

CELL BIOLOGY AND MEMBRANE BIOCHEMISTRY

Structure and function of cell and its organelles

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CONTENTS

[Introduction](#)

[Categories of cells](#)

[Prokaryotes](#)

[Eukaryotes](#)

[Fungus cells](#)

[Why majority of cells are small?](#)

[Cell shape](#)

[Cells](#)

[Cell organelles](#)

[Plasma membrane](#)

[Cytoplasm](#)

[Endoplasmic reticulum](#)

[Golgi Apparatus](#)

[Lysosomes](#)

[Peroxisomes](#)

[Nucleus](#)

[Cytoskeleton](#)

[Mitochondria](#)

[Centrosomes](#)

[Plant cell organelles](#)

Types of cells

[Epithelial cells](#)

[Connective tissue cells](#)

[Nerve cells](#)

[Blood cells](#)

[Differentiated cells](#)

Cell division

[Mitosis](#)

[Meiosis](#)

[Isolation of cell components](#)

Keywords

Cell; Cell organelles; Cell division; Separation of organelles

Introduction

Origin of the earth took place nearly 4.5 billion years back, but it took a billion years to appear first sign of life on this earth. Nucleated cells originated only 1.5 billion years back and multi cellular organisms showed their first appearance before 0.5 billion years (Fig. 1).

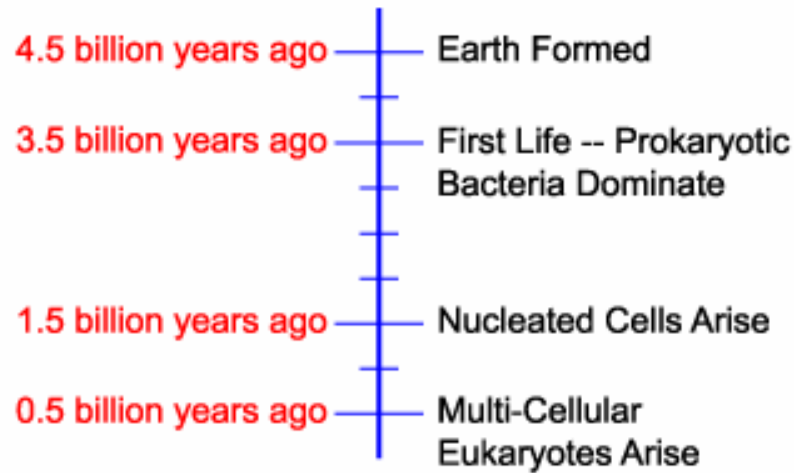


Fig. 1: Time scale for origin of cell

In 1665, Robert Hooke first time coined the term "cell" following his observations of a piece of cork with a simple light microscope, these honey combed structures were named as cell. The word [cell](#) comes from the [Latin](#) word *cella*, meaning a small room (Fig. 2). Each cell is a dynamic, three-dimensional, micro living factory. It is the smallest living unit that can carry out the basic functions of life viz. growth, metabolism and reproduction.



Fig. 2: Thomas Hooke's microscope and section of cork sheet. Honey combs structures named as "cells"

Each cell has its own role to play in the life of the plant or animal and is adapted to perform that particular function; about 300 different types of cell have been identified so far. Schleiden and Schwann proposed cell doctrine in 1830, which stated that all living organism are composed of one or more cells. All cells come from preexisting cells only. Each cell is an amazing world unto itself. It takes in nutrients, convert these nutrients into energy, carry out specialized functions, and reproduce as necessary. Even more amazing is

that each cell stores its own set of instructions for carrying out each of these activities. The cell can thus be defined as the fundamental structural unit of life, which houses the genetic material-genes, in the nucleus. The nucleus is lodged in a jelly like semi-solid substance-the cytoplasm, enclosed in the membrane bag that regulates life.

Categories of Cells

There are two general categories of cells (Fig. 3):

1. Prokaryotes
2. Eukaryotes.
- 3.

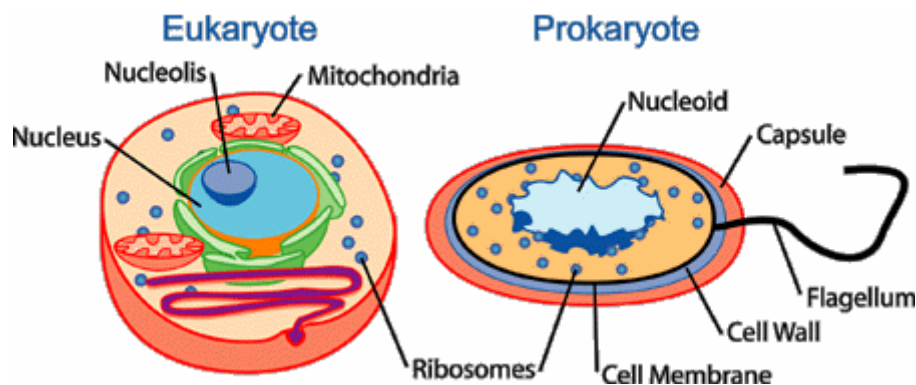


Fig. 3: Prokaryote and Eukaryote cells and their organelles

Prokaryotes

The simplest of and the first types of cells to evolve were prokaryotic cells-organisms that lack a nuclear membrane, the membrane that surrounds the nucleus of a cell. Bacteria are the best known and most studied form of prokaryotic organisms, although the recent discovery of a second group of prokaryotes, called *archaea*, has provided evidence of a third cellular domain of life and new insights into the origin of life itself.

Prokaryotes are unicellular organisms that do not develop or differentiate into multi cellular forms. Some bacteria grow in filaments, or masses of cells, but each cell in the colony is identical and capable of independent existence. If the cells remain in group, it may be due to:

1. The cells may be adjacent to one another because they do not separate completely after cell division, or
2. Because they remain enclosed in a common sheath or slime secreted by the cells.

However, there is no continuity or communication between these cells. Prokaryotes are capable of inhabiting almost every place on the earth, from the deep ocean, to the edges of hot springs, and even in polar regions. They can also grow well just about every surface of plant, animal and our bodies.

Prokaryotes are distinguished from eukaryotes on the basis of nuclear organization, specifically their lack of a nuclear membrane. Prokaryotes also lack any of the intracellular organelles and structures that are characteristic of eukaryotic cells (Fig 4). Most of the functions of organelles, such as mitochondria, chloroplasts, and the Golgi apparatus are taken over by the prokaryotic plasma membrane. Prokaryotic cells have three architectural

regions: appendages called flagella and pili—proteins attached to the cell surface; a cell envelope consisting of a capsule, a cell wall and a plasma membrane; and a cytoplasmic region that contains the cell genome (DNA) and ribosomes and various sorts of inclusions.

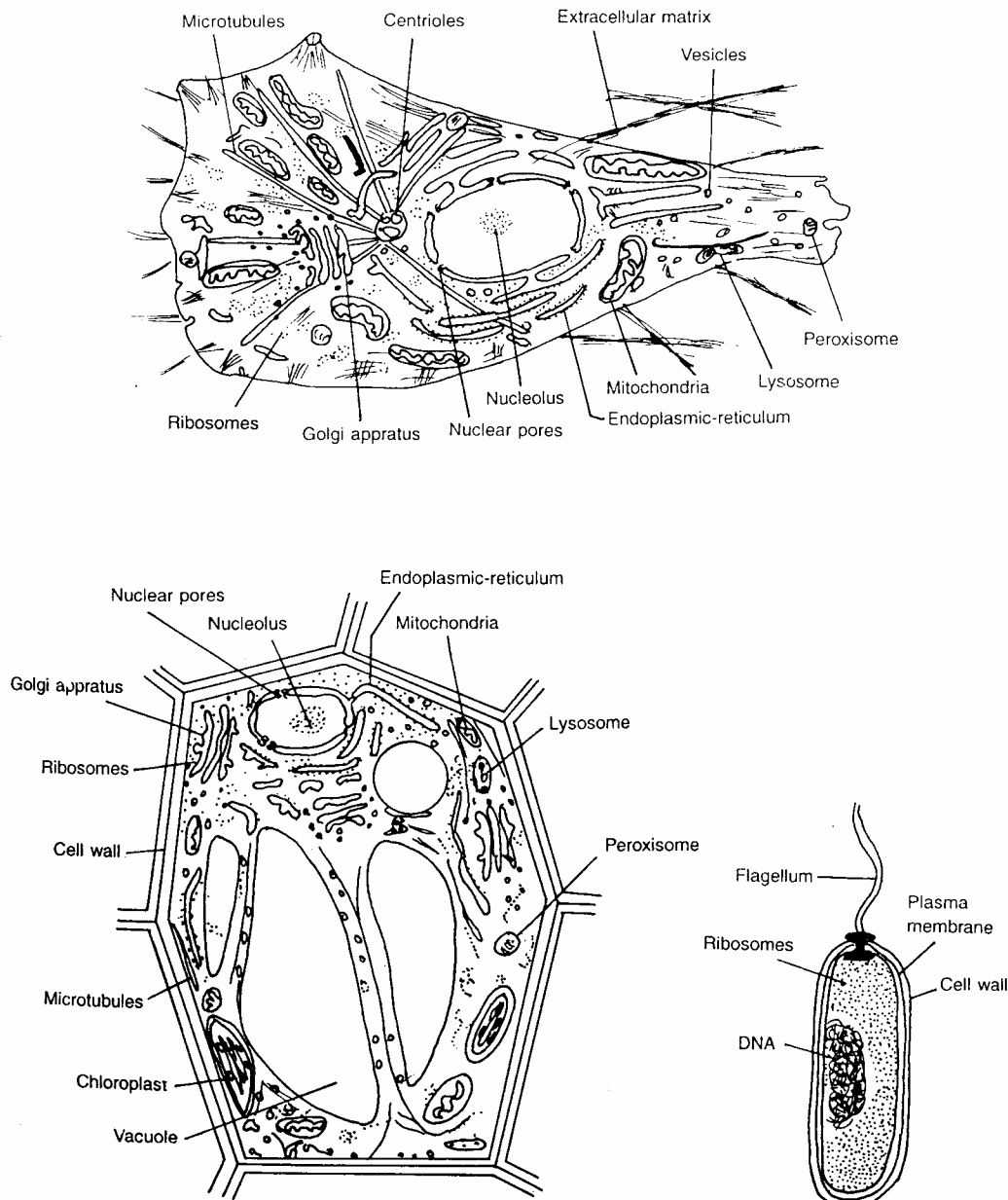


Fig. 4: Comparison between animal, plant and bacterial cells

Eukaryotes

Eukaryotic cells are about 10 times the size of a prokaryote and can be as much as 1000 times greater in volume. The major and extremely significant difference between prokaryotes and eukaryotes is that eukaryotic cells contain membrane-bound compartments in which specific metabolic activities take place. Most important among these is the presence of a nucleus, a membrane-delineated compartment that houses the eukaryotic cell's DNA. It is this nucleus that gives the eukaryote, literally, true nucleus.

Eukaryotic organisms also have other specialized structures, called organelles, which are small structures within cells that perform dedicated functions. As the name implies, cell organelles are like organs in the body. There are a dozen different types of organelles commonly found in eukaryotic cells.

The origin of the eukaryotic cell was a milestone in the evolution of life. Although eukaryotes use the same genetic code and metabolic processes as prokaryotes, their higher level of organizational complexity has permitted the development of truly multi cellular organisms. Without eukaryotes, the world would lack mushrooms, plants, invertebrates, fish, birds and mammals.

The Fig. 3 illustrates a typical eukaryote and a typical prokaryote. The drawing on the left highlights the internal structures of eukaryotic cell, including the nucleus, the nucleolus, mitochondria and ribosomes. The drawing on the right demonstrates how bacterial DNA is housed in a structure called the nucleoid, as well as other structures normally found in a prokaryotic cell, including the cell membrane, the cell wall, the capsule, ribosomes and a flagellum.

Comparison of features of prokaryotic and eukaryotic cells

Both cells are similar in protein synthetic machinery, the presence of DNA, ribosomes, membrane component, etc., nevertheless, these also differ in molecular organization. The other structural components are quite different (Fig. 3 and Table 1).

