subunit like a cap. The 80S ribosome also consists of two subunits, 60S and 40S. The 60S ribosomal subunit is dome-shaped and larger in size. In the ribosomes which remain attached with the membranes of endoplasmic reticulum and nucleus, the 60S subunit remains attached with the membranes. The 40S ribosomal subunit is smaller in size and occurs above the 60s subunit forming a cap-like structure. Both the subunits remain separated by a narrow cleft. The two ribosomal subunits remain united with each other due to high concentration of the Mg++ (.001M) ions. When the concentration of Mg++ions reduces in the matrix, both ribosomal subunits get separated. Actually in bacterial cells the two subunits are found to occur freely in the cytoplasm and they unite only during the process of protein synthesis. At high concentration of Mg++ ions in the

matrix, the two ribosomes (monosomes) become associated with each other and known as the

dimer. Further, during protein synthesis many ribosomes are aggregated due to common messenger RNA and form the polyribosomes or polysomes.

The actual three-dimensional structures of bacterial rRNAs from *Thermus thermopolis* recently have been determined by x-ray crystallography of the 70S ribosome. The multiple, much smaller ribosomal proteins for the most part are associated with the surface of the rRNAs. During translation, a ribosome moves along an mRNA chain, interacting with various protein factors and tRNAs and very likely undergoing large conformational changes **(see Figure 2)**. Despite the complexity of the ribosome, great progress has been made in determining the overall structure of bacterial ribosomes and in identifying various reactive sites. X-ray crystallographic studies on the *T. thermophilus* 70S ribosome, for instance, not only have revealed the dimensions and overall shape of

the ribosomal subunits but also have localized the positions of tRNAs bound to the ribosome during elongation of a growing protein chain. In addition, powerful chemical techniques such as footprinting, have been used to identify specific nucleotide sequences in rRNAs that bind to protein or another RNA. Figure 1 illustrates the ribosomes.

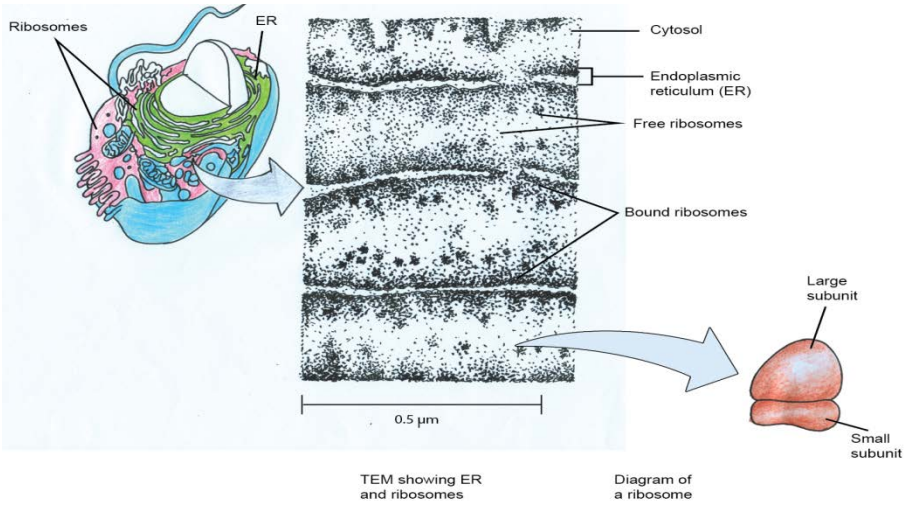

Figure 1: Schematic representation of the ribosome.

