ENZYME REGULATION

Metabolic pathways are controlled by regulating enzyme activity. If enzyme activity is not regulated, it can harm cellular activities and may lead to the development of diseases. Alteration of enzyme regulation is one of the cause for cancer development. Over production of tyrosine kinase is associated with alteration of cell shape in tumour cells. Enzyme regulation can alter when drugs are used. Enzyme regulation can be altered by environmental toxins or pollutants.

Enzyme activity can be regulated by:

(*a*) changing catalytic efficiency.

(*b*) altering the amount or quantity of enzyme in cell or body.

(*a*) Catalytic efficiency of enzymes can be regulated

1. By subjecting enzyme to feedback inhibition
2. By allosteric regulation or inhibition
3. By covalent modification of enzyme molecule
4. By synthesizing enzyme in inactive form

Allosteric Inhibition

Inhibition of activity of allosteric enzymes by allosteric inhibitor is called as allosteric inhibition. Allosteric inhibition is seen in pathways that are subject to regulation. Allosteric inhibitors are not structurally similar to substrates of allosteric enzymes. They bind to enzyme at allosteric site which is different from active site. The activity of an allosteric enzyme is raised by allosteric activator. Most of the allosteric enzymes are oligomeric proteins *i.e.,* they consist of many subunits.

The most extensively studied allosteric enzyme is aspartate transcarbamolyase (Aspartate carbamolytransferase). It catalyzes first reaction unique to pyrimidine nucleotide biosynthesis.

Carbamoylphosphate + Aspartic acid = Carbamoylaspartate + Pi

The enzyme consist of catalytic and regulatory subunits. It exists in less active form and high active form. Binding of CTP to regulatory subunit converts high active form to less active form. So CTP is called as negative effector or allosteric inhibitor. In contrast, binding of ATP to regulatory subunit convert less active form to high active form. So ATP is called as positive effector or allosteric activator.

Aspartate carbamoyl transferase Asparatate carbamoyl transferase

Less active form High active form

Kinetics of Allosteric Enzyme

1. These enzymes does not exhibit Michaelis-Menten Kinetics. A plot of (*v*S) velocity versus substrate concentration is sigmoidal or S shaped curve rather than rectangular hyperbola
2. The sigmoidal curve indicates a rapid increase in velocity after a particular substrate concentration. It is due to phenomenon of co-operativity.
3. To explain co-operativity of allosteric enzymes 'T' and 'R' model was proposed. According

to this model, the oligomer (allosteric enzyme) exist in two states. A tense (T) state and relaxed (R) state. Binding of substrate (ligand) to ‘T’ form which is intially slow causes a conformational change in subunits resulting in ‘R’ form. Further binding of ligand (substrate) to the subunits is rapid.

4. Allosteric inhibitor stabilizes the enzyme in ‘T’ form, so the enzyme is less active. In contrast, allosteric activator stabilizes the enzyme in ‘R’ form, so the enzyme is highly active.

Enzyme Regulation by Covalent Modification

Enzyme activity is regulated by covalent attachment of a group to the enzyme molecule. Phosphate group is most commonly used to modify enzyme activity. Other group involved in regulation of enzyme activity by covalent modification is nucleotide. Enzymes which undergo regulation by covalent modification exist in two forms, a less active and a high active form. Depending on the enzyme, the phospho or dephospho enzyme may be less or more active, respectively. The phosphorylation (attachment of phosphate) and dephosphorylation are catalyzed by protein kinases and phosphatases, respectively. The –OH group of serine residue of the protein is the site of phosphorylation. ATP serve as donor of phosphate group.

Many hormones influence the activities of proteinkinases and phosphatases.

Examples:

1. Phosphorylation of glycogen phosphorylase converts less active to high active form. Dephosphorylation converts high active to less active form.
2. Phosphorylation of glycogen synthase converts high active to less active form
3. HMG-CoA reductase, hormone sensitive lipase and acetyl-CoA carboxylase are also regulated by phosphorylation and de-phosphorylation.

Enzyme Regulation by Covalent Attachment of Nucleotide

The activity of glutamine synthetase of E. Coli is regulated by covalent attachment of nucleotide to the enzyme molecule. The attachment of nucleotide to the –OH group of tyrosine residue of enzyme molecule converts more active enzyme to less active enzyme. Adenyl transferase catalyzes addition of nucleotide to enzyme molecule.

PRO-ENZYMES

One way of regulating catalytic activity of an enzyme is synthesizing enzyme in inactive (precursor) form or pro-enzyme or zymogen. They are converted to active form later when need arises. The conversion of pro-enzyme to active enzyme involves limited proteolysis. Limited proteolysis removes few aminoacids from proenzyme which results in conversion of inactive enzyme to active enzyme. So, conversion of pro-enzyme to active enzyme accompanies decrease in molecular weight of pro-enzyme due to removal of amino acids (peptides).

