

CYTOLOGY

Dr. Paul W. L. Kwan
Tufts University School of Medicine

Learning Objectives

1. The plasma membrane is made up of a lipid bilayer with proteins and carbohydrates in it.
2. The lipids include phospholipids and cholesterol.
3. Two kinds of proteins are found in the plasma membrane: integral and peripheral.
4. The integral proteins have important involvements in metabolic, regulatory and integration of cell function.
5. The fluid mosaic model describes the composition of plasma membrane as well as the movements of some of the components in the membrane.
6. Several kinds of carbohydrates are associated with the cell membranes.
7. The functional roles of the plasma membrane including how materials enter and leave the cell.
8. The differences between the plasma membrane and the intracellular membranes.
9. Functions of intracellular membranes.
10. Nucleus and its components.
11. Movements of materials in and out of the nucleus.
12. Organelles involved in protein synthesis and transport.
13. Role of Golgi in material processing.
14. Structure and functions of lysosomes.
15. Structure and functions of peroxisomes.
16. Differences between lysosomes and proteasomes.
17. Smooth endoplasmic reticulum has varied functions from detoxification to steroid metabolism.
18. The role of mitochondria in energy production.
19. Mitochondria is important in apoptosis.
20. Microfilaments made up of actin.
21. Different kinds of intermediate filaments and their functions.
22. The composition and functions of microtubules.
23. Routine inclusions found in the cytoplasm.

INTRODUCTION

In a multicellular organism, the cell is the smallest structural unit of living material.

Each cell has a nucleus and variable amount of cytoplasm containing organelles. A plasma membrane (plasmalemma) surrounds the cell and serves as a structural and functional barrier between its contents from the external environment.

Some of the organelles inside the cell are also bonded by membranes (cytomembranes/cell membranes), thus setting up microenvironments in the intracellular milieu (nucleus, mitochondria, lysosomes etc.).

An exception to the above is the mature mammalian red blood cell that does not contain a nucleus or organelles.

CELL MEMBRANES

The Plasma Membrane (plasmalemma)

Appearance

The thickness of the plasma membrane is about 8 to 10 nm (nanometer). This structure is too thin to be seen by itself under the light microscope. However, in routinely stained sections, there is usually enough stain bound to the membrane and its coat for the cell membrane to be visible. The organelles and their membranes are too small to be resolved with light microscopy.

In routine transmission electron microscopy, the cell membrane is characteristically trilaminar --- two electron dense layers sandwiching an electron lucent layer, like railroad tracks.

Composition

Each plasma membrane consists of two phospholipid layers (bilayer) with the molecules arranged perpendicular to the cell surface. The hydrophilic ends of the phospholipids face the extracellular and the cytoplasmic surfaces. The hydrophobic nonpolar fatty acid tails are located in the center of the membranes. The membrane is impermeable to water-soluble molecules due to the presence of the hydrophobic ends of the phospholipids in the bilayer.

Embedded within the phospholipid layers are many proteins.

The Phospholipids

The major phospholipids in the plasma membrane are phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, and sphingomyelin. These four make up more than half of the lipids in most membranes. The distribution of these phospholipids in each half of the bilayer is different. In the inner leaflet of the plasma membrane is a fifth phospholipid, phosphatidylinositol.

Cholesterol is a major component of cellular membranes and is present in about the same amounts as the phospholipids. It is inserted into the phospholipid bilayer and modulates membrane fluidity.

Also present in the outer leaflet of the plasma membrane are glycolipids with the oligosaccharide chains sticking out from the surface.

The Membrane Proteins

Embedded in the lipid bilayer are proteins that play important roles in the functions of the plasma membrane.

Integral membrane proteins --- with nonpolar part that lies within the lipid bilayer. Some of these are transmembrane proteins that extend through the membrane and stick out at both surfaces. Others may have only one end sticking out of the surface. Still another type called multi-pass transmembrane protein weaves “in-and-out” of the membrane like a snake. The integral membrane proteins are released from the lipid bilayer with the help of detergents that are quite harsh.