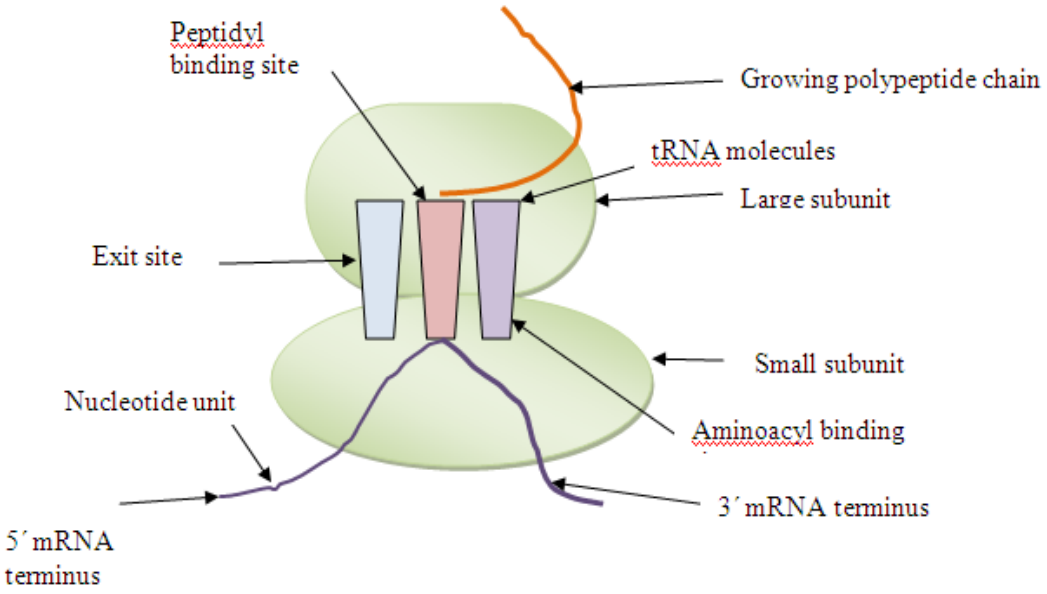

Figure 2: The detailed structure of a ribosome involved in protein synthesis. The figure is not upto the scale of ribosome.

Endoplasmic reticulum:

Endoplasmic reticulum is a network of interconnected internal membranes generally, the largest membrane in a eukaryotic cell—an extensive network of closed, flattened membrane-bounded sacs called cisternae (Figure 3). The name "endoplasmic reticulum" was coined in 1953 by Porter, who had observed it in electron micrographs of liver cells. The endoplasmic reticulum has a number of functions in the cell but is particularly important in the synthesis of lipids, membrane proteins, and secreted proteins.

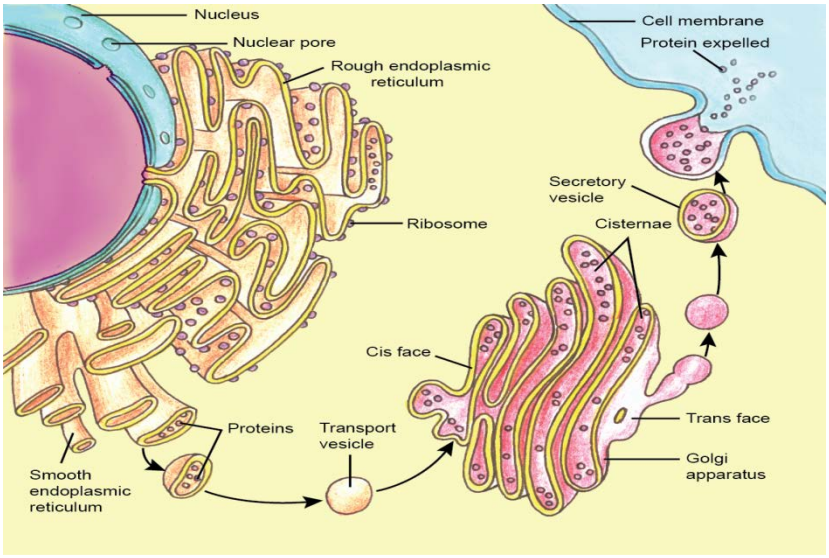

Figure 3. The Endoplasmic reticulum.

Occurrence:

The occurrence of the endoplasmic reticulum is in eukaryotic cells with variation in its position from cell to cell. The erythrocytes (RBC), egg and embryonic cells lack in endoplasmic reticulum. ER is poorly developed in certain cells as the RBC which produces only proteins to be retained in the cytoplasmic matrix (haemoglobin), although the cell may contain many ribosomes). The spermatocytes also have poorly developed endoplasmic reticulum.