Most of the protein digesting enzymes of pancreas are synthesized in inactive forms to protect pancreatic cells from destructive action of proteases. Likewise, pepsin of stomach is also synthesized in pro-enzyme form to protect gastric mucosa from pepsin attack. Most of the blood clotting enzymes are also synthesized in inactive form. They are converted to

active forms only at the time of blood coagulation.

Pro-enzymes of Digestive Tract and their Conversion to Enzymes

In the stomach pepsin is synthesized in inactive pepsinogen form. At the acidic pH of stomach pepsinogen undergo limited proteolysis, which results in the formation of pepsin. When once pepsin is formed it catalyzes its own formation from pepsinogen. This process is called as *autocatalysis*.

The protein splitting enzymes of pancreas are synthesized in inactive forms. They are trypsinogen, chymotrypsinogen, procarboxy peptidase and proelastase. A lipid digesting enzyme is also produced in pancreatic cells as a zymogen. It is prophospholipase. The conversion of these pro-enzymes to active enzymes is initiated by enterokinase produced by mucosal cells of duodenum. Enterokinase removes a hexapeptide from trypsinogen by hydrolysing-Lys-Ile bond.

The removal of hexapeptide converts trypsinogen to trypsin. When once few molecules of trypsin are formed it further catalyzes not only formation from trypsinogen but also the conversion of other proenzymes to active enzymes . Since single molecule of trypsin can trigger the formation of battery of protein digesting enzymes, pancreas has another self protecting mechanism. It contains trypsin inhibitor in small amounts.

The formation of blood clot involves activation of (zymogens) blood clotting factors. Prothrombin is converted to active thrombin by factor X and V. Thrombin in turn converts fibrinogen to fibrin.

Medical Importance

Though there are two in-built defensive mechanisms in the pancreas to avoid activation of pro-enzymes, in acute pancreatitis the pro-enzymes get activated and cause damage to pancreas and severe abdominal pain.

The quantity of enzyme in cell or body is regulated by

1. Enzyme degradation
2. Enzyme induction and repression

Regulation of Enzyme Activity by Degradation

Enzymes produced as a part of development or enzymes produced to overcome certain environmental conditions or enzymes produced to remove toxins are not needed any more later. Their continued presence may be harmful to the body. So, if enzymes were immortal, then it leads to creation of unwanted side effects in the body. Hence, enzymes undergo turnover. They are synthesized and degraded. Individual enzymes have life spans. Some enzymes may last few seconds or minutes in the cell. However, some enzymes may last few days in the body. There are specific mechanisms for degradation of enzymes. Enzymes that control key metabolic events are degraded very fast. Likewise if a defective enzyme is produced, it is degraded very rapidly because it is not useful any more to the body.

Enzyme Regulation by Induction and Repression

The quantity of the enzyme can be increased by increasing its synthesis and quantity of the enzyme can be decreased by decreasing its synthesis. Depending on cell needs quantity of

the enzyme increases or decreases. Enzymes which are regulated in this manner are called as *inducible enzymes*. It take place at nuclear level of the cell.

Inducible Enzymes

Normally these enzymes are present in small concentration but in presence of certain substance called as inducer their quantity increases.

Induction

Increased synthesis of an inducible enzyme in response to inducer is known as induction.

Constitutive Enzymes

These are present in fixed quantities. They are not inducible.

Examples for enzyme induction: When *E. Coli* is grown on medium containing lactose, they produce more of β-galactosidase or lactase required for lactose utilization. When the cells are transferred to medium free of lactose, formation of lactase decreases. Thus, lactose induces the synthesis of lactase. So, in this case lactose is inducer and lactase is an inducible enzyme.

Repression

Certain substances blocks their own synthesis by decreasing synthesis of enzymes, which are required for their formation. This process is called as repression. Substances are called repressors.

Examples for repression: When histidine is added to the S. Typhi. containing medium synthesis of all the enzymes required for histidine formation is blocked. In this case, histidine is repressor molecule.

In humans also, induction and repression of enzymes takes place. They are called as adaptable enzymes.

Examples:

1. Arginase, an enzyme of urea-cycle formation is more in starvation and on high protein diet.
2. Pyruvate carboxylase an enzyme of gluconeogenesis is induced by glucocorticoids and repressed by insulin.
3. Phenobarbitol and anti-convulsive drug induces alkaline phosphatase.

ISO-ENZYMES OR ISOZYMES

1. They are multiple forms of enzymes.
2. They catalyze same reaction but differ in physiochemical properties. They occur in same species or in same individual.
3. They are tissue specific or species specific.
4. They are present in serum and other biological fluids and tissues.
5. Iso-enzyme for dehydrogenases, transaminases and phosphotases have been reported.