Peripheral membrane proteins are located on one or the other surface of the membrane. They are not inserted into the lipid bilayer but are associated with the membrane via protein-protein ionic bonds. These proteins can be detached by gentler methods such as the use of extreme pH or solutions of high salt concentration.

The membrane proteins and lipids are not fixed in rigid positions in the membrane but can change their positions within the plane of the membrane. The Singer and Nicolson fluid mosaic model of cell membrane depicts the proteins as icebergs in a sea of lipid molecules. Membrane proteins can “drift” in this sea but usually do not “flip over.” Phospholipids can also move laterally within the mosaic.

The freedom of movement for some membrane proteins is limited because they are associated with the cytoskeleton. This restriction in the movement of membrane proteins also explains the polarized nature of some cells since different areas of the membrane contain different proteins with different functions.

Carbohydrates

Carbohydrate moieties attach to the membrane lipids or the proteins at the external surface of the plasma membrane. Carbohydrates are also present in the luminal aspects of the membrane of organelles.

Oligosaccharides are bound to membrane proteins (glycoproteins) and lipids (glycolipids).

Polysaccharides are bound to proteins only (proteoglycans). These form the glycocalyx, an extrinsic coat on the surface of the cell.

Glycolipids --- oligosaccharides attached to lipids in the outer layer of the membrane.

Most membranes are 50% protein and 50% lipid. About 5 to 10% of the membrane mass come from the carbohydrate moieties in the glycoproteins and glycolipids.

Functions of the Plasma Membranes

The plasma membranes carry out vital cellular functions. It separates the contents of the cells from the surrounding environment. Everything that enters or leaves a cell has to transverse a membrane.

Selective Barrier --- separates the contents of the cell from the surrounding environment.

Semipermeably barrier

- Protein channels
- Passive Transport
- Active Transport

Uptake and Release

Pinocytosis (cell drinking): uptake of fluid and small molecules via small vesicles (<150 nm). Found in practically in every cell type.

Endocytosis (receptor-mediated): receptors for specific molecules are present at locations on the cell membrane. When specific molecules bind to these receptors, the complex is taken into the cell in the form of 'coated vesicles'. Clathrin molecules are involved in forming a basket-like 'coat' around the plasma membrane invagination. Once inside the cell, the coat comes off and the ingested material is processed. This is also known as clathrin-dependent endocytosis.

Phagocytosis (cell eating): involves the ingestion of larger particles such as bacteria, tissue debris, foreign substances etc. See section on phagosomes later.

Exocytosis: vesicles containing materials move from the cytoplasm toward the plasma membrane. Fusion of the vesicles with the plasma membrane releases the material into the extracellular space.

Site of Interactions with Environmental Factors

- Receptors
- Catalysts
- Junctions
- Intercellular recognition

Intracellular Membranes

Intracellular membranes are slightly thinner than the plasma membrane. They are also formed of lipid bilayer with associated proteins.

Carbohydrate moieties are present in the luminal surface of the membranes that are part of the endoplasmic reticulum (ER), Golgi complex and vesicles.

Intracellular membranes are not covered by a glycocalyx.

Intracellular membranes compartmentalize the cell and increase the surface areas where important metabolic processes take place.

Depending on the organelles, the functions of the intracellular membranes vary greatly. Some of the membrane associated proteins in the intracellular membrane function as enzymes that are specific to the organelle.

NUCLEUS

The nucleus is the largest organelle in the cell. It is the main depository of genetic information. It specializes in DNA replication and transcription, RNA processing and the assembly of ribonucleoprotein particles.

Shape --- Variable, depending on the cell type. Spherical / ovoid most common, other shapes include spindle, bean, kidney, cock-screw or multilobed.