Morphology:

The endoplasmic reticulum occurs in three forms: 1. Lamellar form or cisternae which is a closed, fluid-filled sac, vesicle or cavity is called cisternae; 2. vesicular form or vesicle and 3. tubular form or tubules.

1. Cisternae: The cisternae are long, flattened, sac-like, unbranched tubules having diameter of 40 to 50 μm. They remain arranged parallely in bundles or stakes. RER mostly exists as cisternae which occur in those cells which have synthetic roles as the cells of pancreas, notochord and brain.

2. Vesicles: The vesicles are oval, membrane-bound vacuolar structures having diameter of 25 to 500 μm. They often remain isolated in the cytoplasm and occur in most cells but especially abundant in the SER.

3. Tubules: The tubules are branched structures forming the reticular system along with the cisternae and vesicles. They usually have the diameter from 50 to 190 μm and occur almost in all the cells. Tubular form of ER is often found in SER and is dynamic in nature, *i.e.*, it is associated with membrane movements, fission and fusion between membranes of cytocavity network.

Ultrastructure:

The cavities of cisternae, vesicles and tubules of the endoplasmic reticulum are bounded by a

thin membrane of 50 to 60 Aº thickness. The membrane of endoplasmic reticulum is fluid-mosaic like the unit membrane of the plasma membrane, nucleus, Golgi apparatus. The membrane of endoplasmic reticulum remains continuous with the membranes of plasma membrane, nuclear membrane and Golgi apparatus. The cavity of the endoplasmic reticulum is well developed and acts as a passage for the secretory products. Palade in the year 1956 has observed secretory granules in the cavity of endoplasmic reticulum amking it rough in appearance. Sometimes, the cavity of RER is very narrow with two membranes closely apposed and is much distended in certain cells which are actively engaged in protein synthesis (acinar cells, plasma cells and goblet cells). The membranes of the endoplasmic reticulum contains many kinds of enzymes which are needed for various important synthetic activities. Some of the most common enzymes are found to have different transverse distribution in the ER membranes. The most important

enzymes are the stearases, NADH-cytochrome C reductase, NADH diaphorase, glucose-6-phosphotase and Mg++ activated ATPase. Certain enzymes of the endoplasmic reticulum such as nucleotide diphosphate are involved in the biosynthesis of phospholipid, ascorbic acid, glucuronide, steroids and hexose metabolism.

Types of endoplasmic reticulum:

Agranular or smooth endoplasmic reticulum:

ER with no studded ribosomes makes it smooth in appearance. The adipose tissues, brown fat cells and adrenocortical cells, interstitial cells of testes and cells of corpus luteum of ovaries, sebaceous cells and retinal pigment cells contain only smooth endoplasmic reticulum (SER). The synthesis of fatty acids and phospholipids takes place in the smooth ER. It is abundant in hepatocytes. Enzymes in the smooth ER of the liver modify or detoxify hydrophobic chemicals such as pesticides and carcinogens by chemically converting them into more water-soluble, conjugated products that can be excreted from the body. High doses of such compounds result in a large proliferation of the smooth ER in liver cells.

Granular or rough endoplasmic reticulum:

Ribosomes bound to the endoplasmic reticulum make it appear rough. The rough ER synthesizes certain membrane and organelle proteins and virtually all proteins to be secreted from the cell. A ribosome that fabricates such a protein is bound to the rough ER by the nascent polypeptide chain of the protein. As the growing polypeptide emerges from the ribosome, it passes through the rough ER membrane, with the help of specific proteins in the membrane. Newly made membrane proteins remain associated with the rough ER membrane, and proteins to be secreted accumulate in the lumen of the organelle. All eukaryotic cells contain a discernible amount of rough ER because it is needed for the synthesis of plasma membrane proteins and proteins of the extracellular matrix. Rough ER is particularly abundant in specialized cells that produce an abundance of specific proteins to be secreted. The cells of those organs which are actively engaged in the synthesis of proteins such as acinar cells of pancreas, plasma cells, goblet cells and cells of some endocrine glands are found to contain rough endoplasmic reticulum (RER) which is highly developed.