Separation of Iso-enzymes

Most commonly used technique for the separation of iso-enzymes is electrophoresis. The

serum lactate dehydrogenase (LDH) iso-enzyme pattern is obtained by subjecting serum to electrophoresis at pH 8.6. On electrophoresis, iso-enzymes of lactate dehydrogenase separates into five bands. Each band exhibits same catalytic activity . The five isoenzymes of LDH are LDH1, LDH2, LDH3, LDH4 and LDH5.

Structure of LDH Iso-enzymes

Lactate dehydrogenase isoenzymes differ at the level of quaternary structure. The LDH consist of 4 subunits of two types. They are H and M subunits. The subunit composition of different LDH isoenzymes are shown below:

Name of isoenzyme	**Subunit composition**
LDH1	HHHH or H4
LDH2	HHHM or H3M
LDH3	HHMM or H2M2
LDH4	HMMM or HM3
LDH5	MMMM or M4

The synthesis of two subunits H and M is controlled by different genes. H is acidic and M is basic in nature. The molecule weight of each subunit is 35,000.

Alkaline Phosphatase Iso-enzymes

Electrophoresis is used for the separation of isoenzymes of alkaline phosphatase in serum. On electrophoresis, iso-enzymes of alkaline phosphatase separates into four bands. The four iso-enzymes of alkaline phosphatase are tissue specific. They differ in their carbohydrate content. The four isoenzymes originate from bone, liver, placenta and intestine.

Creatine Phosphokinase (CK) Iso-enzyme

CK iso-enzymes can be separated by electrophoresis. CK has three isoenzymic forms. They are CK1, CK2 and CK3. They differ in subunit composition. CK is a dimer. It consist of two subunits M and B. The subunit composition of three isoenzymes of CK are BB, MB and MM for CK1, CK2 and CK3 respectively.

Carbonic Anhydrase Iso-enzymes

On electrophoresis carbonic anhydrase gives three bands. The three isoenzymes differ in amino acid composition.

CLINICAL ENZYMOLOGY

1. It deals with quantitative estimation of enzymes in body fluids in normal and diseases conditions.
2. Depending on pathological conditions different body fluids are used for enzyme measurement.
3. Serum and plasma are most commonly used.
4. Other body fluids used for enzyme measurement are cerebrospinal fluid, amniotic fluid, pleural fluid, peritonial fluid and synovial fluid.
5. Quantitative estimation of enzymes in serum is used to confirm the diagnosis which is made by observing clinical symptoms. Sometimes it is used to know the effectiveness of treatment *i.e.,* prognosis.

6. Hence measurement of serum enzyme levels is of both diagnostic and prognostic importance. Blood plasma contains several enzymes. Depending on their role they are divided into two groups.

A. Functional Enzymes

They are present in plasma at higher level than in most of tissues and they perform functions in plasma. They include lipoprotein lipase, choline esterase and enzymes of blood coagulation etc.

B. Non-functional Enzymes

They are present only at minimal concentrations in normal and have no known function in plasma. They mainly arises from normal destruction of various blood and tissues cells. So, they are mainly contributed by turnover of tissues. Increased concentration of these enzymes in plasma indicates increased tissue breakdown or damage to tissues due to disease or injury. If the plasma level of secretory enzyme is increased it indicates block in the secretory pathway. Further distribution of enzymes among tissues varies from one organ to another organ. If an organ is rich in an enzyme, injury or damage to that organ leads to release of the enzyme into plasma in significant amounts. Some diseases or cancers of that organ also causes release of the enzyme into plasma. Quantitative measurement of the enzymes in plasma under such conditions serve as good index of disease of that organ. Furthermore, the amount of enzyme released is proportional to the mass of the affected tissue.

Some of the clinically important enzymes which are routinely measured in clinical chemistry laboratory are:

1. Transminases

Aspartate aminoa transferase (AST) and alanine amino transferase (ALT) are two transaminases most frequently measured. Normal levels are 3-20 U/L for AST and 4-20 U/L for ALT (Units-U). The former enzyme is also referred as GOT (Glutamate oxalo acetate transaminase) and latter is referred as GPT (Glutamate Pyruvate Transminase). These two enzymes differ in distribution. Heart is rich in AST where as liver contains both of them in equal amounts. Hence, AST estimation is most commonly done in diseases that affect heart. AST level increases in plasma following heart attack or myocardial infarction. Since liver contains more of ALT, its elevation in plasma is specific indicator of liver damage. Plasma ALT level is more in liver diseases like alcoholic cirrhosis, biliary obstruction, cancer and toxic hepatitis. Both the enzymes are elevated in acute infective hepatitis because liver contains both of them in significant amount. After the onset of viral hepatitis, the levels of these enzymes reaches peak rapidly and come back to normal reference level within a week. Since the skeletal muscle contains appreciate amounts of ALT, its level is increased in muscle damage as in severe trauma and in muscular dystrophy. Serum transaminases are also elevated in lung disease.