Table 1: Comparison of features of prokaryotic and eukaryotic cells

	Prokaryotes	Eukaryotes
Typical organisms	Bacteria, archaea	Protists, fungi, plants, animals
Typical size	~ 1-10µm	~ 10-100µm (sperm cells, apart from the tail, are smaller)
Type of the nucleus	Nucleoid region; no real nucleus	Real nucleus with double membrane
DNA	Circular (usually)	Linear molecules (chromosomes) with histone proteins
RNA-/protein-synthesis	Coupled in cytoplasm	RNA-synthesis inside the nucleus protein synthesis in cytoplasm
Ribosomes	50S+30S	60S+40S
Cytoplasmic structures	Very few structures	Highly structured by endomembranes and a cytoskeleton
Cell movement	Flagella made of flagellin	Flagella and cilia made of tubulin
Mitochondria	None	One to several dozen (though some lack mitochondria)
Chloroplasts	None	In algae and plants
Organization	Usually single cells	Single cells, colonies, higher multi cellular organisms with specialized cells
Cell division	Binary fission (simple division)	Mitosis Meiosis

Fungus cells

Despite common beliefs, fungi are not plants. Biologically speaking, they are more animal-like than plant-like. They look like plants, but no photosynthesis is observed in them and they have no plastids. They have cell wall, but it is different from plant cell wall, and is a clear case of a separate evolutionary development. The cell walls of fungi are typically composed of a material called chitin, which is a part of endoskeletons of animal phyla, the Arthropoda (which includes spiders, insects, crabs and lobsters). Another odd aspect of cell structure in many fungi is that, in some groups, the concept of a "cell" is only very loosely applicable. There are fungi, which are largely composed of a single, huge structure with many nuclei and no subdivisions into cellular chambers. Further, there are fungi whose bodies are divided by incomplete subdivision, with continuous cytoplasm connecting all the "cells" into one giant super-cell.

Why majority of cells are small?

Many cells never have a large increase in size after their formation from a parental cell. Typical stem cells reproduce, double in size and then divide again to come to its original size. If a cell becomes too large, the normal cellular amount of DNA may not be adequate to keep the cell supplied with RNA. First, as a cell gets larger, the volume of the cell increases more rapidly than the surface area if the cell maintains its same shape. For example, 1mm cube volume is 1mm^3 and its surface area is 6mm^2 . But it is 2mm on a side, now the volume is 8mm^3 and the surface area 24mm^2 . The volume has increased eight fold but the surface area has increased only four fold.

The cell's ability to either get substances from the outside or eliminate waste is related to the surface area. In other words, how much food and other material from the outside and how much waste the cell has to get rid of, is related to the volume. Therefore, as a cell gets bigger, a time will come when its surface area is insufficient to meet the demands of the cell's volume and the cell stops growing.

However, certain cells have to grow; therefore, there are ways to get around this problem. Oocytes can be unusually large cells in species for which embryonic development takes place away from the mother's body. Their large size can be achieved either by pumping in cytosolic components from adjacent cells through cytoplasmic bridges (*Drosophila*) or by internalization of nutrient storage granules (yolk granules) by endocytosis, *e.g.*, frogs and birds. Bird eggs are much larger than typical cells, but they have a storehouse of food and also rapidly divide to give size to multi-celled embryos. Large cells that are primarily for nutrient storage can have a smooth surface membrane. Most cytosolic contents such as the endomembrane system and the cytoplasm easily scale to larger sizes in larger cells. Another way to get around limitations of surface area is to make the cell long and thin or skinny and flat. Certain cells in your body such as nerve cells and muscle cells are long and skinny. Metabolically active large cells, *e.g.*, nerve cells often have some sort of folding of the cell surface membrane in order to increase the surface area available for transport functions. Neuron size is often a reflection of the number of synaptic contacts onto the neuron or from a neuron onto other cells. Other common means to produce very large cells is by cell fusion to form syncytia. For example, very long (several inches) skeletal muscle cells are formed by fusion of thousands of myocytes.

Increase in the size of plant cells is complicated by the fact that almost all plant cells are inside of a solid cell wall. Under the influence of certain plant hormones the cell wall can be remodeled, allowing an increase in the cell size that is important for the growth of some plant tissues.

In order for the cell to survive, the overall rate of consumption of nutrients (or oxygen) should match the overall rate of uptake. In general, for a given type of cell, we may assume that the following intrinsic properties are determined by the type of cell membrane, the rate of metabolism of the cell, etc, and are therefore constant. Rate of absorption per unit area per unit time should be equal to rate of consumption per unit volume per unit time

The ability to avoid excessive changes of cell volume is one of the most obvious requirements for cell survival. For cell volume constancy osmotic gradients across the cell membrane must remain small despite ever changing intra-cellular and extra-cellular concentrations of osmotically active substances. Any osmotic gradient, across human and animal cell membranes, is followed by respective movement of water, since most cell membranes are highly permeable to water and not rigid enough to build up significant transmembrane hydrostatic pressure gradients. Obviously, excessive cell swelling will eventually lead to disruption of the fragile cell membranes and thus to cell death. Cell volume regulatory mechanisms participate in the regulation of diverse cellular functions such as epithelial transport, metabolism, excitability, phagocytosis, migration, cell proliferation, necrotic and apoptotic cell death.

The relationship between cell size and cell division has been extensively studied. Cell division may be regulated in part by dilution of a protein (Wee1) in cells as they grow larger as in yeast cell whereas in mammals the protein mTOR is a serine/threonine kinase that regulates translation and cell division. Nutrient availability influences mTOR so that when cells are not able to grow to normal size they will not undergo cell division.

Cell size controls in a variety of diseases

Cell volume regulatory mechanisms are highly interesting targets for diagnostic and therapeutic approaches. Deranged cell volume plays a major pathophysiological part in a variety of clinical conditions, such as ischemia, hypernatremia, hyponatremia, hepatic encephalopathy, diabetes mellitus, chronic renal failure and uremia as well as various hypercatabolic states.

Basic research into a tumor suppressor gene that controls cell size has uncovered a link between three different genetic diseases and points to a possible treatment for all of them. The tie that binds these three seemingly disparate medical conditions is a biochemical chain of events that govern cell size. At the end of this chain, a known drug may work to replace missing or broken parts of the biochemical chain.

Cell shape

The shape of a cell can tell much about its physiological status. The fate of a cell, whether it will divide, differentiate, or undergo apoptosis, is closely dependent on whether it is spread or rounded. However, it is still unclear how cell shape is sensed and how the signal is transduced. In recent years, various forms of photolithography and micro-fabrication technology have made it possible to precisely control the shape of a cell. These

technologies involve creating micron scale patterns of cell adhesive islands on a non-adhesive background. Cells seeded on patterned surfaces are only able to attach to the adhesive areas and consequently adopt the shape of the adhesive island. However, the current micro fabrication technology only allows patterning on rigid surfaces. Thus, after the cell has attached, the experimenter can neither change the shape of the cell nor control the rate of cell spreading.

A better understanding of the pathway cell use to sense and respond to shape changes may lead to new treatments for various diseases. By artificially changing the levels of signaling molecules used by the cell to sense shape, it may be possible to trick cells to behave in a manner not consistent with its real shape. As such, spread cancer cells may respond to “rounded shape” signals by initiating apoptosis. Similarly, a “spread signal” may coax rounded quiescent cells to proliferate and thereby replacing their damaged neighbours. The relationship between a cell's shape and the cell's fate is an intriguing and relevant story. Recent micro fabrication technologies have provided new experimental approaches to the problem. .

Plant cells are almost universally surrounded by a rigid cell wall. This cell wall is generally a box-like structure and serves the function of an exoskeleton (made rigid by turgor pressure). In contrast, animal cells are not surrounded by a rigid cell wall, but rather by a flexible and rather variable extra- cellular matrix (which can range from a thin jelly-like coat to bone). This allows them to assume a much wider variety of shapes.

A protein discovered by scientists at the University of North Carolina at Chapel Hill appears to play a key role in determining the shape of cells and allowing them to move. The newly identified protein, palladin, is being explored for its influence on a number of biological processes including the invasive spread of cancer, wound healing, brain development, and the implantation of the embryo in the uterus.

Prof. Otey named the new protein after Andrea Palladio, the influential 16th century architect. Palladin appears to be very involved in the architecture of cells, specifically via the actin cytoskeleton, a polymer protein complex that provides much of the basis for cell shape. "Cells have a shape that is related to their function," Otey explains. "A good example of specialized cell shape is the neuron. They must be very long and skinny to allow the nervous system to function. Another example is epithelial cells (including skin cells) which bind tightly to one another to form a continuous sheet." According to Otey's findings, palladin belongs to a small group of cytoskeletal adhesion proteins that seem to provide molecular 'glue' for maintaining cellular shape and for the attachment of cells to one another via their plasma membranes. For example, fibroblasts are spindle-shaped cells involved in connective tissue, collagen formation and are also crucial to wound healing. In these cells, palladin is very concentrated near attachment points to the plasma membrane.

On the other hand, palladin is absent, not expressed, in some undifferentiated cells; that is, in cells which haven't achieved their genetically predetermined shape. Thus, the protein is absent in precursor stem cells. "So this indicates that palladin plays a role in forming the new cytoskeleton of cells that start differentiating and take on their specialized shape," she said.

According to Otey, an exciting thing about palladin is its presence in different forms with different molecular weights. In many different types of cells, one form of palladin may be necessary for tight adhesion and another for migration, or movement. A heavier form of

palladin is more highly present, or expressed, in metastatic cancer cells the tumor cells that spread beyond their point of origin. This form of palladin is highly expressed in the early placenta, which of course is an 'invasive' organ.

Further, it is during the first half of the ovulatory cycle that the womb prepares for embryo implantation by undergoing very dramatic changes in cell shape. A palladin form of greater molecular mass occurs during that time and then diminishes later in the cycle. Cell shape and integrity depend, at least partially, on the equilibrium of intra-cellular and extra-cellular mechanical forces applied to that cell. In contrast to a complete organism under near weightlessness conditions where e.g. bones and muscles are highly unloaded, gravity is a nearly insignificant force at the scale of a single cell. Past space flight and ground based cell biological studies in hypogravity or hypergravity environments showed changes in the cell behaviour (signal transduction) cell shape (cytoskeleton) or proliferation. From these studies it is not always clear whether these result emerged from direct or indirect effects or (micro-) gravity. No doubt physical forces play a critical role in cell integrity and development, but little is known how cells convert mechanical signals into biochemical responses. However, it is established that vinculin is presented as a mechanical coupling protein that contributes to the integrity of the cytoskeleton and cell shape control.

Cells

Three cell types, one each from plant animal and bacteria, are drawn in a more realistic manner than the schematic drawing in figure and the table give the comparative account of animal, plant and bacterial cells (Fig. 4). The animal cell drawing is based on a fibroblast, a cell that crawls through connective tissue, depositing extra-cellular matrix. The plant cell drawing is typical of a young leave cells, containing chloroplast and large fluid-filled vacuole. The bacterium is a rod shaped Bacillus with a single flagellum for motility.

Three basic categories of cells make up the mammalian body, such as germ cells, somatic cells and stem cells. The majority of these cells is diploid or has two copy of each chromosome. These cells are called somatic cells. This category of cells includes most of the cells that make up our body, such as skin and muscle cells.

Germ line cells are any line of cells that give rise to gametes, viz. eggs and sperm and continue through the generations.

An adult human body is composed of about 10^{14} cells which are of over 200 types, viz. the covering or lining cells, the epithelial cells, free floating cells, haemopoietic cells, with long appendages, the neurons, hard calcified, the bone cells, contractile protein containing, the muscle cells, germ cells and sensory cells for specialized functions. Stem cells on the other hand, have the ability to divide for indefinite period and to give rise to specialized cells. They are best describes the context of the normal human development.

A totipotent stem cell, the zygote gives rise to many cells on proliferation; these cells differentiate into various types of cells, some act as stem cell for that particular lineage where as majority of them become end cells or terminally differentiated cells. These terminally differentiated cells under go programmed cell death (PCD). To maintain homeostasis of the number of the cells in the organ, a new set of similar type of cells develop by proliferation.

Cell organelles

The human body contains many different [organs](#), such as the heart, lungs, and kidneys, with each organ performing a different function. Cells also have a set of "little organs", called [organelles](#) that are adapted and/or specialized for carrying out one or more vital functions. They are of two types:

1. Membrane-bound and
2. Non-membranous organelles.

Membrane-bound are found only in eukaryotes.