Rough endoplasmic reticulum and protein secretion:

George Palade and his colleagues in the 1960s were the first to demonstrate the role of endoplasmic reticulum in protein secretion. The defined pathway taken by secreted protein is: Rough ER - Golgi - secretory vesicles- cell exterior. The entrance of proteins into the ER represents a major branch point for the traffic of proteins within eukaryotic cells. In mammalian cells most proteins are transferred into the ER while they are being translated on membrane bound ribosomes. Proteins that are destined for secretion are then targeted to the endoplasmic reticulum by a signal sequence (short stretch of hydrophobic amino acid residues) at the amino terminus of the growing polypeptide chain. The signal sequence is K/HDEL which is Lys/His-Asp-Glu-Leu. This signal peptide is recognized by a signal recognition particle consisting of six polypeptides and srpRNA. The SRP binds the ribosome as well as the signal sequence, inhibiting further translation and targeting the entire complex (the SRP, ribosome, and growing polypeptide chain) to the rough ER by binding to the SRP receptor on the ER membrane. Binding to the receptor releases the SRP from both the ribosome and the signal sequence of the growing polypeptide chain. The ribosome then binds to a protein translocation complex in the ER membrane, and the signal sequence is inserted into a membrane channel or translocon with the aid of GTP. Transfer of the ribosome mRNA complex from the SRP to the translocon opens the gate on the translocon and allows translation to resume, and the growing polypeptide chain is transferred directly into the translocon channel and across the ER membrane as translation proceeds. As translocation proceeds, the signal sequence is cleaved by signal peptidase and the polypeptide is released into the lumen of the ER.

Smooth endoplasmic reticulum and lipid synthesis:

Hydrophobic lipids are synthesized in the ER and then they are then transported from the ER to their ultimate destinations either in vesicles or by carrier proteins. Phospholipids are synthesized in the cytosolic side of the ER membrane from water-soluble cytosolic precursors. Other lipids that are synthesized in the ER are cholesterol and ceramide which is further converted to either glycolipids or sphingomyelin in the golgi apparatus. Smooth ER are also the site for the synthesis of the steroid hormones from cholesterol. Thus steroid producing cells in the testis and ovaries are abundant in smooth ER.

Common functions of SER and RER:

1. The endoplasmic reticulum provides an ultrastructural skeletal framework to the cell and gives mechanical support to the colloidal cytoplasmic martix.

2. The exchange of molecules by the process of osmosis, diffusion and active transport occurs through the membranes of endoplasmic reticulum. The ER membrane has permeases and carriers.

3. The endoplasmic membranes contain many enzymes which perform various synthetic and

metabolic activities and provides increased surface for various enzymatic reactions.

4. The endoplasmic reticulum acts as an intracellular circulatory or transporting system. Various secretory products of granular endoplasmic reticulum are transported to various organelles as follows: Granular ER– agranular ER – Golgi membrane–lysosomes, transport vesicles or secretory granules. Membrane flow may also be an important mechanism for carrying particles, molecules and ions into and out of the cells. Export of RNA and nucleoproteins from nucleus to cytoplasm may also occur by this type of flow.

5. The ER membranes are found to conduct intra-cellular impulses. For example, the sarcoplasmic reticulum transmits impulses from the surface membrane into the deep region of the muscle fibres.

6. The sarcoplasmic reticulum plays a role in releasing calcium when the muscle is stimulated and actively transporting calcium back into the sarcoplasmic reticulum when the stimulation stops and the muscle must be relaxed.

Lysosomes:

C. de Duve, in 1955, named these organelles as 'lysosomes'. Lysosomes is an organelle which provides an excellent example of the ability of intracellular membranes to form closed compartments in which the composition of the lumen (the aqueous interior of the compartment) differs substantially from that of the surrounding cytosol. Found exclusively in animal cells, lysosomes are responsible for degrading certain components that have become obsolete for the cell or organism. Lysosomes are often budded from the membrane of the Golgi apparatus, but in some cases they develop gradually from late endosomes, which are vesicles that carry materials brought into the cell by a process known as endocytosis. The biogenesis of the lysosomes requires the synthesis of specialized lysosomal hydrolases and membrane proteins. Both classes of proteins are synthesized in the ER and transported through the Golgi apparatus, then transported from the trans Golgi network to an intermediate compartment (an endolysosome) by means of transport vesicles (which are coated by clathrin protein).