2. Alkaline phosphatase

This enzyme catalyzes the hydrolysis of organic esters at alkaline pH 9.0, hence the name

alkaline phosphatase. The normal level is 20-90 units/L. The level of the enzyme is elevated in rickets, obstructive jaundice, hyperparathyroidism, metastatic cancer, bone cancer and osteomalacia. In obstructive jaundice, its level is 10 times the normal level because its secretion is blocked due to obstruction. Its level also increases in some non-specific diseases like leukemia, lung and kidney damages and congestive heart failure, Hodgkin's disease and intestinal disorders.

3. Acid phosphatase

This enzyme catalyzes the hydrolysis of organic esters at acidic pH (5.0) hence the name acid phosphatase. The normal level of the enzyme is 2.5-12.0 units/L. It is increased in prostrate cancer. Small increase are seen in bone disease and breast cancer.

4. γ-glutamyl trans peptidase (GGT)

It is involved in the degradation of glutathione. Its level is increased in alcoholic cirrhosis. The normal plasma level of GGT is less than 30 units/L. Since this enzyme is secreted into bile by liver, like alkaline phosphatase γ-glutamyl trans peptidase level increases in cholestatic or obstructive jaundice. It is also elevated in brain lesions.

5. Creatine phosphokinase (CK)

The normal level of this enzyme in plasma is 12-60 U/l. Since skeletal muscle is rich in CK serum CK level raises in disease effecting skeletal muscle. Its level is elevated in muscular dystrophy, polymyositis, severe muscle exercise, muscle injury, hypothyroidism, epileptic seizures and in tetanus.

CK level is also elevated in diseases affecting cardiac muscle because of its high content in it. CK level is elevated in myocardial infarction.

6. Lactate dehydrogenase (LDH)

The LDH normal level is 70-90 units/L. LDH levels are elevated in myocardial infraction. The serum LDH level raises within 24 hours after infraction, reaches peak level around 2-3 days and returns to normal in a week. Serum LDH level is also elevated in pernicious anemia, megaloblastic anemia, acute hepatitis, blood cancer and in progressive muscular dystrophy.

7. Isocitrate dehydrogenase

The normal level of this enzyme in plasma is 1-5 Units/L. Its level is elevated in inflammatory diseases of liver like infective heptatitis, toxic hepatitis. In obstructive jaundice, its level remains normal. This enzyme is found in cerebrospinal fluid. Measurement of enzyme in C.S.F. is a valuable diagnostic aid in the cases of meningitis and brain tumors. In meningitis the level is elevated more than that of in cerebral tumors.

8. Amylase

The normal range of this enzyme in plasma is 800-1800 Units/L. This enzyme is secreted by pancreas and salivary glands. So, its level is elevated mainly in acute pancreatitis and parotitis. Its level raised in other conditions like intestinal obstruction and in mumps.

9. Lipase

It is an enzyme produced by pancreas. It is secreted into duodenum through pancreatic duct.

The normal level of this enzyme is up to 150 Units/L. It is mainly elevated in acute pancreatitis and pancreas cancer. It is also elevated in patients with abdominal lesions, perforated peptic ulcer, intestinal obstruction and in acute peritonitis.

ISOENZYMES IN CLINICAL MEDICINE

1. In some cases, elevated serum enzyme level may not indicate severity and specific organ damaged, because the serum enzyme is derived from routine destruction of cells of various organs.
2. Since isoenzymes are organ specific, iso-enzyme determination gives an indication about the specific organ affected. Further, iso-enzyme distribution varies from one organ to other organ. Hence, if an organ rich in a isoenzyme is damaged or diseased, more of that iso-enzyme enters plasma.
3. By measuring that isoenzymes level in serum the specific organ diseased can be confirmed.
4. Therefore, iso-enzyme determination is useful in differential diagnosis.

(a) LDH Isoenzymes

Serum LDH is the combination of five isoenzymes. Each iso-enzyme is derived from specific organ. LDH1 is derived from heart because heart is rich in LDH1. Similarly, LDH5 is derived from skeletal muscle because it is rich in LDH5. Liver also contain LDH2 to LDH5 isoenzymes in different amounts. LDH isoenzymes are present in different proportions. The proportions of LDH isoenzymes in normal serum are 25%, 35%, 27%, 8% and 5% for LDH1, LDH2, LDH3, LDH4 and LDH5, respectively.

When heart muscle is affected as in myocardial infarction, LDH1 level increases in plasma because of release of LDH1 from damaged heart muscle. So measurement of LDH isoenzyme in serum in myocardial infraction is more sensitive index of myocardial necrosis than the measurement of total LDH activity. Similarly, elevated levels of LDH5 is more specific of muscle lesions and liver inflammation of hepatitis.