Number --- Usually, only one in each cell. In some tissues, bi-nucleated cells may be present (liver, bladder). In other tissues, the cells may have multiple nuclei (e.g. skeletal muscles, osteoclasts). On the other hand, the mature red blood cells of mammals do not contain nuclei.

Contents of the Nucleus --- Chromatin, nucleolus and matrix enclosed in an envelope

Chromatin

Made of DNA (genetic information) and basic proteins, histones (modulate transcription).

Euchromatin --- Uncoiled DNA being transcribed. Site of synthesis of ribosomal RNAs (mRNA, tRNA). Electron lucent in EM, pale areas in LM.

Heterochromatin --- Condensed DNA, not involved in transcription. Electron dense in EM, darkly stained in routine LM (basophilic).

Barr body --- Condensed, inactive, second sex chromatin in females. May appear as a nuclear appendage ('drumstick') in some neutrophils, or as a dark spot attached to the nuclear envelope in other cells. Seen only in a percentage of the cells in the tissue.

Nucleosome is the basic structural unit of chromatin. Each nucleosome consists of a center of four types of histones, wrapped by a strand of 166 base pair DNA. An

additional strand of DNA (with an additional piece of histone) links this to another nucleosome. A number of nucleosomes like this form an 11 nm filament.

The 11 nm filament coils around an axis and form a 30 nm chromatin fiber.

Further coiling and packing of the chromatin fibers lead to the familiar image of chromosomes seen under the microscope.

The ratio of euchromatin to heterochromatin reflects on the level of protein synthesis taking place in the cell. The more euchromatin in the cell, the more DNA surface is available for transcription and the cell is considered more active.

Chromosomes are replicated for cell division. The chromosomes are most condensed at the time of metaphase. These chromosomes can be isolated, stained, and studied in great details.

Karyotype describes the number and appearances of chromosomes present in the cell of an individual animal. This number differs from species to species.

After being prepared for karyotyping, the chromosomes have specific “banding” patterns unique for each chromosome. Careful study of the banding can reveal abnormalities such as deletions, duplication, translocations etc. in the chromosomes. These changes can sometimes be directly correlated with pathology seen in the animal.

Nucleolus

Appearance --- Dark staining structure in LM (basophilic). This is the site of synthesis of ribosomal RNAs. Electron dense fibrils and granules along with electron lucent areas in EM.

Number and Size --- Usually one to two. Size and number increase with the level of protein synthesis.

Composition --- Ribosomal RNA (rRNA) and associated proteins.

Under the electron microscope, the nucleolus has three components:

Nucleolar organizer DNA that encodes for rRNA. There may be one to several of these lightly stained structures in the nucleolus. Electron-lucent structure in the nucleolus.

Pars fibrosa contains tightly packed, electron-dense, ribonucleoprotein fibers (primary transcript of rRNA genes).

Pars granulosa containing electron-dense granules that are mature ribosomes.

The nucleolus is involved in the production of ribosomes. Ribosomal RNAs are synthesized in the nucleus. In the nucleolus, the rRNA becomes associated with proteins subunits (manufactured in the cytoplasm and transported into the nucleus). The resultant ribosomes move into the cytoplasm via the nuclear pores.

A cell that has a large nucleolus is considered actively involved in protein synthesis.

Nuclear Matrix

The nuclear matrix (nucleoplasm) contains proteins, the chromatin, the nucleolus and a nucleoskeleton made up of fibrillar components.

Nuclear Envelope

The nuclear envelope is made up of two parallel membranes separated by a perinuclear space.

The outer membrane is considered as a continuation of the endoplasmic reticulum (RER). Thus, ribosomes are seen attached to part of the outer nuclear membrane.

Associated with the inner membrane is a fibrous lamina containing lamins. The lamina plays a role in stabilizing the nuclear envelope as well as providing places for interactions with chromosomes.