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 cells or in acinar cells of the pancreas. Leucocytes, especially granulocytes are a particularly rich source of lysosomes. Their lysosomes are so large-sized that they can be observed under the light microscope. They are also numerous in epithelial cells of absorptive, secretory and excretory organs (intestine, liver, and kidney). They occur in abundance in the epithelial cells of lungs and uterus. Phagocytic cells and cells of reticuloendothelial system (bone marrow, spleen and liver) are also rich in lysosomes.

Structure:

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

Isolation and chemical composition:

Lysosomes are very delicate and fragile organelles. Lysosomal fractions have been isolated by

sucrose-density centrifugation (Isopycnic centrifugation) after mild methods of homogenization.

The location of the lysosomes in the cell can also be pinpointed by various histochemical or cytochemical methods. For example, lysosomes give a positive test for acid Schiff reaction.

Certain lysosomal enzymes are good histochemical markers. For example, acid phosphatase is the principal enzyme which is used as a marker for the lysosomes by the use of Gomori'staining technique. Specific stains are also used for other lysosomal enzymes such as B- glucuronidase,

aryl sulphatatase, N-acetyl-B-glucosaminidase and 5-bromo-4-chloroindolacetate esterase. A lysosome may contain up to 40 types of hydrolytic enzymes. They include proteases (cathepsin for protein digestion), nucleases, glycosidases (for digestion of polysaccharides and glycosides), lipases, phospholipases, phosphatases and sulphatases. All lysosomal enzymes are acid hydrolases, optimally active at the pH5. The membrane of the lysosome normally keeps the enzymes latent and out of the cytoplasmic matrix or cytosol (pH is ~7.2), but the acid dependency of lysosomal enzymes protects the contents of the cytosol (cytoplasmic matrix) against any damage even if leakage of lysosomal enzymes occur. The latency of the lysosomal enzymes is due to the presence of the membrane which is resistant to the enzymes that it encloses. Most probably this is due to the fact that most lysosomal hydrolases are membrane-bound, which may prevent the active centres of enzymes to gain access to

susceptible groups in the membrane.

Lysosomal Membrane:

The lysosomal membrane is slightly thicker than that of mitochondria. It contains substantial amounts of carbohydrate material, particularly sialic acid. In fact, most lysosomal membrane proteins are unusually highly glycosylated, which may help protect them from the lysosomal proteases in the lumen. The lysosomal membrane has another unique property of fusing with other membranes of the cell. This property of fusion has been attributed to the high proportion of membrane lipids present in the micellar configuration. Surface active agents such as liposoluble vitamins (A,K,D and E) and steroid sex hormones have a destabilizing influence, causing release of lysosomal enzymes due to rupture of lysosomal membranes. Drugs like cortisone, hydrocortisone and others tend to stabilize the lysosomal membrane and have an anti-inflammatory effect on the tissue. The entire process of digestion is carried out within the lysosome. Most lysosomal enzymes act in an acid medium. Acidification of lysosomal contents depends on an ATP-dependent proton pump which is present in the membrane of the lysosome and accumulates H+ inside the organelle. Lysosomal membrane also contains transport proteins that allow the final products of digestion of macromolecules to escape so that they can be either excreted or reutilized by the cell.

Functions:

1. Lysosomes serve as digestion compartments for cellular materials that have exceeded their lifetime or are otherwise no longer useful by 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,

e.g., gills, fins, tail, etc., are digested by the lysosomes and utilized by the other cells.

2. Lysosomes break down cellular waste products, fats, carbohydrates, proteins, and other macromolecules into simple compounds, which are then transferred back into the cytoplasm as new cell-building materials. To accomplish the tasks associated with digestion, the lysosomes utilize about 40 different types of hydrolytic enzymes, all of which are manufactured in the endoplasmic reticulum and modified in the Golgi apparatus.