(b) CK Isoenzymes

The normal serum CK is composed of CK1, CK2 and CK3. In normal persons, CK2 accounts only 2% of total CK but it accounts for 20% of CK in a patient within 4 hours after heart attack.

(c) Alkaline Phosphatase Isoenzymes

The normal serum alkaline phosphatase is composed of 4 isoenzymes. They are derived from bone, liver, placenta and intestine. Measurement of isoenzymes of alkaline phosphatase is used to distinguish liver lesions from bone lesions in metastatic carcinoma.

SERUM ENZYME PROFILES

1. It involves estimations of different serum enzymes for few days following the onset of a disorder.
2. Multi enzyme determinations for a short span of time serve as good index of disorder. More over determination of more than one enzyme in a particular disease is more useful in prognosis.

3. Several serum enzymes serve as diagnostic indices of myocardial infarction. Serum AST level starts increasing by 6 hours after heart attack, reaches peak value around one to second day and returns to normal by sixth day. CK level follows a pattern similar to AST. It contrast, LDH levels raises within 24 hours of heart attack, reaches peak around 2-3 day and level remain increased even after a week.
Serum enzyme levels are also determined to detect inherited disorders associated with altered enzyme levels like galactosemia, glucose-6-phosphate dehydrogenase deficiency etc.

ENZYME-LINKED IMMUNO ADSORBENT ASSAY

It is popularly known as ELISA technique. The technique combines enzymology with immunology and photometry. It is used for detection and estimation of substances which are either antigens or antibodies. It is based on immune complex formation. The immune complex consist of an antibody, antigen and second antibody with bound enzyme (antibodyantigen-antibody2-enzyme). Enzyme linked to second antibody has a crucial role in detection and estimation of antigen present in sample. When it reacts with substrate color is produced.
Intensity of the color is proportional to amount of antigen present in sample.
Steps of this techniques are given below:
1. Antibodies specific to an antigen of interest are produced. They are fixed to support materials using coupling agent. The support materials are cellulose, plastic, polystyrene or glass. Plastic plates containing wells (depressions) which are coated with antibodies are commonly used.
2. Sample (serum) containing antigen is allowed to combine with antibody by placing sample in the well.
3. Unbound molecules of sample are removed by washing.
4. A second antibody linked to an enzyme is added. This also binds to antigen to form antibody-antigen-antibody2-enzyme complex. Thus, second antibody linked to enzyme is fixed to support material.
5. Unbound antibody2-enzyme complex is removed by washing.
6. In the final step, substrate is added. Enzyme linked to antibody2 convert substrate to colored product which is measured.

Medical Importance

1. Using this technique, antigens or antibodies that are present in very small amounts (picograms) in biological fluids are detected and estimated.
2. Several hormones like insulin, TSH, hCG, Calcitonin etc. are determined world wide using this technique.
3. Antibodies are detected using this technique by fixing antigen to support material.
4. Detection of highly infectious diseases like AIDS, Hepatitis, Malaria etc. World wide involves use of this technique.
5. Some tumor markers in biological fluids are detected and estimated using this

technique.

CELL

Cell is the universal functional unit of all forms of life. On the basis of differences in cell structure, all life forms are divided into two major classes. They are prokaryotes and eukaryotes. *Prokaryotes* are simple cells and in most cases, individual cell itself is the organism. They contain cell wall and cytosol is not divided into compartments. Examples for prokaryotes are bacteria, primitive green algae and archae bacteria. All other organisms are called *eukaryotes*. They are multicellular organisms. They are plants, animals, fungi, protozoa, uni-cellular yeast and true algae.

MEDICAL AND BIOLOGICAL IMPORTANCE

1. All higher living organisms including humans are made up of cells.
2. Human body contains wide variety of cells that differ in structure and function.
3. Human cell contains subcellular structures like nucleus, mitochondria, lysosomes and peroxisomes etc.
4. Each subcellular structure has unique shape and function.
5. Some diseases are due to a lack of subcellular structures.
6. Zellwegers syndrome is due to lack of peroxisomes.
7. Lysosomal enzymes are involved in spreading of cancer.
8. Lack of lysosomes or its enzymes results in lysosomal diseases.
9. Growth of cells requires cell divisions. Cell cycle encompasses all the events of cell division.
10. Cells are not immortal. They have finite life span. Because of this humans are not immortal.
11. Cell death is crucial for shaping of organs during development and for recovery from injuries.
12. Biochemistry explores molecular mechanisms of normal cellular processes as well as diseases.
13. Mitochondria is involved in apoptosis.
14. Endoplasic reticulum, lysosomes and golgi complex are involved in the integration of pro-apoptic signals.