Plasma membrane

The outer boundary of the cell is the plasma membrane, a continuous sheet of the phospholipids molecules, about 4-5nm thick in which various proteins are embedded (Fig. 5A, B).

Some of these proteins serve as pumps and channels for transporting molecules into and out of the cells. The cell membrane protects the cell and gives it, its shape. It also controls what goes in and out the cell. All the nutrients and wastes need to go through the cell membrane to enter and exit the cell. The cell membrane is permeable to some substances, and impermeable for few substances. The phospholipid molecules are so close together that only very small molecules can pass through the membrane freely, such as water and oxygen. Other substances have to be carried by the proteins (receptors) to enter the cell.

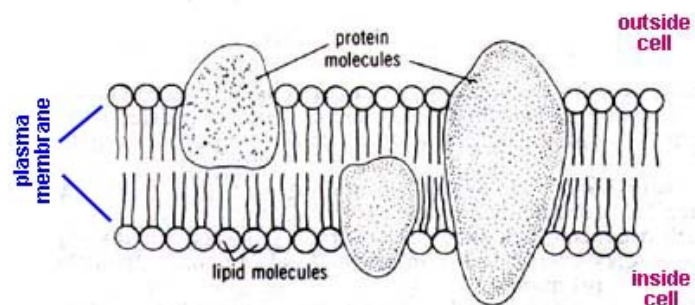


Fig. 5A: Plasma Membrane: Lipid layer intercepted with varying sizes of protein molecules (Schematic drawing)

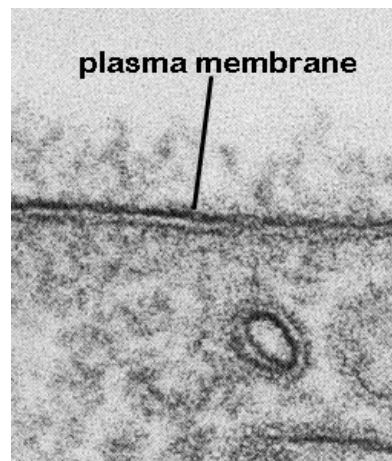


Fig. 5B: Electron Micrograph of Plasma Membrane

Table 2: A comparison between animal and plant cells in relation to their cell organelles

Cell organelles	Animal cell	Plant cell	Bacterial cell
Cell wall	-	+	-
Plasma Membrane	+	+	+
Cytoplasm-Nucleus	+	+	+
Chromatin	+	+	-
Nuclear envelop	+	+	-
DNA	+	+	+
Mitochondria	+	+	-
Golgi apparatus	+	+	-
Lysosomes	+	+	-
Peroxisomes	+	+	-
Ribosomes	+	+	+
Endoplasmic reticulum	+	+	-
Vesicles	+	+	-
Chloroplast	-	+	-
Microfillaments	+	+	-
Microtubules	+	+	-
Intermediatefillaments	+	-	-
Microsomes	-	-	+

Cytoplasm

The jelly-like substance inside the cell is called cytoplasm. Most of the living material is made in the cytoplasm. The cytoplasm forms the true internal milieu of the cells that has sub-cellular organelles suspended in it. The cytoplasmic fluid or cytosol (a biochemical term meaning, the cytoplasmic clear fluid free from all suspended major cell organelles) occupies nearly half of the total cell volume. The organelles inside the cell are like the organs inside the human body. It also consists of granular bodies such as, free ribosomes, endoplasmic reticulum with ribosomes attached to it, glycogen granules and fat droplets. In addition to suspended particles, the cytosol also contains soluble proteins, enzymes of glycolytic pathway and those required for amino acid activation during protein biosynthesis. Besides these, the cytosol also contains many other soluble enzymes, ions and metabolites. This jelly-like fluid is also responsible for the colloidal properties of the cell. Cytoplasm acts as a store room of the molecules for growing and the repairing structure inside the cells.

Endoplasmic reticulum

Flattened sheets, sacs and tubes of membrane extend throughout the cytoplasm of eukaryotic cells, enclosing a large intracellular space. The ER membrane is in structural continuity with the outer membrane of the nuclear envelope and it specializes in the synthesis and transport of lipids and membrane proteins. The rough endoplasmic reticulum (rough ER) generally occurs as flattened sheets and studded on its outer face with ribosomes engaged in protein synthesis. The smooth endoplasmic reticulum (smooth ER) is generally more tubular and lacks attached ribosomes.

It is involved in glycogenolysis and detoxification of many endogenous and exogenous compounds. Endoplasmic reticulum also serves certain special function, e.g. sequestration

of calcium in muscle cells and synthesis of steroid in the testes and other organs (Fig. 6 A, B).

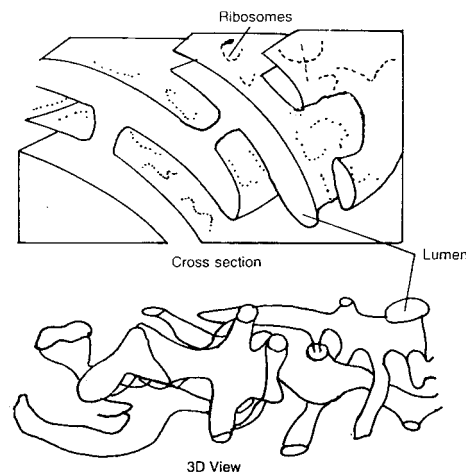


Fig. 6A: Endoplasmic reticulum (ER): Sectional and three-dimensional views. The membranes loaded with ribosomes are known as rough ER and devoid of ribosomes are called on smooth ER

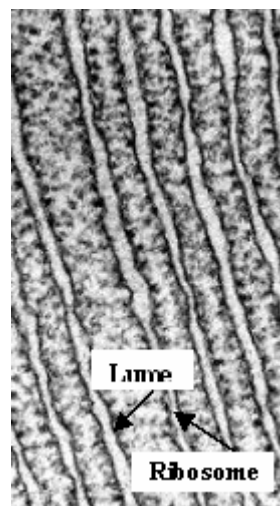


Fig. 6B: Electron micrograph of endoplasmic reticulum. The membranes loaded with ribosomes are known as rough ER and devoid of ribosomes called smooth ER.

Golgi apparatus

A system of stacked, membrane bound, flattened sacs involved in modifying, sorting and packaging secretory macromolecules or for delivers to other organelles are called Golgi bodies, which are made up of a stacked of 6-20 saucer-shapes membrane bags. Membranes bud from the ER that is arranged facing a central zone at one end of the Golgi complex (Fig. 7A, B). These buds become vesicles and are coated with COPII protein cores. The ER faces a central zone called a vesicular-tubular cluster (VTC). After they lose their COPII, they merge with the VTCs a carrying the soluble and membrane proteins to the Golgi complex. That's entire complex unique in the cytoplasm. It is termed as the export complex and contains unique proteins that suggest that is specialized for the information flow to and from

ER and the Golgi complex. Transport of material in and out of the Golgi complex involves budding and fusion of vesicles. The cartoon figure (Fig. 7A) shows that the membranes of each join and align themselves during the process so that the inside face remains in the lumen and the outside face remains towards the cytoplasm. The functional differentiation of the Golgi complex can be studied with the Electron Microscope with specific techniques that detects enzymes. The *cis* region is rich in lipid-bearing membranes and can be delineated by osmium tetroxide labeling. The middle regions label for enzymes that add carbohydrates or other groups on the product. The inner or *trans* region is the area where the lysosomes are sorted. Therefore, it is heavily labeled for acid phosphatase.

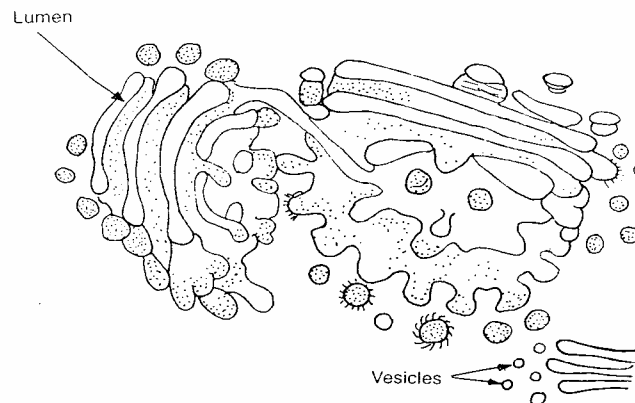


Fig. 7A: Golgi bodies: Line drawing

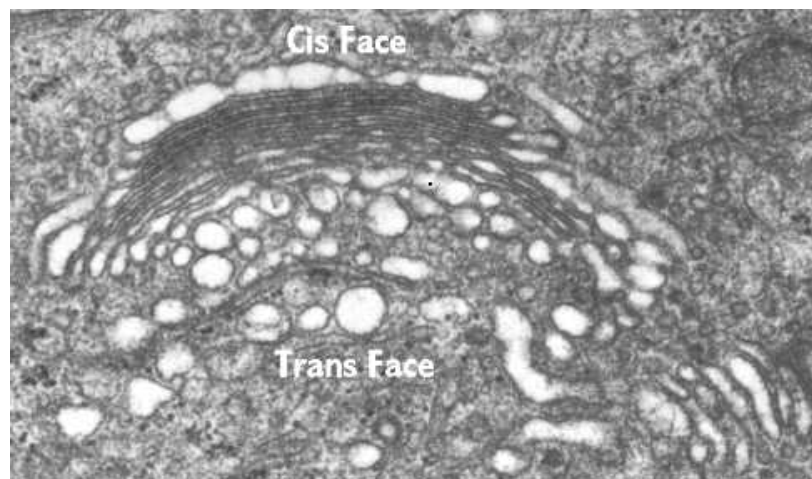


Fig. 7B: Electron micrograph of Golgi bodies showing *trans* and *cis* regions. Smooth membranes stacked always associated with membrane bound vesicles of varying sizes and shapes

What types of secretions are controlled by the Golgi complex?

The important role of the Golgi complex is to make certain the plasma membrane proteins reach their destination. It may be noted the orientation of the protein is maintained so that the region destined to project outside the cell (a receptor binding site, for example), ends up in that place. In order to do this it may be placed in such a way that it faces inside the vesicle.

How does the Golgi complex add carbohydrate groups to a glycoprotein?

The Golgi complex is compartmentalized. Phosphorylation occurs in the *cis* region. In other regions, different types of carbohydrates are added as a glycoprotein passes through the cisternae. This figure illustrates the different regions where sugars like mannose (man), galactose (gal), etc. are added. The final sorting is done in the *trans* Golgi complex.

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There is much interest in understanding how the different Golgi cisternae are organized and differentiated. A number of models exist, however a favorite is called the "Maturation model". This model suggests that the new vesicles from the ER enter the *cis* Golgi network and retrograde vesicles (bearing COPI) coats move to merge with the *cis* region cisternae. The cisternae carry Golgi complex processed enzymes; the action of these processed enzymes depends on their concentration in the *cis* region. Then, as processing continues, the middle cisternae contain more mature product and lower amounts of the enzymes needed in the beginning. Finally, the *trans* region is specialized for sorting, containing receptors to sort and isolate lysosomal enzymes.

Can proteins be transported back to the rough endoplasmic reticulum?

Sometimes vital proteins needed in the rough endoplasmic reticulum are transported along with the other proteins in the Golgi complex. The Golgi complex has a mechanism for trapping them and sending them back to the rough endoplasmic reticulum.

This cartoon shows the process. The protein destined for secretion is marked in red. The protein marked in blue must remain in the rough endoplasmic reticulum. The rough endoplasmic reticulum has inserted a receptor protein on the membrane it sends to the Golgi complex in the transitional vesicles (shown in green). These are retrograde vesicles and are therefore coated with "COPI" (coatamer). The ER protein receptor captures all of the protein that carries the ER residency signal; vesicles then bud from the Golgi complex and move back to the rough endoplasmic reticulum. The receptor can circulate and continue to return the proteins needed by the endoplasmic reticulum.

A drug called "brefeldin A" blocks the transfer of protein to the Golgi complex; however the reverse transport is not blocked.