3. 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.

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 penetra 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.

The Golgi Complex: Processes and Sorts Secreted and Membrane Proteins

The golgi complex was discovered by Camillo Golgi during an investigation of the nervous system and he named it the "internal reticular apparatus". Functionally it is also known as the post office of the cell. Certain important cellular functions such as biosynthesis of polysaccharides, packaging (compartmentalizing) of cellular synthetic products (proteins), production of exocytotic (secretory) vesicles and differentiation of cellular membranes, occurs in the Golgi complex or Golgi apparatus located in the cytoplasm of animal and plant cells.

Occurrence:

The Golgi apparatus occurs in all eukaryotic cells. The exceptions are the prokaryotic cells (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 (*i.e.*, more than 25,000 in algal rhizoids, Sievers,1965), to a single organelle in some algae. 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. *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.

Morphology

The Golgi apparatus is morphologically very similar in both plant and animal cells. However, it is extremely pleomorphic: in some cell types it appears compact and limited, in others spread out and reticular (net-like). Its shape and form may vary depending on cell type. It appears as a complex array of interconnecting tubules, vesicles and cisternae. There has been much debate concerning the terminology of the Golgi's parts. The simplest unit of the Golgi apparatus is the cisterna. This is a membrane bound space in which various materials and secretions may accumulate. Numerous cisternae are associated with each other and appear in a stack-like (lamellar) aggregation. A group of these cisternae is called the dictyosome, and a group of dictyosomes makes up the cell's

Golgi apparatus. All dictyosomes of a cell have a common function. The detailed structure of three basic components of the Golgi apparatus are as follows:

1. Flattened Sac or Cisternae

Cisternae of the golgi apparatus are about 1 μm in diameter, flattened, plate-like or saucer-like closed compartments which are held in parallel bundles or stacks one above the other. In each stack, cisternae are separated by a space of 20 to 30 nm which may contain rod-like elements or fibres. Each stack of cisternae forms a dictyosome which may contain 5 to 6 Golgi cisternae in animal cells or 20 or more cisternae in plant cells. Each cisterna is bounded by a smooth unit membrane (7.5 nm thick), having a lumen varying in width from about 500 to 1000 nm. Polarity. The margins of each cisterna are gently curved so that the entire dictyosome of Golgi apparatus takes on a bow-like appearance. The cisternae at the convex end of the dictyosome comprise proximal, forming or cis-face and the cisternae at the concave end of the dictyosome comprise the distal, maturing or trans-face. The forming or cis face of Golgi is located next to either the nucleus or a specialized portion of rough ER that lacks bound ribosomes and is called “transitional” ER. Trans face of Golgi is located near the plasma membrane. This polarization is called cis-trans axis of the Golgi apparatus.

2. Tubules

A complex array of associated vesicles and tubules (30 to 50 nm diameter) surround the dictyosome and radiate from it. The peripheral area of dictyosome is fenestrated or lace-like in structure.

3. Vesicles

The vesicles are 60 nm in diameter and are of three types : (i) Transitional vesicles are small membrane limited vesicles which are form as blebs from the transitional ER to migrate and converge to cis face of Golgi, where they coalasce to form new cisternae.

(ii) Secretory vesicles are varied-sized membrane-limited vesicles which discharge from margins of cisternae of Golgi. They, often, occur between the maturing face of Golgi and the plasma membrane.

(iii) Clathrin-coated vesicles are spherical protuberances, about 50 μm in diameter and with a rough surface. They are found at the periphery of the organelle, usually at the ends of single tubules, and are morphologically quite distinct from the secretory vesicles. The

clathrin-coated vesicles are known to play a role in intra-cellular traffic of membranes and of secretory products.