MOLECULAR COMPOSITION OF CELL

Water

Water accounts for about 70-75% of the weight of the cell. Other cellular constituents are either dissolved or suspended in water.

Organic Compounds

1. Organic compounds accounts for 25-30% of the cell weight.
2. They are nucleic acids, proteins, polysaccharides (carbohydrates) and lipids. Proteins accounts 10-20% of the weight of the cell. Nucleic acids account 7-10% of the cell weight.

Polysaccharides usually account for 2-5% of the cell weight. About 3% of cell weight is due to lipids. Lipids content may be higher in adipocytes or fat cells. Proteins may account more of cell weight in cells like erythrocytes.
3. Other low molecular weight organic compounds may account for 4% of cell weight. They are monosaccharides, aminoacids, fatty acids, purine and pyrimidine nucleotides, peptides, hormones, vitamins and coenzymes.

Inorganic Compounds

1. Inorganic compounds account for the rest of the cell weight.
2. They are cations like sodium, potassium, calcium, magnesium, copper, iron and anions like chloride, phosphate, bicarbonate, sulfate, iodide and fluoride.

EUKARYOTIC CELL STRUCTURE AND FUNCTION

In eukaryotes, cells aggregate to form tissues or organs and these are further organized to form whole organism. In humans, eukaryotic cells exist in large number of sizes and shapes to perform varieties of functions. For example, nerve cells differ from liver cell which differ from muscle cell and they differ in function also. Though the eukaryotic cells differ in sizes and shapes they have certain common structural features. Further, eukaryotes contain subcellular structures and well defined nucleus. Cells are surrounded by membranes. It separates the cells from surrounding and it is called as *plasma* or *cell membrane*. The other subcellular organelles are also composed in parts by membranes.

SUBCELLULAR STRUCTURES AND THEIR FUNCTIONS

Cell Membrane

Structure

1. The outermost structure of the cell that decides its contour is the cell membrane.
2. It is a lipid bi-layer. It also consist of proteins and small amounts of carbohydrate

Functions

1. It is fluid and dynamic.
2. It is semi permeable, only selected compounds are allowed to pass through from outside. The selective permeability is responsible for the maintenance of internal environment of the cell and for creating potential difference across the membrane.
3. The modification of the cell membrane results in formation of specialized structures like axon of nerves, microvilli of intestinal epithelium and tail of spermatids.

Nucleus

Structure

1. Centre of the cell is nucleus.
2. It is surrounded by double-layer membrane of about 250-400 Å thick.
3. The two layers of nuclear membrane are an outer and inner membrane (layer). The two membranes fuse periodically to produce nuclear pores. Exchange of material between nucleus and rest of the cell occurs through nuclear pores.
4. The outer nuclear membrane continuous with other cytomembranes. In some eukaryotic

cells, like erythrocyte nucleus is absent. In spermatozoa, nucleus accounts for 90% of cell whereas in other cell's nucleus accounts for less than 10% of the cell. In prokaryotes, nucleus is not well defined.

Functions

1. Nucleus is the information centre of eukaryotic cell. More than 90% of the cellular DNA is present in the nucleus. It is mainly concentrated in the form of chromosomes.
2. Human cell contains 46 chromosomes. These chromosomes are composed of nucleoprotein chromatin, which consist of DNA and proteins histones. Some RNA may also present in the nucleus.
3. In prokaryotes, the DNA is present as thread in the cytosol.

Nucleolus

Structure and Function

These are small dense bodies present in the nucleus. Their number varies from cell to cell. There is no membrane surrounding them. They are continuous with nucleoplasm. Protein accounts for 80% of nucleolus remainder is DNA and RNA.

Nucleoplasm

It is also called as nuclear matrix. It contains enzymes involved in the synthesis of DNA and RNA.

Cytosol, Cytoplasm or Cell Sap

Structure

1. The extra nuclear cell content that possess both orgenelles and other material constitutes cytoplasm. Material other than subcellular components in the cytoplasm makes up the cytosol or cell sap.
2. Sometimes soluble portion of the cell is referred as cytosol. Cytoplasm accounts for 70-75% weight of the cytosol.

Functions

1. Numerous enzymes, proteins and many other solutes are found in cytosol.
2. Cytosol is the main site for glycolysis, HMP shunt, activation of aminoacids and fattyacid synthesis.

Mitochondria

Structure

1. Are the second largest structures in the cell.
2. Generally mitochondria are ellipsoidal in shape and can assume variety of shapes.
3. The length of a mitochondrion is about 7 microns and has a diameter of 1 micron.
4. Mitochondria consist of outer and inner membranes. The outer membrane is composed of equal amount of protein and lipids.
5. The lipids are mainly phospholipids and cholesterol. The outer membrane functions as a limiting membrane and permeable to many compounds.
6. The inner membrane consist of 75% protein and remainder is lipid.
7. Cardiolipin is the important phospholipid of inner mitochondrial membrane.