Lysosomes

Membrane-bound vesicles that contain hydrolytic enzymes, mainly acid hydrolases (proteases, nucleases, gangliosidases lipases, phosphatases, phospholipases, sulfatases etc.) are involved in intra-cellular digestion. Lysosomes are the cells' garbage disposal system. They degrade the products of ingestion, such as the bacterium that has been taken in by

phagocytosis seen in the above cartoon. After the bacterium is enclosed in a vacuole, vesicles containing lysosomal enzymes (sometimes called primary lysosomes) fuse with it. The pH becomes more acidic and this activates the enzymes. The vacuole thus becomes a secondary lysosome and degrades the bacterium (Fig. 8A, B).

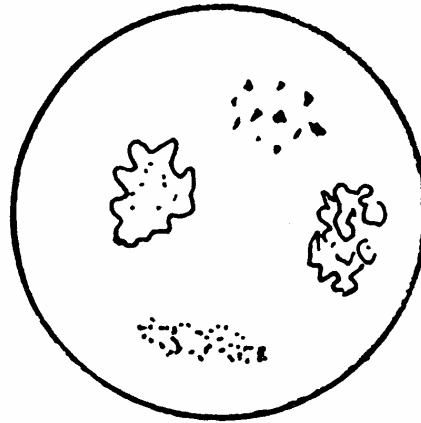


Fig. 8A: Lysosome line drawing



Fig. 8B: Electron micrograph of lysosome: Membrane bound vesicles containing enzymes, degraded cell organelles, bacteria or viruses

Lysosomes also degrade worn out organelles such as mitochondria. In this cartoon, a section of rough endoplasmic reticulum wraps itself around a mitochondrion and forms a vacuole. Then, vesicles carrying lysosomal enzymes fuse with the vesicle and the vacuole becomes an active secondary lysosome.

A third function for lysosomes is to handle the products of receptor-mediated endocytosis such as the receptor, ligand and associated membrane. In this case, the early coalescence of vesicles bringing in the receptor and ligand produces an endosome. Then, the introduction of lysosomal enzymes and the lower pH causes release, and degradation of the contents. This can be used for recycling of the receptor and other membrane components. Lysosomes carry hydrolases that degrade nucleotides, proteins, lipids, phospholipids, and also remove carbohydrate, sulfate, or phosphate groups from molecules. The hydrolases are active at an acid pH , which is fortunate because if they leak out of the lysosome, they are not likely to

do damage (at pH 7.2) unless the cell becomes acidic. A hydrogen ion ATPase is found in the membrane of lysosomes to acidify the environment.

Lysosomal morphology varies with the state of the cell and its degree of degradative activity. Lysosomes have pieces of membranes, vacuoles, granules and parts of mitochondria inside. Phagolysosomes may have parts of bacteria or the cell it has ingested. This electron micrograph shows typical secondary lysosomes. They have been detected by cytochemical labeling for acid phosphatase. This is a good marker for lysosomes. It may be recalled that it is also used as a marker for the *trans* Golgi cisternae.

How does the Golgi complex sort lysosomal enzymes?

The Golgi complex sorts the lysosomal enzyme in the *Trans* region. It is received from the rough endoplasmic reticulum (RER in this cartoon) in the *cis* region. There it has a phosphate radical attached to the mannose residue. This mannose-6 phosphate forms a sorting signal that moves through the cisternae to the *trans* region where it binds to a specific receptor. Then it begins to bud and a "cage" or "coat" made of clathrin forms around the bud (to strengthen it). It moves away to fuse with a developing lysosome, such as the vacuoles seen in the figure. This lysosome contains a hydrogen ion pump on its surface. The pump works to acidify the environment inside the lysosome. This removes the phosphate and dissociates the hydrolase from the receptor. The receptor is then recycled back to the Golgi complex.

Lysosomes can actually be detected by pH indicator dyes. This photograph shows dyes that indicate different pH's with different colours. The red lysosomes (pH 5.0) are probably typical lysosomes. The blue and green lysosomes are probably endosomes. This change can be detected if the ligand is linked to fluorescein. Fluorescein will not fluoresce at pH's lower than 6.0. Therefore, one can follow entry of the receptor-ligand complex and then see the fluorescence disappears as the endosome containing the complex is acidified

Peroxisomes

Membrane-bound vesicles containing oxidative enzymes that generate and destroy hydrogen peroxide.

Nucleus

About 3-4µm in diameter, the nucleus is the most conspicuous organelle in the eukaryotic cell. It is separated from the cytoplasm by a nuclear envelop, made by differentiation of the rough endoplasmic reticulum, consisting of double membrane. It is perforated at intervals by nuclear pores. The nucleus communicates with the cytosol by these pores which contain many different types of proteins that regulate the passage of ions and macromolecules-mRNA, ribosomal subunits etc. in and out of the nucleus (Fig. 9A, B). The chromosomal DNA is packaged into chromatin fibers by an equal mass of histone proteins. However, only 20% DNA has all the information in the form of genes to regulate body functions, the rest of it are junk DNA (Fig. 10). Nucleolus is a factory in the nucleus where the ribosomes are assembled.

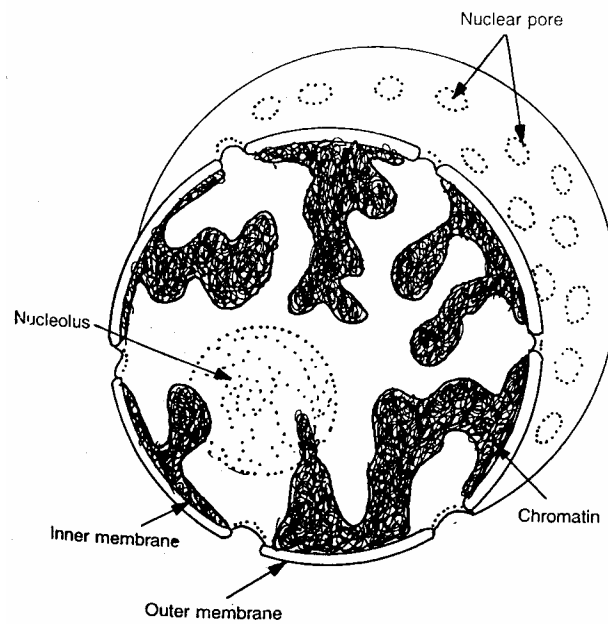


Fig. 9A: 3D sketch of animal cell nucleus.

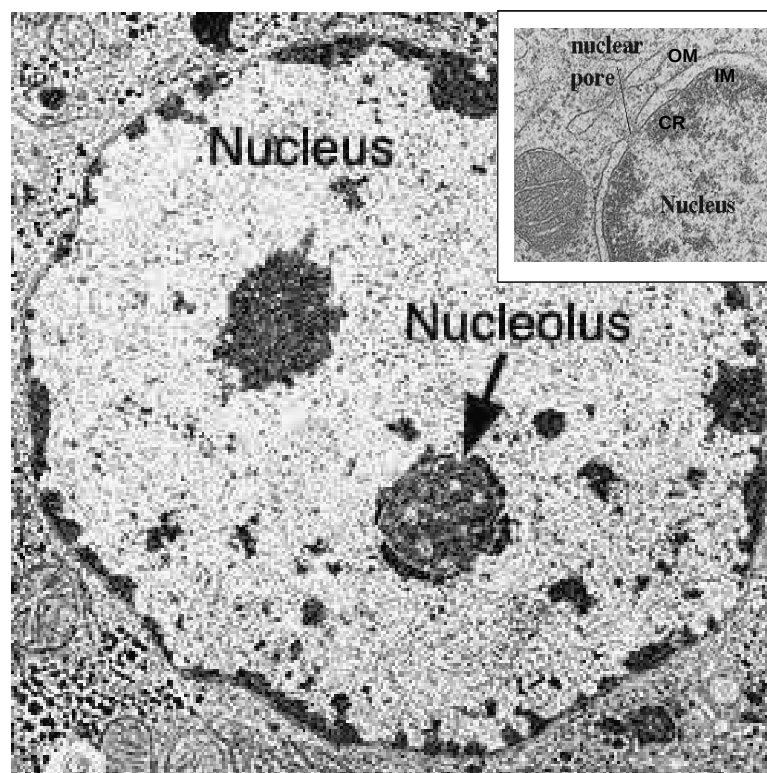


Fig. 9B: 2D electron micrograph of animal cell nucleus. Inset shows outer (OM) and Inner (IM) nuclear membranes, the nuclear pore and chromatin (CR). The nucleus bound by double membranes, perforated by nuclear pores. Chromatin and nucleolus are embedded in nucleoplasm.

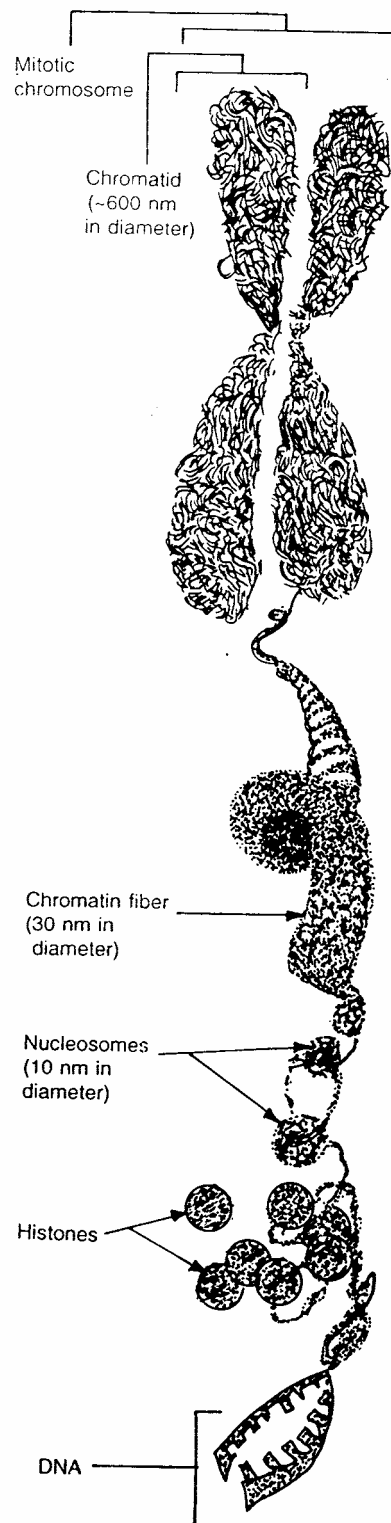


Fig. 10: Packing of DNA in a chromosome. Long DNA thread is coiled on histone protein called nucleosome.

Cytoskeleton

In the cytosol, arrays of protein filaments form networks that give the cell its shape and provide a basis for its movement. In animal cells the cytoskeleton is often organized from an area near the nucleus that contains the pair of centrioles.

Three main kinds of cytoskeletal filaments are:

- (1) *Microtubules*: These are hollow, thin and rigid tubular structures, long and about 30nm in diameter. During cell division, microtubules play an important role by forming the mitotic spindle, which distributes chromosomes equally between the two daughter cells.
- (2) *Microfilaments*: These are rod like structures about 5-10 nm in diameter and form part of the contractile machinery in both muscle and non-muscle cells. These are composed mainly of the protein actin and may be associated with myosin or other proteins required for contraction. Microfilaments are associated with non-muscle cell and helps in cell motility in form of amoeboid movements.
- (3) *Intermediate filaments*: These are long fibers, straight or slightly bent, which provide the mechanical strength and reinforcement.

Cytoskeleton is a cell's scaffold and an important, complex, and dynamic cell component. It acts to organize and maintain the cell's shape; anchors organelles in place; helps during endocytosis, the uptake of external materials by a cell; and moves parts of the cell in processes of growth and motility. There are great numbers of proteins associated with the cytoskeleton, each controlling a cell's structure by directing, bundling, and aligning filaments.

Over more than three decades, sporadic and circumstantial evidence has accumulated to suggest that nuclear actin has crucial functions in RNA polymerase II-based transcription. Now, using a biochemical approach, actin has been identified as a highly specific, constitutive component of the active transcriptional complex required for formation of the pre-initiation transcription complex (Fig. 11).