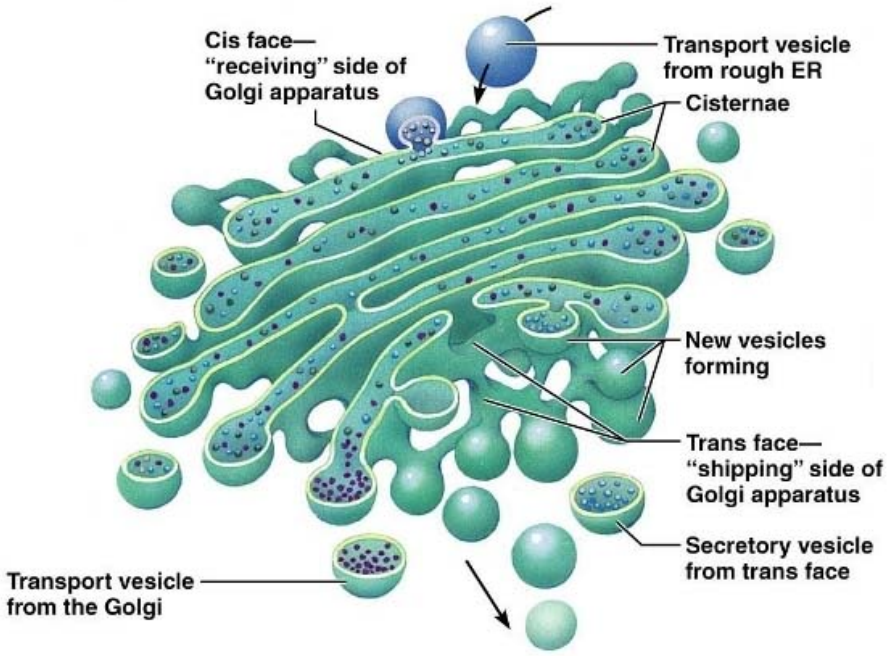

Figure 5: The Golgi complex.

Functions:

1. Modifying, sorting, and packaging of macromolecules for cell secretion: The golgi complex is involved in the transport of lipids around the cell, and the creation of lysosomes. Proteins are modified by enzymes in cisternae by glycosylation and phosphorylation by identifying the signal sequence of the protein in question. For example, the Golgi apparatus adds a mannose-6-phosphate label to proteins destined for lysosomes. One molecule that is phosphorylated in the Golgi is Apolipoprotein, which forms a molecule known as VLDL that is a constituent of blood serum. The phosphorylation of these molecules is important to help aid in their sorting for secretion into the blood serum.

2. Proteoglycans and carbohydrate synthesis: This includes the production of glycosaminoglycans (GAGs), long unbranched polysaccharides which the Golgi then attaches to a protein synthesised in the endoplasmic reticulum to form proteoglycans.

3. Golgi Functions in Animals:

In animals, Golgi apparatus is involved in the packaging and exocytosis of the following: Zymogen of exocrine pancreatic cells; Mucus (a glycoprotein) secretion by goblet cells of intestine; Lactoprotein (casein) secretion by mammary gland cells (Merocrine secretion); Secretion of compounds (thyroglobulins) of thyroxine hormone by thyroid cells; Secretion of tropocollagen and collagen; Formation of melanin granules and other pigments; and Formation of yolk and vitelline membrane of growing primary oocytes. It is also involved in the formation of certain cellular organelles such as plasma membrane, lysosomes, acrosome of spermatozoa and cortical granules of a variety of oocytes.

4. Golgi Functions in Plants:

In plants, Golgi apparatus is mainly involved in the secretion of materials of primary and secondary cell walls (formation and export of glycoproteins, lipids, pectins and monomers for hemicellulose, cellulose, lignin). During cytokinesis of mitosis or meiosis, the vesicles originating from the periphery of Golgi apparatus, coalesce in the phragmoplast area to form a semisolid layer, called cell plate. The unit membrane of Golgi vesicles fuses during cell plate formation and becomes part of plasma membrane of daughter

Interesting Facts:

- George Palade, a Romanian-born naturalized American and cell biologist, was the first to describe free ribosomes.
- An example of an animal cell with many Golgi bodies is an epithelial cell that secretes mucus.
- The cell wall of plant cells is exported to the outside of the membrane by Golgi bodies.