8. The inner membrane is convoluted to form number of invaginations known as cristae extending to matrix.
9. These cristae are covered with knob like structures, which are composed of head piece, stalk and a base piece.
Functions
1. The number of mitochondria ranges from 1-100 per cell depending on type of cell and its function. Several factors influence the size and number of mitochondria in cells. In yeast, mitochondria is present in aerobic state and absent in anaerobic state. Exposure to cold increases mitochondria by 20-30% in liver cells.
2. In highly metabolically active cells mitochondria are more and large.
3. Location of mitochondria in cell also depends on types and functions of cell. In liver cell mitochondria are scattered. In muscles they are parellely arranged. Mitochondria in liver cell may range up to 2000 whereas in kidney they may range up to 300.
4. Mitochondria is the *power house* of the cell. It is responsible for the production of energy in the form of ATP. The knob like structures function in electron transport and oxidative phosphorylation.
5. Mitochondria also contain other energy producing pathways like citric-acid cycle, fatty acid oxidation and ketone-body oxidation.
6. Some reactions of gluconeogenesis and urea cycle also occurs in mitochondria. Mitochondria is capable of synthesizing some of its proteins.
7. Mitochondria contains some DNA known as mitochondrial DNA and ribosomes.
8. Mitochondria which are essential for life because of their involvement in ATP production, also pay key role in programmed cell death of several types of cells. During apoptosis, mitochondrial membrane potential drops. This leads to permeabilization of mitochondrial membrane. Cytochrome-C or mitochondrial proteins are released into cytosol which activates death enzymes. Further alterations in mitochondrial morphology also occur during apoptosis.
9. In humans, mitochondria is derived from mother only. Hence, origin of mother of humans have been traced.
10. Outer and inner mitochondrial membranes contain translocase enzymes. They are involved in sorting of nuclear encoded proteins into mitochondrial sub-compartments as well as for their import into mitochondria. The inter mitochondrial membrane space is home for several lethal proteins like pro-death enzymes.

Lysosomes

Structure
1. They are small vesicles present in cytoplasm.
2. They are surrounded by a membrane. Lysosomes are called as ‘Suicidel bags’ of the cell.
Functions
1. Lysosomes are rich in hydrolytic enzymes, which are active at acidic pH. The lysosomal enzymes digest the molecules brought into the cell by phagocytosis.

2. Macrophages are rich in lysosomes.
Medical Importance
1. Lysosomal enzymes are involved in bone remodelling and intracellular digestion.

2. Disease, shock or cell death causes rupture of lysosomes and release of enzymes. In some organisms, lysosomal enzymes are responsible for cell death of larval tissues.
3. Lack of one or more of lysosomal enzymes cause accumulation of materials in the cell resulting in lysosomal diseases.
4. In some disease like arthritis and muscular dystrophy, lysosomal enzymes are released to cause uncontrolled destruction of surrounding tissues. Lysosomal proteases cathepsins are involved in spreading of cancer (metastasis).
5. As the age advances in digestible material an age pigment 'lipofuscin' occurs in some cells.
6. Lysosomol cystine transporter cystinosin is defective in cystinosis, which is a lysosomol disease. Hence, cystine transport into cytosol from lysosome is blocked.
7. Lysosomes are involved in integration of pro-apoptic signals.

Peroxisomes

Structure
1. Are also small vesicles surrounded by a membrane. They are also called as *microbodies.*
Functions
1. They contain enzymes of H_2O_2 metabolism. The concentration of protein in peroxisomes is very high and they may occur in crystallines form. The enzymes of H_2O_2 catabolism present in peroxisomes are peroxidase and catalase.
2. Peroxisomes also contain other enzymes like D, L-amino acid oxidase, uric acid oxidase and L-hydroxy fatty acid oxidation that generates H_2O_2. Glycerophospholipids are also synthesized in peroxisomes.

Medical Importance
1. Lack of peroxisomes result in Zellwegers syndrome.

Cytomembranes

There is an extensive network of membranes in the cytoplasm. These membranes are called as cytomembranes. They are divided into endoplasmic reticulum and golgi complex or apparatus. The endoplasmic reticulum is further subdivided into rough endoplasmic reticulum (RFR) and smooth endoplasmic reticulum (SER).

Rough Endoplasmic Reticulum

Structure
1. It is continuous with outer nuclear membrane.
2. The cytoplasmic surface of rough endoplasmic reticulum is coated with ribosomes. Membrane enclosed channels of endoplasmic reticulam are called *cisternae*. The ribosomes

are complexes of RNA and protein.

Functions

1. Ribosomes and rough endoplasmic reticulum are involved in protein synthesis.
2. Protein synthesized, enters cisternae and later extruded.