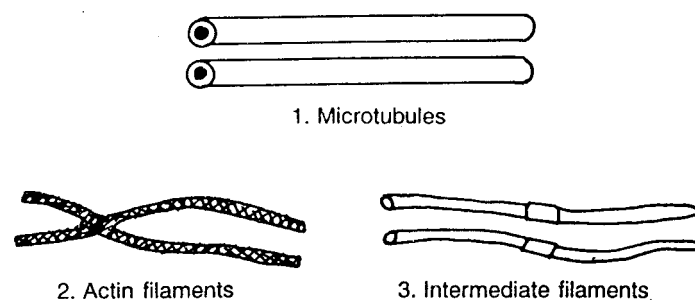


Fig. 11: Cytoskeleton filament in a cell

Mitochondria

Mitochondria are dynamic structures, which may grow in length, branch, divide and coalesce. Each mitochondrion consists of a smooth outer membrane, an inner membrane space and a highly structured inner membrane. The inner membrane is convoluted into elongated folds called cristae and has numerous knob-like projections extending into the inner-most compartment, the matrix of the mitochondria (Fig. 12 A, B). The advantage of such a structure is tremendous increase in the surface area. The matrix contains RNA and DNA, which is similar to that of the bacteria (circular in nature), and suspended granules,

fibres and crystals. The presence of genetic material in mitochondria (extra chromosomal DNA) indicates that they may have evolved from the entry of small prokaryotes into larger eukaryotes. Mitochondria are the power plants of all eukaryotic cells, harnessing energy obtained by combining oxygen with food molecules to make ATP. It is store house for Ca^{+2} . The inner membranous space and the matrix contains enzymes of the citric acid cyclic, certain accessory enzymes pyruvate dehydrogenase complex, pyruvate carboxylase, L-glutamate dehydrogenase and certain transaminases which link carbohydrate and protein metabolism to the citric acid cyclic, and the fatty acid β -oxidation enzyme sequence.

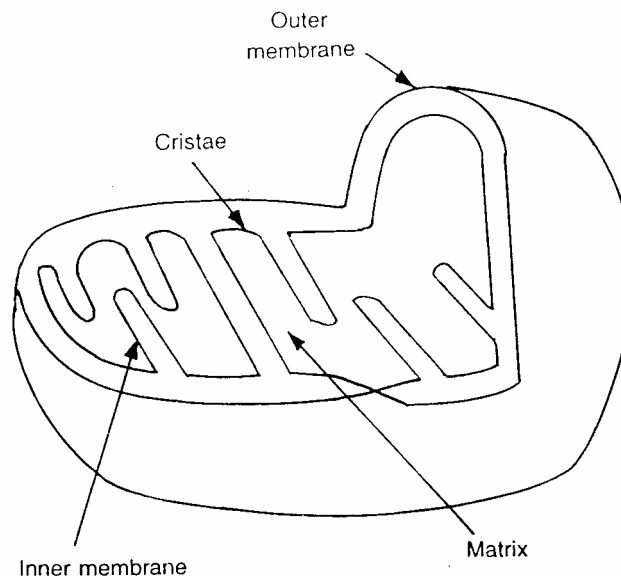


Fig. 12A: 3D stucture of mitochondrion

If the inner membrane is so permeable, how do proteins enter?

The outer membrane of the mitochondria contains the protein "porin". This forms an aqueous channel through which proteins up to 10,000 daltons can pass and go into the inter membrane space. Indeed, the small molecules actually equilibrate between the outer membrane and the cytosol. However, most proteins cannot get into the matrix unless they pass through the inner membrane. This membrane contains cardiolipin, which renders it virtually impermeable. Transport across the mitochondrial membranes requires the concerted action of a number of translocation machineries. The machinery in the outer membrane is called the Tom complex (Translocator outer membrane) and that for the inner membrane is called the Tim complex (Trans locator inner membrane). Proteins that have to go all the way to the matrix have an NH_2 cleavable signal sequence.

Most proteins must be uncoiled or stretched out to go through the translocators. This involves ATP binding and is monitored and stabilized by chaperone proteins, including hsp70. Thus, before the protein can go through Tom complex, it must become "translocation competent" (Fig. 12 A, B).



Fig. 12B: Electron micrograph of mitochondrion showing all the structures marked in 12A and dark Ca granules in the mitochondrial matrix

Centrosome and the centrioles

Boveri was the first to coin the terms 'centrosome' and 'centriole', and as early as 1887 he determined that inheritance and heritable elements were intimately connected with chromosomes. These studies were carried out jointly with Edouard van Beneden in the horse nematode *Ascaris megalocephala*.

The centrosome, also called the "microtubule organizing center", is an area in the cell where microtubules are produced. Within an animal cell centrosome there is a pair of small organelles, the centrioles, each made up of a ring of nine groups of microtubules. There are three fused microtubules in each group. The two centrioles are arranged such that one is perpendicular to the other.

During animal cell division, the centrosome divides and the centrioles replicate (make new copies). The result is two centrosomes, each with its own pair of centrioles. The two centrosomes move to opposite ends of the nucleus, and from each centrosome, microtubules grow into a "spindle" which is responsible for separating replicated chromosomes into the two daughter cells.

Plant cells have centrosomes that function much like animal cell centrosomes. However, unlike centrosomes in animal cells, they do not have centrioles. An interesting observation about centrioles is that there are none in the cells of higher plants, even though these plants perform mitosis and meiosis just fine. The explanation for this turns out to be pretty simple. One of the important functions that centrioles perform is the generation of cilia and flagella for cells. These are surface features that cells use for movement. The cells of higher plants have no centrioles because there's no cell anywhere throughout their life cycles, which makes cilia or flagella. A cell can make a spindle without centrioles, but it can't make cilia or flagella.

Special plant cell organelles

Cell wall Plant cells are surrounded by a rigid wall called cell wall is composed of tough fibrils of cellulose laid down in a matrix of other polysaccharides.

Chloroplasts In contrast to animal and fungal cells, green plant cells contain one or multiple chloroplasts, the organelle(s) in which photosynthetic reactions take place. These chlorophyll containing plastids are double membrane bound organelles found in all higher plants. An elaborate membrane system in the interior of the chloroplast contains the photosynthetic apparatus. Chloroplasts are believed to have originated from an endosymbiotic event and contain DNA that codes for some of their proteins. Most chloroplast proteins are encoded by the nuclear genome and imported with the help of sorting signals that are intrinsic parts of the polypeptides. Here, it is shown that a chloroplast-located protein in higher plants takes an alternative route through the secretory pathway, and becomes N-glycosylated before entering the chloroplast (Fig. 13).

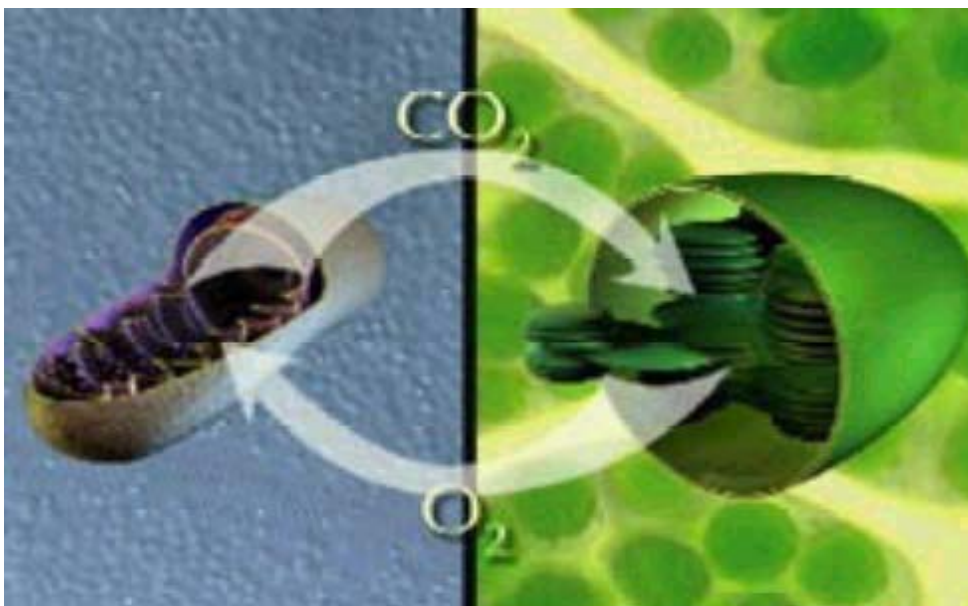


Fig. 13: Physiological and structural comparison between mitochondria and chloroplast

Vacuole It is large single membrane bounded vesicle occupying up to 90% of the cell volume, the vacuole functions in space-filled and also in intracellular digestion.

Mesosome They are membraneous whorls extended from the plasma membrane in the cytoplasm. They show the presence of respiratory chain enzymes. They are found only in bacteria and some fungi.

Types of cells

Epithelial cells

These cells are used in lining the inner and outer surfaces of the body and internal organs. There are many specialized types of epithelial cells (Fig. 14).

1. **Absorptive epithelial cells:** These cells have numerous hair-like projections called microvilli on their free surfaces to increase the area for absorption. Adjacent epithelial cells are bound together by cell junctions that give mechanical strength to the sheath and also make it impermeable to small molecules. The epithelial

sheet rests on a basal lamina- the basement membrane. These cells are present in the small intestine.

Table 3: Comparison between animal and plant cells in relation to their cell organelles

Typical Animal cell	Typical Plant cell
Organelles	
Plasma membrane	Plasma membrane
Nucleus	Nucleus
Nucleolus	Nucleolus
Rough Endoplasmic reticulum (ER)	Rough ER
Smooth ER	Smooth ER
Ribosomes	Ribosomes
Cytoskeleton	Cytoskeleton
Golgi apparatus	Golgi apparatus
Cytoplasm	Cytoplasm
Mitochondria	Mitochondria
Vesicles	Vesicles
Vacuoles	Vacuoles
Lysosomes	Peroxisome, Glyoxysome
Centrioles	Centrioles
Additional Structures	
Cilium	Flagellum (only in gametes) Cell wall Plasmodesmata Chloroplast and plastid Tonoplast
Flagellum	

2. *Ciliated cells*: These cells have cilia on their free surface that beat in synchrony to move substances (such as mucus) over the epithelial sheet. These cells are present in the lining of fallopian tubes and trachea.
3. *Secretory cells*: These cells are found in most epithelial layers. These specialized cells secrete substances on to the surface of the cell sheet. Secretory epithelial cells are often collected together to form a gland that specializes in the secretion of a particular substance. As illustrated, exocrine glands secrete their products such as tears, mucus, and gastric juices into ducts. Endocrine glands (ductless glands) secrete hormones directly into the blood.
4. *Germinative cell*: These are special types of cells, which divide mitotically to begin with, subsequently undergo reduction division (meiosis) to form haploid germ cell. A sperm from the male fuses with an egg from the female forms a new diploid zygote, which then forms an organism by successive divisions.
5. *Sensory epithelial cells*: These are highly specialized cells dedicated to sensory function such as smell. Among the most strikingly specialized cells in the human

body are those that detect external stimuli. Hair cells of the inner ear are primary detectors of sound. They are modified epithelial cells that carry special microvilli on their surface. The movement of these in response to sound vibrations causes an electrical signal to pass to the brain.