Nuclear pores complexes, located in the nuclear envelope, control the flow of materials in and out of the nucleus. Each nuclear pore is made up of three parts. There is an outer ring containing eight proteins and associated fibrils sticking into the cytoplasm. There is an inner ring of eight proteins with fibrils sticking into the nucleoplasm forming a basket-like arrangement. In the middle is a central cylinder with radiating spokes connecting with the outer and inner rings.

There is a continuous stream of materials entering and leaving the nucleus. The nucleus has to import every protein from the cytoplasm. The cytoplasm relies on the nucleus for a steady supply of tRNAs, mRNAs and ribosomes. Small molecules can enter or leave the nucleus via passive diffusion through the pores. Larger molecules, including proteins, require a receptor-mediated, energy-dependent process to get into the nucleus. The importin- β superfamily of protein is involved in the process. A protein “cargo” contains a “nuclear localization sequence” is recognized by importin. The protein then translocates through the nuclear pore complex. Ran-GTP separates the incoming cargo from importin. An export cycle involving Ran-GTP and exportin moves protein out of the nucleus.

The nuclear envelope disappears at the time of cell division and then reforms (from the ER) after mitosis.

ORGANELLES OF PROTEIN SYNTHESIS

RNA Molecules

Messenger RNA (mRNA)

Ribosomal RNA (rRNA)

Transfer RNA (tRNA)

Ribosomes

Each ribosome is formed by two subunits and interacts with a molecule of mRNA. Four types of rRNA and many different proteins are present in a ribosome. A number of binding and recognition sites are present on each ribosome.

The ribosomal RNAs are synthesized in the nucleoli while the proteins are synthesized in the cytoplasm. The proteins migrate into the nucleus and join up with the rRNA. The resulting subunits emigrate through the nuclear pores into the cytoplasm.

A strand of mRNA holds a number of ribosomes together to form polysomes. Some polysomes are attached to the membranes of the ER. Proteins (polypeptides) synthesized by these ribosomes extend into the cisternae of the RER via pores in the membrane. These proteins are packaged and can be secreted (secretory granules containing digestive enzymes) or used inside the cell (lysosomes). Other proteins synthesized by RER include integral proteins of the plasma membrane.

Free, unattached, polyribosomes synthesize proteins for internal use within the cytoplasm.

Presence of a large amount of ribosomes in the cell renders the cytoplasm basophilic in H&E sections (e.g. nerve cell body, exocrine pancreas).

Rough Endoplasmic Reticulum (RER)

This is an interconnecting network of ribosome-studded membranes with the enclosed space called cisterna. It is involved in the synthesis of proteins for export or for the intracellular compartment. It also synthesizes carbohydrates, proteins and lipids for cellular membranes.

Protein synthesis first takes place in a free ribosome. The peptide contains a signal sequence that interacts with a signal-recognition particle. The complex binds to a receptor protein on the membrane of the RER. The peptide then elongates in the cisterna. The protein product is further modified by the removal of the signal sequence, folding and the addition of oligosaccharides to most proteins.

Cellular membranes are also assembled by the RER. The proteins of the membrane are produced by the ribosomes. Integral proteins present in the ER membrane itself produce the carbohydrate and lipid components.

Due to presence of the attached ribosomes, the RER, if present in significant amount, will appear as basophilic patches in the cytoplasm (e.g. Nissl substance in nerve

cells). The amount of this basophilia reflects the level of protein synthesis going on in the cell.

Golgi Complex

This organelle is made up of 3-15 parallel stacks of smooth, membrane-bounded cisternae and associated vesicles. The stacks are organized in a “directional” manner. The convex surface of the cisternae closer to the nucleus is the forming or cis face. The stack facing the surface of the cells is called the mature or trans face. Transition vesicles pinch off from the RER, carry materials to, and fuse with the forming face. On the other side of the stack, secretory vesicles and condensing vesicles form at the mature face. Secretory vesicles move toward the cell surface for release. Condensing vesicles further process their contents before they become secretory.