Smooth Endoplasmic Reticulum

Structure

1. It is continuous with rough endoplasmic reticulum. It differs from RER by the absence of ribosomes. When isolated SER is called as microsomes.

Functions

1. SER of intestinal cells is involved in formation of triglycerides.
2. In the adrenal cortex, SER is the site of steroid formation.
3. Cytochrome P450 dependent monooxygenases are present in liver cell SER.

Golgi Apparatus

Structure

1. It consist of cluster of paired cytomembranes. The margins of these cytomembranes are flattened.
2. It also contains several small vesicles, which are pinched off from the flattened margins of membranes.

Functions

1. The golgi bodies are well developed in cells, which are involved in secretion. Material produced in the cell for export is processed by golgi body and is packaged as vesicle and is pinched off. The vesicles fuse with plasma membrane and their content is released to exterior by the process known as exocytosis. The digestive enzymes of pancreas and insulin are produced and released in this way.
2. Golgi apparatus helps in the formation of other subcellular organelles like lysosomes and peroxisomes.
3. Golgi apparatus is involved in protein targeting. It directs proteins to be incorporated into membranes of other subcellular structures. It is also involved in glycosylation and sulfation of proteins.
4. Golgi apparatus is involved in integration of proapoptic signal. It generates preapoptic mediator ganglioside GD3.

Medical Importance

Some cases of diabetes are due to defective processing of insulin in golgi complex.

Intracellular Ion Channels

Membrane of endoplasmic reticulum, golgi complex and nucleus has ion channels. They are involved in transport of ions between cytosol and these intracellular components. Calcium and chloride ion channels which are involved in their transport from these components into cytosol are known.

Vacuoles. Some animal cells contain vacuoles. They are membrane enclosed vesicles

containing fluid. Mostly they contain nutrients.

Cell Coat. Some mammalian cells contain thin coat known as cell coat on the outer surface of the cell membrane. The cell coat is flexible and sticky. It is composed of mucopolysaccharides, glycolipids and glycoproteins. The adhesive properties of cell and organization of tissue is controlled by cell coat

Cytoskeletons

These are filament like structures made up of proteins present in cytoplasm. Non-muscle cells perform mechanical work with these intracellular network of proteins.

(*a*) **Microfilaments.** They are actin like filaments. They form loose web beneath cell membrane.

(*b*) **Myosin Fibres.** Same as that of myosin of skeletal muscle.

(*c*) **Microtubules.** Tubulin is the building block of microtubules. Dendrites, axons of nerve cells and sperm cells contain microtubules. The sperm cell moves with the help of flagellum, a microtubule. These cyto skeletons are involved in the maintenance of cell shape, cell division, cell motility, phagocytosis, endocytosis and exocytosis.

(*d*) **Intermediate Filaments.** They are not involved in movement of cell. They are stable components of cytoskeleton. Neurofilament of neurons, glial filaments of glial cells and keratin of epithelial cells are some examples of intermediary filaments.

MEDICAL IMPORTANCE

1. In all forms of life growth requires cell division.
2. However, some cells divide even after growth like erythrocytes and epithelial cells of intestine.

Sequence of events associated with cell division occur in cyclic manner. Hence, cell cycle consist of sequence of events, which occur in cyclic manner during cell division. There are four stages (phases) in cell cycle. They are

1. *S (Synthesis)-Phase*
2. *G1 (Gap 1)-Phase*
3. *G2 (Gap 2)-Phase*
4. *M (Mitosis)-Phase*

Sometimes, cell cycle is considered in two main events. They are mitosis and inter phase which consist of G1, G2 and S-phases.

1. *S (Synthesis)-Phase:* Division of a cell into two daughter cells requires duplication of DNA. During S-phase concentration of DNA precursors increases nearly 10-20 folds. In S-phase DNA synthesis occurs. Period of DNA synthesis is almost constant in all adult cells. (1 Hour)
2. *G1 and G2-Phases:* G1 and G2-phases are gaps or breaks in cell cycle. No special events occur during these phases except the size of the cell may increase. However, there may be many biochemical reactions taking place preparing the cell for division and checking that all appropriate steps are completed. The period of S1, G2 and M-Phases may range

from 12-18 hours. But the period of G1-phase varies, it can be few hours to months or even years.

3. *Mitosis (M)-Phase:* Many events take place in this phase of cell cycle. At the end mitosis cell divides into two daughter cells. The daughter cells are in G1-phase.

Check Points in Cell Cycle

1. It is essential that during cell cycle, the synthesis of DNA, chromosomal segregation and cytoplasm division takes place in proper order. So, controls or check points within the cell cycle exist for all organisms.

2. During cell cycle, oscillation of cell from mitosis to interphase is controlled by many cellular proteins. Further check points exist at the G1/S and G2/M boundaries of cell cycle