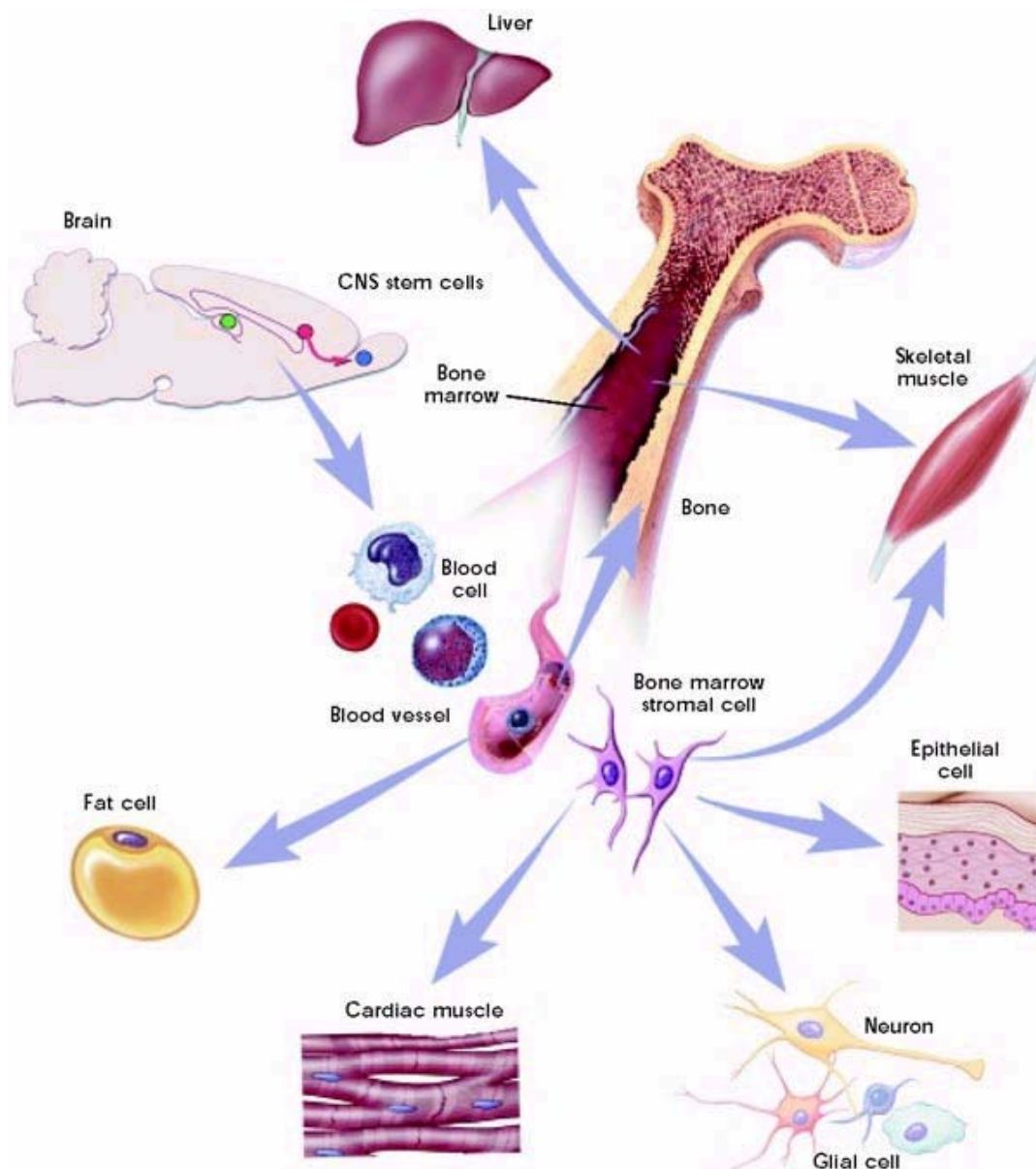


Fig. 14: Types of cells in adult body

Connective tissue cells

The groups of similar cells put together are known as 'Tissues'. The space between organs and tissues in the body are filled with connective tissues made up of principally of a network of tough protein fibres embedded in a glycoprotein gel. This extra cellular matrix is secreted mainly by fibroblasts. Two main types of extra-cellular protein fibres are present in the body tissues, collagen and elastin. Bone is made up of the cells called the osteoblasts. The osteoblasts secrete an extra-cellular matrix in which crystals of calcium phosphate are later deposited.

Fat cells (or adipose cell): These *cells* are among the largest cells in the body and are responsible for the production and storage of fat. In these cells large lipid droplet squeezes nucleus and cytoplasm.

Muscle cells: Muscle cells produce mechanical force by their contractions. In vertebrates there are three main types of muscles;

Skeletal muscles: This moves joints by its strong and rapid contractions. Each muscle is a bundle of muscle fiber, each of which is multinucleated cell.

Smooth muscles: It is present in digestive tract, bladder, arteries and veins. It is composed of thin elongated cells (not striated), each of which has single nucleus.

Cardiac muscle: It is an intermediate in character between skeletal and smooth muscles by its constrictions and relaxations and produce heart beat. Adjacent cells are linked by electrically conducting junctions that cause the cells to contract in synchrony.

Nerve cells

Nerve cells or neurons are specialized for communication. The brain and spinal cord for example, are composed of a network of neurons with supporting glial cell. The axon conducts electrical signals away from the cell body. These signals are produced by a flux of ions across the nerve cell plasma membrane. Specialized cells wrap around an axon to form a multilayered membrane sheath called myelin sheath.

A synapse is specialized junction between the two neurons (or with a muscle cell). At synapse, signals pass from one neuron to another through neurotransmitters (e.g. acetylcholine).

Blood cells

The fluid plasma contains many types of cells, the red colour of the blood is due to erythrocytes (red blood cells, RBC), which are very small cells, and in mammals these cells have no nucleus or intra-cytoplasmic membranous structures. When mature, they are full of the oxygen-binding protein haemoglobin. The leucocytes (white blood cells, WBC) protect against infections. Blood contains about one WBC for every 100 RBCs. Although leucocytes travel in the circulation, they can pass through the walls of the blood vessels to do their work in the surrounding tissues. There are several different kinds of WBCs including the various types of lymphocytes (T, B, Helper and Killer cells) responsible for immune responses such as the production of antibodies. The macrophages and neutrophils also belong to the WBC category, move to sites of infection where they ingest bacteria and cell debris. The platelets or thrombocytes help in the clotting the blood.

Differentiation of cells

Undifferentiated cell, zygote is capable of differentiating about 200 different types of cells, found in the human body. They are totipotent. In adults undifferentiated cells found among differentiated cells in a tissue or organ, can renew themselves and can differentiate to yield the major specialized cell types of the tissue or organ. They are not totipotent but they are

pluripotent. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. They are also known as somatic stem cells instead of adult stem cell. Unlike embryonic stem cells, which are defined by their origin (the inner cell mass of the blastocyst), the origin of the adult stem cells in mature tissues is unknown. Criteria for identifying and testing the adult stem cells are yet not unified universally. However, the following three methods are used (Figs. 15 and 16).

1. Labeling the cells in a living tissue with molecular markers and then determining the specialized cell types they generate;
2. Removing the cells from a living animal, labeling them in cell culture, and transplanting them back into another animal to determine whether the cells repopulate their tissue of origin.
3. Isolating the cells, growing them in cell culture, and manipulating them, often by adding growth factors or introducing new genes to determine what differentiated cells types they can become.

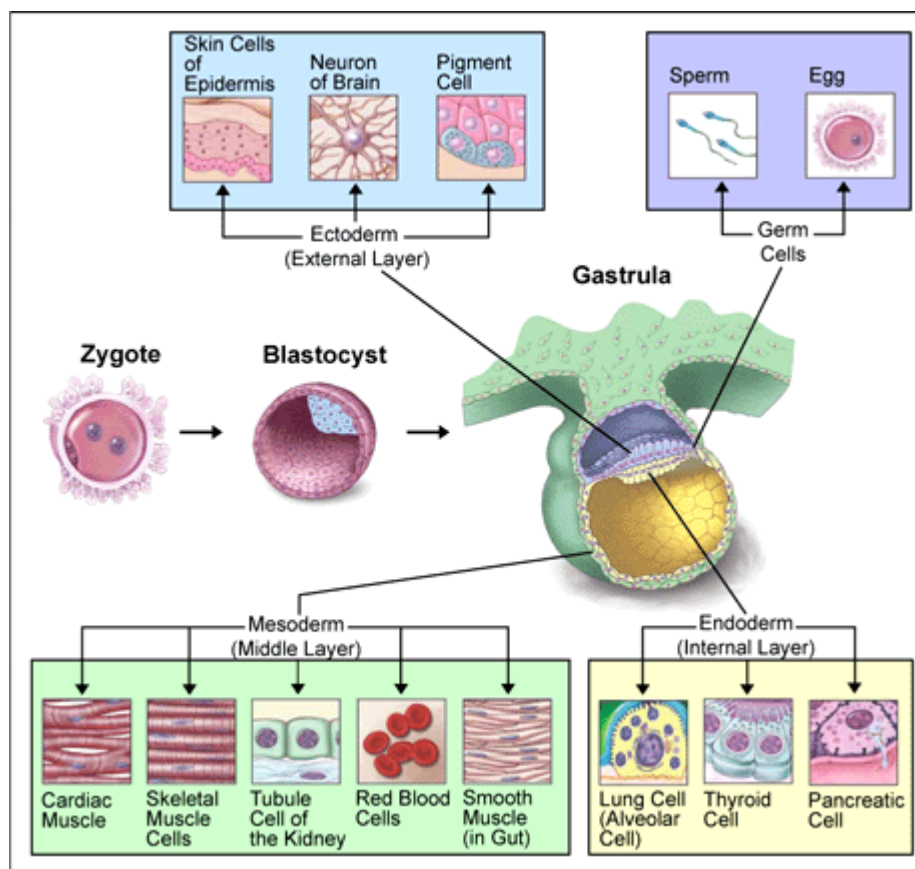


Fig. 15: Differentiation of various types of cells from zygote, the totipotent cell

Research on adult stem cells has recently generated a great deal of excitement. Scientists have found adult stem cells in many more tissues than they once thought possible. This finding has led adult blood forming stem cells from bone marrow have been used in transplants for 30 years. In the 1960s, researchers discovered that the bone marrow contains at least two kinds of stem cells. One population, called hematopoietic stem cells, forms all the types of blood cells in the body. A second population, called bone marrow cells, was discovered a few years later. Stromal cells are a mixed cell population that generates bone, cartilage, fat and fibrous connective tissue. Certain kinds of adult stem cells seem to have the ability to differentiate into a number of different cell types, given the right conditions. [f

this differentiation of adult stem cells can be controlled in the laboratory, these cells may become the basis of therapies for many serious common diseases.

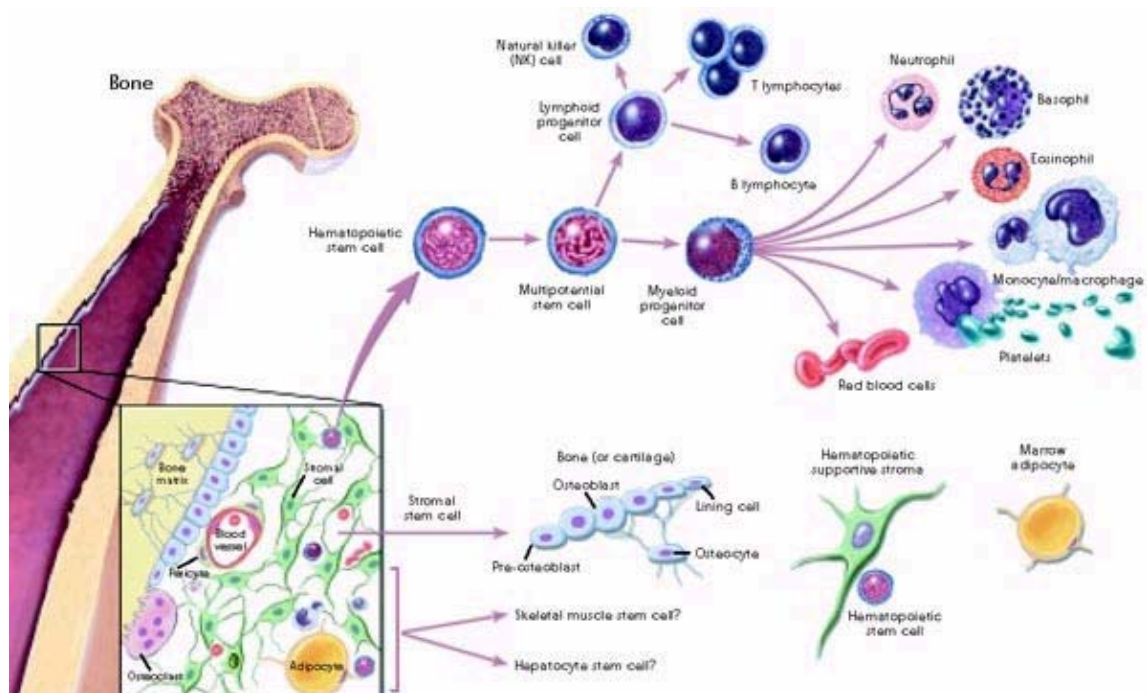


Fig. 16: Differentiation of various types of cells from adult stem cell

Also in the 1960s, scientists who were studying rats discovered two regions of the brain that contained dividing cells, which become nerve cells. Despite these reports most scientists believed that new nerve cells could not be generated in the adult brain. It was not until the 1990s that scientists agreed that the adult brain does contain stem cells that are able to generate the brain's three major cell types-astrocytes and oligodendrocytes, which are non neuronal cells and neurones or nerve cells.