Both synthesis and processing of proteins take place in the cisternae and vesicles of the Golgi complex.

Segments of the newly synthesized peptide chain may be removed. Carbohydrate moieties are attached to proteins to form glycoproteins. On the other hand, carbohydrates may be removed from some proteins. Lipids (synthesized on site or imported from SER) are added to some proteins. The material is then concentrated by the removal of water.

Proteins such as lysosomal enzymes that will be packaged for internal use and not exported have ‘markers’ on them designating the intended destination. The oligosaccharides on these proteins contain mannose 6-phosphate. In the RER and the Golgi complex, special receptors recognize the mannose 6-phosphate and direct the proteins to be packaged into lysosomes.

Secretory proteins lack the mannose 6-phosphate tag. The materials are modified (sulfation, glycosylation etc.), packaged, and concentrated into secretory granules. The granules migrate toward the cell surface, fuse with the plasma membrane and release the contents into the extracellular space. This secretory process is called exocytosis.

The Golgi complex also processes membrane. Some membranes are altered so that they can fuse with the plasma membrane. For newly synthesized plasma membrane components, a glycocalyx is added to the surface.

The Golgi complex by itself is difficult to see under light microscopy. Special metal stains (Golgi stains) can be used to turn the complex darkly stained. The complex can also be demonstrated by immunohistochemistry. Sometimes, it is possible to detect the location of the Golgi apparatus in a cell active in protein synthesis. In this case, the Golgi complex may appear as a pale halo in a background of darker cytoplasm (e.g. in plasma cells). The pancake-like stacks of cisternae stand out clearly under EM.

OTHER MEMBRANOUS ORGANELLES

Lysosomes

These are membrane bound spherical structures containing hydrolytic enzymes and other components. They are involved in normal metabolism, tissue repair, infection, inflammation and other defense mechanisms.

Lysosomal enzymes include acid phosphatases, esterases, glycosidases, ribonuclease, sulfatase, lipases, glucuronidase, etc. They function best in the acidic pH inside the lysosome. The enzymes are synthesized in the RER, modified and packaged in the Golgi apparatus. Vesicles pinch off from the trans face and become primary lysosomes.

Lysosomes fuse with other vesicles containing materials to be digested. Source of material can be endogenous (autophagy) or exogenous (heterophagy).

Phagocytosis is the process of ingesting particulate matters. The vesicle containing the material is a phagosome.

Pinocytosis is the ingestion of fluid in small pinocytic vesicles.

Fusion of a primary lysosome and a phagosome forms a phagolysosome.

Sometimes part of the cytoplasm with organelles is walled off by a membrane forming an autosome. Fusion with a primary lysosome results in an autophagosome.

A secondary lysosome is a composite structure that contains lysosomal enzymes and materials in the process of being digested. The material may be of endogenous or exogenous origin.

Residual body is a structure that contains materials that cannot be digested by the lysosomal enzymes. Lipofuscin is a brownish-yellow material that accumulates in residual bodies and is sometimes called “wear and tear pigment”.

Storage diseases --- Deficiency in lysosomal enzymes, usually due to congenital mutations, will lead to the accumulation of undigested substrates in the cell and affect cellular functions.

Defects in the fusion of lysosomes and phagosomes containing bacteria can lead to susceptibility of the animal to severe infection.

Lysosomes are small and usually cannot be observed under the light microscope. However, there are cases where the cells contain very large lysosomes that are easy to see, e.g. azurophilic granules in white blood cells. Lysosomes can be easily demonstrated with immunohistochemistry.

Peroxisomes

These are membrane-bound vesicles containing oxidative enzymes such as peroxidases and catalase.

Under the electron microscope, the peroxisomes have granular contents and, in most mammalian species, have crystalloid cores of urate oxidase.

The formation of peroxide (H_2O_2) is an important step in the function of the peroxisome. This is done by the removal of hydrogen from specific substrates and combining it with molecular oxygen. The oxidative enzymes (L-amino and D-amino oxidases, urate oxidase, and hydroxyacid oxidase) are involved with this. The peroxide thus generated is used by catalase to oxidize other substrates.

The production of peroxide is important in the antibacterial actions of phagocytes.

Lipid peroxidation also takes place in the peroxisomal enzymes.

The enzymes found in peroxisomes are synthesized on free polysomes (with a 'tag') and imported into peroxisomes via a signal-receptor mediated process. These organelles divide by budding.

Peroxisomes are present in most cells but are most abundant in the kidney and the liver. They help to degrade drug metabolites and toxins.

In a way similar to lysosomal diseases, defective or deficient peroxisomes can lead to significant pathology in the animal.

Proteasomes

This is another mechanism of digestion used by the cell. While lysosomes can digest a variety of materials, the proteasomes are geared to the digestion of proteins.

Each proteasome is a cylinder-like structured containing a complex of proteases shaped like four stacked doughnuts. At one end of the cylinder is a regulatory unit that contains ATPase and a recognition site for ubiquitin.

A protein that is destined to be destroyed by the proteasome complex is first tagged by ubiquitin that in itself is a small, 76 aa protein. Other ubiquitin molecules then attached to the first one. This tagging marks the protein for digestion. The recognition site on the proteasome "sees" the tagged protein. The ATPase of the regulatory unit unfolds the protein, using energy from ATP. The stretched protein enters the cylinder and is broken down into peptides of about eight amino acids. These fragments are dumped into the cytosol and the ubiquitin molecules are recycled for further use.

Smooth ER

This organelle is actually a continuation of the RER except the membranous network is not studded with ribosomes. The cisternae vary in size, have many interconnections, and are unlike the flattened stacks seen in RER.

Cells with extensive SER are usually those that are involved in lipid metabolism or are specialized to regulate movements of certain ions (e.g. Ca^{++} , Cl^-).

Steroid secretion and metabolizing cells have extensive SER, e.g. cells of the adrenal cortex.

Liver cells have abundant SER that are involved in lipid metabolism and detoxification of fat-soluble xenobiotics. The amount of SER increases greatly if the animal is challenged with a chemical such as alcohol or insecticide (along with induction of enzymes needed to metabolize these substances). Glycogen metabolism in the liver also involves glucose-6-phosphatase located in the SER.

The SER in the muscles regulate the flux of calcium ions. In this tissue, the SER is also known as the sarcoplasmic reticulum.

Another important function of the SER involves the synthesis of phospholipids for cell membranes. Molecules of phospholipids are transported from the lipid-rich SER to lipid-poor cellular membranes. This process involves transporting proteins.

Mitochondria

This organelle, like the nucleus, has two membranes.

The outer membrane surrounds the whole structure. This membrane resembles other intracellular membrane.

The space between the outer and the inner membrane is called the outer compartment.

The space enclosed by the inner membrane is the inner compartment.

The inner membrane runs parallel to the outer membrane except where it extends into the organelle in folds called cristae. Most cristae are finger-like. In steroid producing cells, the cristae can be tubular or vesicular (e.g. adrenal cortical cells, interstitial cells of Leydig).

The inner membrane (including the cristae) contains the electron transport chain responsible in ATP production.

The inner compartment of the mitochondria contains the amorphous mitochondrial matrix. The matrix contains enzymes for fatty acid beta-oxidation and the citric acid cycle. Matrix granules, made up of calcium phosphate, are present in the matrix.

The mitochondria contain its own DNA and ribosomes, and produce some of their own proteins. The DNA and ribosomes are similar to bacteria and may reflect the phylogeny of the mitochondria. The amount of DNA in the mitochondria can code for only a small number of proteins. The majority of proteins present in the mitochondria are transported into the organelle after being produced in the cytoplasm.