Adult stem cells have been identified in many organs and tissues. Stem cells are thought to reside in a specific area or each tissue where they may remain quiescent (non-dividing) for many years until they are activated by disease or tissue injury. One important point to understand about adult stem cells is that there are a very small number of stem cells in each tissue. The adult tissues reported to contain stem cells include brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin and liver.

Now it is easy to grow adult stem cells in cell culture and manipulate them to generate specific cell types so that they can be used to treat injury or disease. Some examples of potential treatments include replacing the dopamine-producing cells in the brains of Parkinson's patients, developing insulin-producing cells for type I diabetes and repairing damaged heart muscle following a heart attack with cardiac muscle cells.

Also, a single adult stem cell should be able to generate a line of genetically identical cells known as a clone which then gives rise to all the appropriate differentiated cell types of the tissue. Scientists tend to show that either stem cell can give rise to a clone of cells in cell culture, or a purified population of candidate stem cells can repopulate the tissue after transplant into an animal. Recently, by infecting adult stem cells with a virus that gives a unique identifier to each individual cell, scientists have been able to demonstrate that

individual adult stem cell clones have the ability to repopulate injured tissues in a living animal. Adult stem cells may also exhibit the ability to form specialized cell types of other tissues, which is known as transdifferentiation or plasticity.

In a living animal, adult stem cells can divide for a long period and can give rise to mature cell types that have characteristic shapes and specialized structures and functions of a particular tissue. The following are examples of differentiation pathways of adult stem cells.

1. Hematopoietic stem cells give rise to all the types of blood cells namely, red cells, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, macrophages, and platelets.
2. Bone marrow stromal cells (mesenchymal stem cells) give rise to a variety of cell types namely, bone cells (osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes), and other kinds of connective tissue cells such as those in tendons.
3. Neural stem cells in the brain give rise to its three major cell types namely nerve cells (neurons) and two categories of non-neuronal cells-astrocytes and oligodendrocytes.
4. Epithelial stem cells in the lining of the digestive tract occur in deep crypts and give rise to several cell types: absorptive cells, goblet cells, paneth cells, and enteroendocrine cells.
5. Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles. The epidermal stem cells give rise to keratinocytes, which migrate to the surface of the skin and form a protective layer. The follicular stem cells can give rise to both the hair follicle and the epidermis.

A number of experiments have suggested that certain adult stem cell types are pluripotent. This ability to differentiate into multiple cell types is called plasticity or transdifferentiation. Following are the examples of adult stem cell plasticity that have been reported during the past few years:

- (i) Hematopoietic stem cells may differentiate into three major types of brain cells (neurons, oligodendrocytes and astrocytes); skeletal muscle cells; cardiac muscle cells and liver cells.
- (ii) Bone marrow stromal cells may differentiate into cardiac muscle cells and skeletal muscle cells.
- (iii) Brain stem cells may differentiate into blood and skeletal muscle cells.

Current research is aimed at determining the mechanisms that underlie adult stem cell plasticity. If such mechanisms can be identified and controlled, existing stem cells from a healthy tissue might be induced to repopulate and repair a diseased tissue.

Cell division

Mitosis

How does one cell become two? In the early 1950s, the idea that chromosomes are made of DNA was generally agreed on. DNA occurs in well-marked units characteristic of the strain or species. There is a duplication of this number in mitosis and a reduction in meiosis (Fig. 17).

Centrosomes: These are the main microtubule organizing centers of the cell. These structures make sure that, upon division, each cell receives an equal number of chromosomes. This double-helical base-paired structure explained how DNA replication

could occur so precisely? The centrosomes duplicate once per cell cycle, separate and then nucleate the two end points of the mitotic spindle. The function of the microtubule spindle during mitosis is to distribute replicated DNA equally between daughter cells. During mitosis, sister chromatids separate and are reeled into centrosomes at opposite poles of the spindle. The 'bait' that captures them is the kinetochore, a proteinaceous structure that binds both microtubules and centromeric DNA. The kinetochore is a multilayered structure, and can mediate microtubule-DNA interactions. Kinetochores move linked sister chromatids back and forth along the spindle during metaphase and separate sisters to the centrosomes during anaphase. Chromosome movement might be driven by several molecular machines with quite different mechanisms, and that an ordered assembly of kinetochore components might exist.

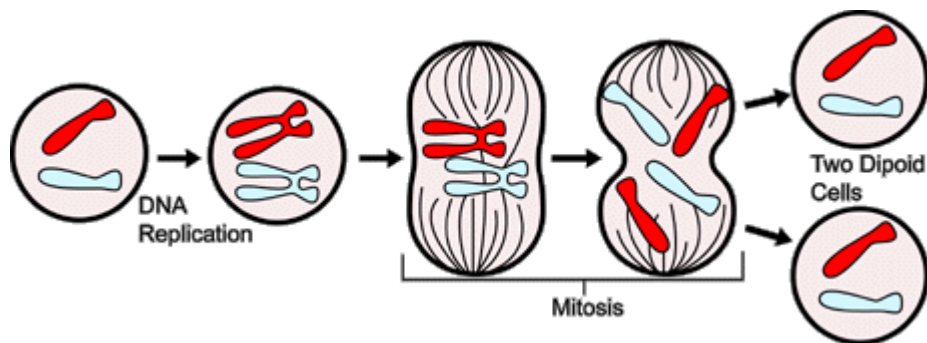


Fig. 17: Mitosis

The cell is thought to use two types of regulators to ensure that this crucial duplication takes place: first, cell-cycle factors, which regulate when centrosome duplication takes place; and second, intrinsic factors, which ensure only one round of duplication occurs per cell cycle. During the cell cycle, a cell goes through a series of sequential events - one step has to be completed before the next is initiated.

In general, cell status can be the process of division or in interphase. During interphase, the cell grows, performs its metabolic function, and replicates its chromosomes in preparation for another division.

These can be subdivided as discussed below:

The division phase is named M (mitotic) phase and include mitosis (nuclear division) and cytokinesis (cell division). The interphase is divided into G1 (*gap 1*) phase in which daughter cells grow and function. The cells are $2n$ and $2c$ since they are diploid and have 2 copies of each of the chromosomes.

Synthesis of DNA replication occurs, making duplicate copies of each of the chromosomes in preparation for cell division (each chromosome consists of 2 sister chromatids which are tightly bound to each other). The cells are $2n$ and $4c$ are diploid and have 4 copies of each chromosome (synthesis). G2 (*gap 2*) phase the cells may grow more at this point. At the end of G2, chromosomes begin to condense in preparation for cell division.

And how is two reduced back to a pair of ones?

The answer to this question is especially important during cell division, when the genome and the centrosome must be precisely duplicated to ensure cells progress correctly through mitosis.

During mitosis, the replicated chromosomes condense, are aligned between the two poles of the cell, then sister chromatids are split and each chromatid is pulled to the opposite poles.

Prophase

The chromosomes condense; revealing sister chromatids attached at the centromere, the nucleolus dissipates the centrosomes form outside of the nucleus.

In animals, each centrosome has a pair of centrioles the cytoskeleton in the cytoplasm disassembles and the microtubules reassemble, starting at the centrosomes, to form the mitotic spindles the centrosomes begin to migrate to opposite poles of the cell.

Prometaphase

Nuclear envelope (membranes) and the nuclear lamina (made up of intermediate filaments) break down.

The spindle microtubules extend to chromosomes and bind to the centromere in a junction called the kinetochore. Centrosomes are now at the opposite poles.

Metaphase

Three types of microtubules (MTs) exist in the mitotic spindle viz. Kinetochore MTs, interpolar MTs, and aster MTs. These three align the chromosomes across the metaphase plate, perpendicular to the spindle, tugging them equally to each pole.

Anaphase

The two sister chromatids separate and are pulled by the kinetochore MTs towards the poles (anaphase A), then the interpolar MTs push the poles apart and the aster MTs pull the poles towards the cell cortex (anaphase B).

This pulling and pushing is accomplished by motor proteins associated with the microtubules: dynein-type motor proteins would pull the kinetochore towards the spindle pole while the kinetochore MTs get shorter; dynein-type motor proteins would also pull the spindle poles towards the cell cortex as the aster MTs get shorter; kinesin-type motor proteins would slide the interpolar MTs opposite each other as the polar MTs get longer. Each pole has a daughter chromosome from each replicated chromosome.

Telophase

The spindle dissociates, the nuclear envelope and nuclear lamina begin to reform, the nucleolus reforms, the chromosomes begin to uncoil.

Cytokinesis (in animals)

A cleavage furrow (puckering of the plasma membrane) forms perpendicular to the orientation of the mitotic spindles.

A belt of actin and myosin microfilaments contracts (similar to muscle contraction) to cleave the cytoplasm in half (usually in half, sometimes the two daughter cells are of different sizes).

Cytokinesis (in plants)

A new cell wall is deposited between the two poles centered at the phragmoplast vesicles from the Golgi carry polysaccharides and cell wall proteins to the phragmoplast in the process of building the new cell wall.

Meiosis

At the beginning of the twenty-first century, we view cell division as a process chiefly concerned with splitting cells, so that the resulting daughters have a complete copy of their mother cell's genetic material in the form of chromosomes (Fig. 18).

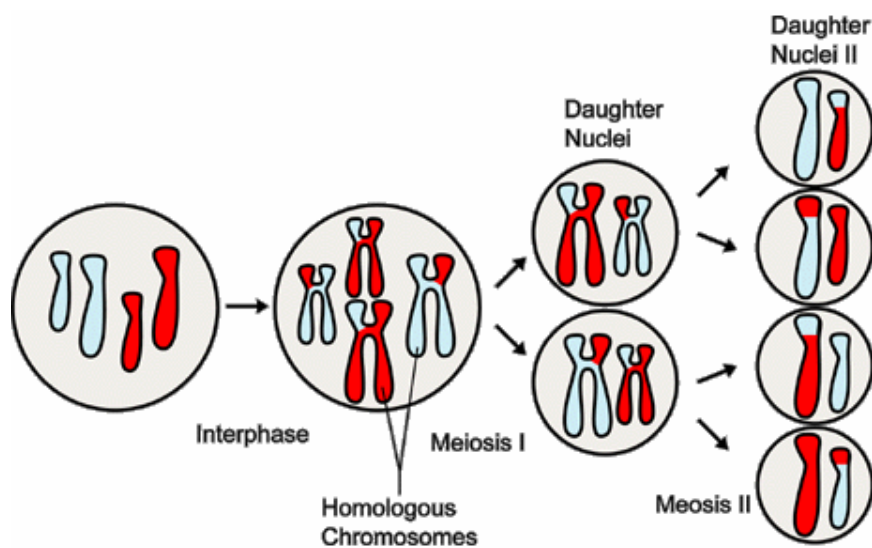


Fig. 18: Meiosis

This central importance of the chromosomes was established at the beginning of the previous century by the German cell biologist, Theodor Boveri (1862- 1915) and Edouard van Beneden in the horse nematode *Ascaris megalocephala* observed that maturing eggs lose half their nuclear material through cell division, effectively presaging the discovery of meiosis. Boveri hypothesized that each cell needed a full set of chromosomes for normal development. If any chromosomes were missing, the cell would lack 'developmental potential', but duplication of chromosomes would have relatively minor effects, for the products of cell division to develop normally, they must receive from their progenitor a full set of the structures that determine inheritance - the chromosomes. With these elegant experiments Boveri set the agenda for cell division research for the century to come.

In the process of cell division of special cells in plants and animals (germ cells) when diploid cell becomes haploid the process is called meiosis. The gametes thus formed contain only one chromosome representative of each chromosome pair. When two gametes fuse together during fertilization, then the diploid state is restored.

Meiosis is completed in two steps meiosis I and meiosis II.

Meiosis I

Prophase I

Similar events as mitotic prophase, such as condensing of the chromosomes, appearance of centrosomes, which migrate to poles, and disassembly of the nuclear envelop. The difference from the mitotic prophase is that the homologous chromosomes (each with 2 sister chromatids) align next to one another to form bivalents (four sister chromatids, 2 from each homologous chromosome). Homologous chromosomes are held together by the synaptonemal complex. When the homologous chromosomes are aligned together, crossing over (recombination) may occur between non-sister chromatids i.e. exchange of portions of each chromatid.