Mitochondria are involved in cellular respiration. They oxidize nutrients to produce ATP and heat. The ATP is passed to the cytosol and used as a source of metabolic energy for other reactions. Thus, it is not surprising to see them concentrated in regions of the cell that have high energy requirement (e.g. basal areas of kidney cells involved in active transport, around the flagellum of the sperm etc.).

Studded in the outer and inner mitochondrial membranes are enzymes and proteins that carry out important functions. Other enzymes are present in the matrix. The 'topographic' arrangements of these components are critical to their functions. The 'translocation' of a component to a different compartment (or into the cytoplasm) can have very deleterious effect on the cell.

Mitochondria also play a very important role in apoptosis (programmed cell death), a subject that will be discussed in great detail in General Pathology.

Mitochondria are inherited only from the maternal parent (all from the mitochondria of the ovum). During mitosis, each daughter cell gets about half of the parental mitochondria. New mitochondria arise from splitting of the organelle (fission), a process easily observed in living cells under a phase-contrast microscope.

Mitochondria can be seen by the light microscope only after the application of special stains (iron hematoxylin, immunohistochemistry) or with the use of phase microscopy.

CYTOSKELETON

The cytoskeleton provides support of the cell and is responsible for cellular movements, phagocytosis, cytokinesis, cell-to-cell and cell-matrix adherence.

Three main types of structures: microfilaments, intermediate filaments, and microtubules.

Microfilaments (F-actin, actin filaments)

These are thin filaments about 6-8 nm in diameter.

These are contractile filamentous structures made of the polymers of globular G-actin. About half of the actin in non-muscle cells exists in filamentous form while the other half is in the form of subunits. Polymerization of the actin dimers, an ATP-dependent process, leads to the formation of an actin filament and increase in the length of the structure at the 'plus' end. Depolymerization at the 'minus' end leads to shortening of the filament.

For most cells, microfilaments are found mainly near the plasma membrane. Other microfilaments are in the cytoplasm and are associated with the movements of certain organelles. The microvilli of epithelial cells in the intestine and the brush border of kidney epithelial cells are filled with microfilaments. Microfilaments may also interact with other types of cytoskeleton, e.g. terminal web of surface epithelial cells.

In special cases, the microfilaments are in association with other special proteins and are arranged in linear arrays, e.g. actin and myosin filaments in cardiac and skeletal muscle cells. This will be discussed in greater details in the chapter on Muscles.

In other cells, microfilaments cause movements of the plasma membrane. Ameboid movements, extension of pseudopods and processes, contraction of microvilli, endocytosis, pinching of the cell at mitosis etc. are some of the examples of microfilament involvements. In these cases, movements may also involve myosin that has different form than the polymerized thick filaments in muscle cells.

Microfilaments are beyond the resolution of light microscopes. The best way to demonstrate microfilaments in cells and tissues is the use of immunohistochemistry and anti-actin antibodies. In the case of muscle cells, the arrangement of thick and thin filaments can be seen by LM where they form cross striations.

Intermediate Filaments

These include a number of heterogeneous filamentous protein forming filaments about 7-11 nm in diameter.

All intermediate filaments are flexible and possess tensile strength. They support and maintain the shapes of the cells.

Keratin filaments (tonofilaments)

Attached to plasma membrane by dense plaques. Found in areas where the cell is subjected to shearing forces, e.g. cell junctions, especially at desmosomes.

There are many kinds of keratin filaments and they are found in many types of cells. Examples include cells of the epidermis, hooves, scales, and feathers.

Desmin filaments

Plentiful in muscle cells, where they hold the thick and thin filaments in place and anchor them to the plasma membrane.

Vimentin filaments

Found in cells derived from the mesenchyme (including developing myoid cells). They help to hold the nucleus in place.

Neurofilaments and Glial fibrillary acidic protein (GFAP)

Present in neurons (NF) and astrocytes (GFAP). Help to support the extensive cell processes found in neural tissues.

Spectrin filaments

Present in red blood cells. Maintains the asymmetric shape of the erythrocytes and protect them from shearing forces encountered in the circulation.

Intermediate filaments are beyond the resolution of the light microscope. Thick bundles of intermediate filaments present in some tissue (e.g. skin, neurons) may be visible by LM. Immunohistochemistry, using type-specific antibodies, has been very helpful in differentiating the various types of intermediate filaments. This is especially true in the tumor diagnosis.

Microtubules

These are unbranching, hollow structures, variable in length, about 25 nm in diameter.

Tubulin --- Structural component of microtubules, a dimer of α -tubulin and β -tubulin. The subunits polymerize into a tight helix to form a microtubule. Each hollow cylinder contains 13 concentrically arranged protofilaments. Each protofilament is made up of a linear array of α - and β -tubulin dimers. Polymerization starts at the microtubule-organizing centers. In response to regulatory signals, microtubule can polymerize and grow at one end (the plus, + end) and depolymerize at the other (minus, -) end. Calcium ions, GTP and some associated proteins are involved in the polymerization process.

Microtubules are present in various locations in the cell either as individually or in bundles.

Mitotic spindle --- Made up of microtubules, essential for cell division. Disassembles after completion of cell division.

Centrioles --- Occur in pairs, each is a short cylinder of nine sets of triple tubules (triplets). Serve as poles during cell division and organize the assembly of the mitotic apparatus. In non-dividing cells, centrioles organize the formation of basal bodies that secure cilia and flagella in the cytoplasm.

Cilia and flagella contain microtubules arranged in a 9+2 set up called an axoneme. The two microtubules in the center are enclosed in a central sheath. The outer ring is made up of nine microtubule doublets. Two dynein (an ATPase) arms are present on each of the doublets and the arms are very close to the next doublet. ATP activation causes the dynein arms to make contact with the adjacent doublet and provide for the sliding of doublets against each other. This results in the movement of the cilia or flagellum. Defects in the 9+2 arrangement, be it in the microtubules or in the dynein arms, leads to cilia or flagella that will not beat properly and can have adverse effects (chronic infection, infertility etc.)

Microtubules have a number of functions. They organize intracellular components and are important in the formation of cytoplasmic processes such as axons and dendrites of nerves. They are major cytoskeletal elements in cells and help to maintain cell shape. Movements of organelles and other components in the cytoplasm are facilitated by microtubules. Transfer of materials and vesicles take place along microtubules, e.g.

axonal transport. In cell division, microtubules move chromosomes to opposite poles of the daughter cells.

Some drugs can interfere with the functions of this organelle (e.g. delayed fusion of lysosomes with phagosomes, interruption of cell division). Paclitaxel, vinblastine and colchicine are good examples of microtubule interferers/disruptors.

CYTOPLASMIC INCLUSIONS

Glycogen --- Stored in the cytoplasm as clusters of granules, serves as source of energy. There is considerable amount of glycogen stored in the liver and this material can be demonstrated with the PAS stain.

Lipid droplets --- Found much in cell type but are especially abundant in cells that metabolize lipids and steroids such as hepatocytes and adrenal cortical cells. Since routine histological processing employs solvents, lipid droplets usually appear as vacuoles in tissue sections. Special techniques are needed to preserve and stain the lipid droplets.

Residual bodies and lipofuscin --- Tertiary lysosomes with materials that cannot be digested.

Pigments

Melanin

Hemoglobin and hemosiderin

Secretory granules --- Membrane-bound structures destined to be released from the cell. Contents variable.

CYTOPLASMIC MATRIX

The cytoplasmic matrix is the material inside the cell that occupies the space between the organelles and the inclusions.

In this matrix are the numerous molecules involved in cellular functions.

The matrix may exist in a gel or sol form and, under the proper conditions, change readily from one to the other.

SPECIALIZATION OF THE CELL SURFACE

This will be discussed further in the lecture dealing with Epithelium.