Metaphase I

The homologous chromosomes are attached to spindle microtubules at the kinetochores, with the 2 kinetochores from a single centromere on a chromosome facing toward one of poles (which one is random), the bivalents align along the metaphase plat.

Anaphase I

The chromosomes separate and are pulled to the poles by the spindle microtubules. The difference from mitosis may be noted. Herein homologous chromosomes are separated from one another, each chromosome, with two sister chromatids, goes to one of the poles.

Telophase I and cytokinesis

The nuclear envelope reforms and the cells divide, essentially as in mitosis except that the daughter cells are now haploid.

Meiosis II

Prophase II

Essentially the same as mitotic prophase except there are half as many chromosomes (there is only one chromosome from each of the pairs).

Metaphase II

The chromosomes align at the metaphase plate and spindle microtubules attach to the kinetochores of each sister chromatid, facing opposite poles.

Anaphase II

The sister chromatids are separated and pulled to the poles.

Telophase II and cytokinesis

The nuclear envelope reforms and the cells divide each cell has a daughter chromosome from only one of the chromosomes in each pair (the cells are haploid). There are points in these phases when a cell will stop and "decide" whether to continue on through the cell cycle.

There are two check points: (i) at the end of G1 (controls the start of chromosome replication) and (ii) at the end of G2 (controls the start of mitosis). The cell cycle can come to a halt at these points (and not finish cell division). The "decision" to proceed can be determined by cell growth (linked to nutrition) environmental factors, and proper replication of DNA.

"Molecular triggers" measure the passage through the cell cycle and control progress through the checkpoints. These triggers are kinases that require an additional protein subunit (cyclins). The cyclin protein accumulates through interphase and is degraded during mitosis (M Phase). The cyclin dependent kinase (Cdk) associates with cyclin to form a complex, the cyclin-Cdk complex become activated by phosphorylation and dephosphorylation of the Cdk at the end of G2. The active cyclin-Cdk complex is called M_{phase}-promoting factor (MPF) and triggers the cell to proceed into M phase.

Cytoplasm from a dividing cell (like a frog oocyte) can be injected into a cell in G1 and MPF will stimulate mitosis. There are actually different Cdks and cyclins active at different points in the Cell cycle. There is a G1 cyclin-Cdk complex that acts early in G1 phase and helps to activate the S phase cyclin-Cdk complex, especially in response to growth factors. There is a S phase cyclin-Cdk complex that triggers the cells to enter S phase. There is a mitotic cyclin-Cdk complex that triggers the cells to enter M phase.

Meselson and Stahl summarized their results and pointed out that their results were "in exact accord with the expectations of the Watson-Crick model for DNA duplication" with the following caveat: 'it must be emphasized that it has not been shown that the molecular subunits found in the present experiment are single polynucleotide chains or even that the DNA molecules studied here correspond to single DNA molecules proposed by Watson and Crick'.

Cells must be protected from a barrage of insults in order to survive. Every day they encounter chemicals and ultraviolet rays that damage their DNA and can lead to their death. So what protects them from this dangerous world? The answer is surprisingly is simple. The check points arrest cells in G2, so giving them time to repair the damage. The first step in the elucidation of this checkpoint pathway was taken in 1988 by Ted Weinert and Leland Hartwell with the discovery of RAD9.

The phase a cell is in when it encounters damaging agents affects its sensitivity to the damage it incurs. Haploid G1 cells have neither a sister chromatid nor a homologous chromosome, so are more sensitive to DNA damage than S phase and G2 cells, which use DNA homology to repair double-strand breaks.

Asymmetric cell division is crucial during many developmental processes to generate two distinct cells from one mother cell. Occasionally this occurs through the activity of localized determinants in the progenitor cell and nowhere is this.

During the cell cycle, a cell goes through a series of sequential events - one step has to be completed before the next is initiated. This linear progression can be achieved in at least two ways: each step could require a product of the preceding step for its initiation, or there might be regulatory feedback mechanisms which ensure that a subsequent step in the cell cycle is not initiated if a crucial event, known as a check point, has not been completed successfully.

By 1991, it was known that entry to mitosis is regulated by a protein kinase, a so-called 'maturation-promoting factor' (MPF). Activation of MPF induces entry into mitosis and assembly of the mitotic spindle; conversely, its inactivation induces interphase, chromosome segregation and cell division.

During mitosis and meiosis the cell cycle is halted at certain time points, when animal cells await fertilization. During the 1960s scientists were furiously searching for the factor that mediated this process, but it wasn't until 1971 that Yoshio Masui and Clement Markert identified the maturation- promoting factor (MPF).

In the early 1970s, the nature and duration of the *G0* phase were a source of controversy. Indeed, some had even proposed that *G0* did not really exist. In 1974, Arthur Pardee argued that cells do go into a *G0* state, and that this is an equivalent state irrespective of the means by which it is induced. He showed that cells re-enter the cell cycle by transiting a 'restriction point' after which they are committed to the cell cycle and argued that this switch is defective in cancer cells.

Again, the cells always seemed to be blocked at a point between the M- and S- phases that is, in *G1*. Pardee then showed that when cells are treated with colchicine and hydroxyurea the non-physiological agents stops cells in M and S phase respectively; they do not stop at this point. Pardee termed this switching point the 'restriction point', and proposed that it "permit [s] normal cells to retain viability by a shift to minimal metabolism upon differentiation, when conditions are suboptimal for growth". Eleven years later, Zetterberg and Larsson followed the behavior of individual cells by time-lapse microscopy to provide a detailed map of cell responses to serum starvation, cyclohexamide treatment or exposure to particular growth factors. They showed that a variable growth-factor-dependent phase preceded a growth-factor-independent phase.

During asymmetric cell division, the precursor cell is polarized to segregate cell fate determinants predominantly into just one daughter cell, and the mitotic spindle is orientated along the appropriate axis before cytokinesis to ensure this. So, defective cell division can result in tetraploid cells with a concomitant amplification of centrosomes.

Isolation of cell components by centrifugation

A major advance in the biochemical study of cells was the development of methods for separating organelles from the cytosol and from each other. In a typical cellular fractionation, cells, or tissues are disrupted by gentle homogenization in a medium containing sucrose (about 0.2M). This treatment ruptures the plasma membrane but leaves most of the organelles intact (the sucrose creates a medium with an osmotic pressure similar to that within organelles, which would cause them to swell, burst, and spill their contents).

Organelles such as nuclei, mitochondria and lysosomes differ in size and therefore sediment at different rates during centrifugation (Fig. 19). They also differ in specific gravity, and they "float" at different levels in a density gradient. Differential centrifugation results in a rough fractionation of the cytoplasmic contents, which may be further purified by isopycnic ("same density") centrifugation. In this procedure, organelles of different buoyant densities (the result of different ratios of lipid and protein in each type of organelle) are separated on a density gradient. By carefully removing material from each region of the gradient and observing it with a microscope, the biochemists can establish the sedimentation position of each organelle and obtain purified organelles for further study. In this way it was established, for example, that lysosomes contain degenerative enzymes, mitochondria contain oxidative enzymes, and chloroplasts contain photosynthetic pigments. The isolation of an organelle enriched in a certain enzyme is often the first step in the purification of that enzyme.

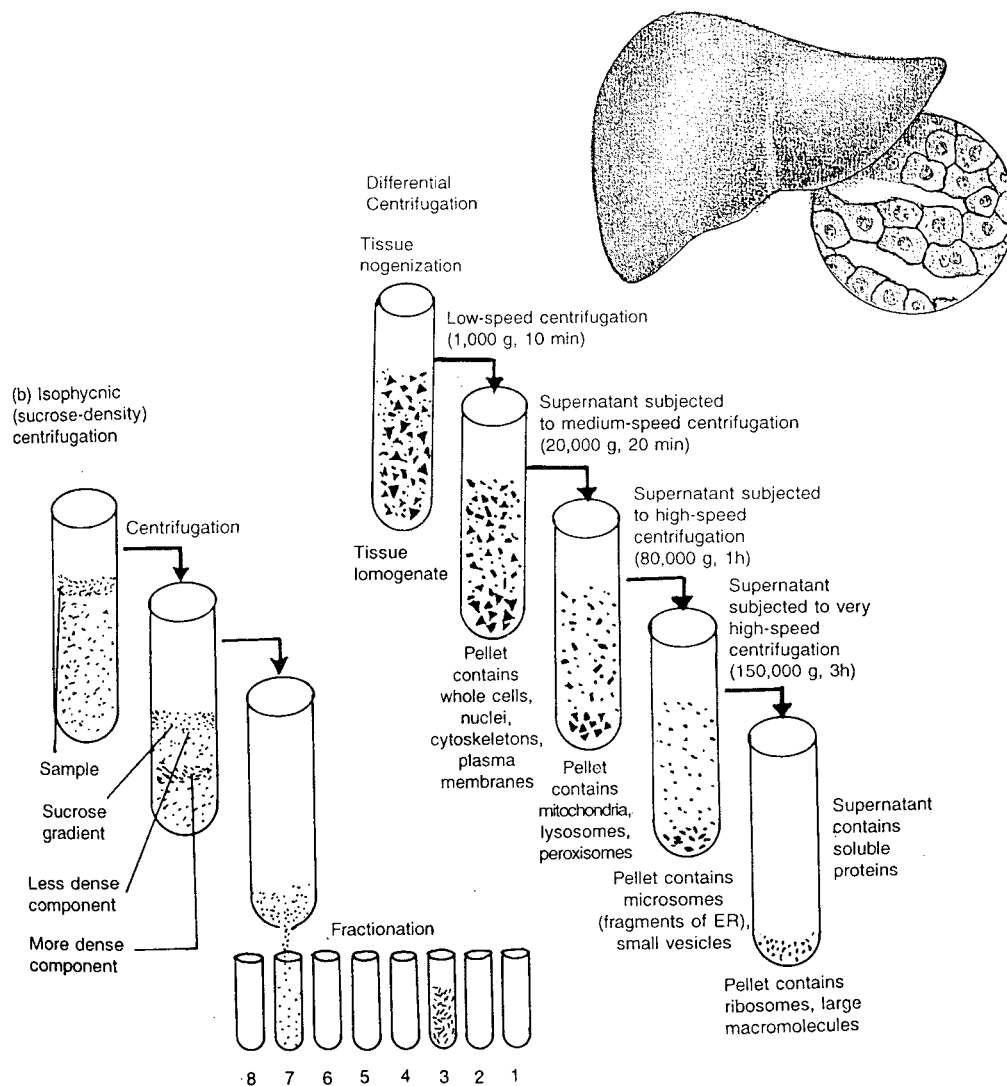


Fig. 19: (a) Differential centrifugation (b) Isopycnic (sucrose-density) centrifugation.

[Subcellular Fractionation of tissue. A tissue such as liver is mechanically homogenised to break cells and disperse contents in an aqueous buffer. The large and small particles in this suspension can be separated by centrifugation at different speeds (a), or particles of different density can be separated by isopycnic centrifugation (b). In isopycnic centrifugation, centrifuge tube is filled with a solution, the density of which increases from top to bottom; a solute such as sucrose is dissolved at different concentrations to produce the density gradient. When a mixture of organelles is layered on top of the density gradient and the tube is centrifuged at high speed, individual organelles sediment until their buoyant density exactly matches that of the gradient. Each layer can be collected separately.]

Conclusion

All cells are made up on a basic fundamental plan, i.e. each cell is bound by thin semipermeable membrane, the plasma membrane. The fluid inside the plasma membrane bag is known as the cytoplasm in which particles of various sizes and shapes are suspended. The biggest of all generally found in the cell cytoplasm is the nucleus, small rod shaped mitochondria, stack of lamellae of membrane, the Golgi bodies, single membrane bound

bag of lytic enzyme, the lysosomes, membrane cisternae the endoplasmic reticulum studded with round particles, the ribosomes, and other non-membraneous particles are present in the cytoplasm of a typical cell. However, terminally differentiated cells, such as, the eye lens fibres, red blood cells, keratinocytes, sperm, etc. do not follow the basic pattern of cell structure but they are differentiated form of a typical cell. For example, long slender oval shaped human sperm is differentiated from cuboidal germinal epithelial cell. Similarly, RBC and karatinocytes are terminally differentiated cells from the erythroblast and epithelial cells respectively.