VII. THE CYTOPLASM

VII.1. GENERAL CONSIDERATIONS

The cytoplasm is a general term for the material inside the cell between the cell membrane and the nucleus. This material can be divided into the cytosol, cytoskeleton, organelles and inclusions.

The ***cytosol*** is the intracellular fluid, which contains dissolved nutrients, ions, soluble and insoluble proteins, and waste products. It differs in composition from the interstitial fluid, the extracellular fluid that surrounds most of the cells in the body. The cytosol contains a high concentration of potassium, whereas extracellular fluid contains a high concentration of sodium. It contains a relatively high concentration of dissolved proteins, many of them enzymes that regulate metabolic operations. These proteins give the cytosol a consistency that varies between that of thin maple syrup (sol stage) and almost -set gelatin (gel stage). The cytosol contains relatively small quantities of carbohydrates and large reserves of amino acids and lipids. The carbohydrates are broken down to provide energy, and the amino acids are used to manufacture proteins. The lipids stored in the cell are primarily used as an energy source when carbohydrates are unavailable.

Structure and ultrastructure

The cytoplasmic ground substance was called ***cytosol*** in older texts because it was believed to be an amorphous fluid. It is now termed ***cytoplasmic matrix*** to emphasize that it has an organized structure. The cytoplasm has a complex three-dimensional architecture that provides a substratum for cytoplasmic functions. The cytoplasmic matrix (ground substance or cytosol) shows little specific structure by light microscopy or conventional transmission electron microscopy and has traditionally been described as a solution containing electrolytes, metabolites, RNA, and synthesized proteins. Studies with high-voltage electron microscopy (HVEM) of 0.25-0.5 µm sections, reveal a very complex three-dimensional structural network of thin ***microtrabeculae.***

This ***microtrabecular lattice*** or ***cytoskeleton*** is composed, in part, of filaments and tubules and perform the following roles:

-appears to anchor most of the other cytoplasmic organelles;
-it is a structural substratum on which cytoplasmic reactions occur;
-regulates the cytoplasmic transport;
-participates to the division;
-forms the intracytoplasmic currents.

VII.2. THE INCLUSIONS

The inclusions are materials in the cytoplasm that may or may not be surrounded by a membrane. These materials are represented by secretory granules, pigment granules, neutral fat and other lipid droplets, glycogen, and crystalline inclusions.

Secretory granules and pigments granules are surrounded by a membrane; lipid droplets and glycogen are not surrounded by a membrane.

Secretory granules and neutral fat may often fill most of the cytoplasmatic volume, compressing the other formed organelles into a thin rim at the margin of the cell (it is typical for intestinal goblet cell, and for the adipose cell of connective tissue).

Glycogen - represents the storage form of the carbohydrates.

In L.M. may be seen only after special fixation and staining procedures-PAS staining (periodic acid Schiff). In E.M., glycogen appears as granules of 25-30 nm diameter, or clusters of such granules that often occupy significant portions of the cytoplasm. Liver and striated muscle cells contain large amounts of glycogen.

Lipid inclusions (fat droplets*)* -can appear by:

- transitory storage in hepatocytes (after eating) or in secretory cells of mamallian gland (during lactation);
- constant physiological storage in the adipose cell of connective tissue;
- constant pathological storage -in genetic diseases like alterations in lipidic metabolism (lysosomal lipidose), or other diseases-chronic alcoholism.

The number of those lipid droplets is significant in cells with high activity in steroids metabolism (the cortical region of adrenal glands and gonads).

Protein inclusions - are secretory granules found in endocrine and exocrine glands, just at the moment of the highest activity.

Pigment granules are represented by:

- lipofuscin granules or "age pigment" that remain in cells for the life of the cell, as in nerve cells;
- melanin granules produce by melanocytes.

Crystalline inclusions - are recognized with the light microscope. In the human, such inclusions are found in the sustentacular (Sertoli) and interstitial (Leydig) cells of the testis. With the electron microscope, crystalline inclusions have been found in many cell types and in virtually all parts of the cell, including the nucleus and most cytoplasmic organelles.

VII.3. MICROFILAMENTS

VII.3.1. ACTIN MICROFILAMENTS

Actin, first isolated from skeletal muscle, was originally thought to be a protein found exclusively in muscle tissue. It is a component of all cells, representing 5% to 30% of the total protein in nonmuscle cells. Although present in all eukaryotic cells, actin isolated from nonmuscle cells (brain cells) is different from that found in skeletal muscle.

Six different isoforms of actin have been described in humans: α -skeletal, in skeletal muscle; α -cardiac, in heart muscle; α -vascular, in smooth muscle of the vasculature; γ -enteric, in smooth muscle of viscera; β -cytoplasmic and γ -cytoplasmic, preponderantly in nonmuscle cells. The differences consist in the amino acid sequence at the NH_2-terminal end of the acting is form and seems to have little effect on the rate of actin monomers to polymerize into filaments.

Actin exists as a globular protein called **G-actin** (about 5.5 nm in diameter), represents the globular actin molecular with 43 kDa molecular weight. G-actin polymerize into filaments called **F-actin.** Each F-actin microfilaments (filamentous) appears as two helically interwined

chains of G-actin monomers, for which a complete turn of the helix occurs over a distance of 37 nm or 14 G-actin monomers.

Each G-actin monomer must have an ATP molecule bound to polymerize onto an actin filament. If we added ATP and Mg^{2+} (or physiologic salt concentrations) to G-actin at high enough concentration, it would spontaneously polymerize to F-actin. The polymerization of actin occurs in three stages:

1. ***a lag phase*** in which an actin trimer nucleation site is formed;
2. ***a polymerization phase***, during which G-actin monomers are added preferentially at the plus and of the actin filament;
3. ***a steady state***, at which actin monomers have being added at the plus end at the same rate they are being removed at the minus end.

So actin microfilaments have a polarity, with a fast-growing plus end and a slow-growing minus end. Shortly after the addition of each G-actin monomer to the actin filament, ATP is cleaved to ADP, with release of inorganic phosphate.

F-acting is the preponderant protein of the skeletal muscle thin filament, these filaments also contain other regulatory proteins tropomyosin, troponin, and alfa – actinin.

Tropomyosin is a long rod-shaped molecule (41 nm in length), so called because of its similarities with myosin, specifically the rodlike tail domain of the myosin molecule. Tropomyosin is formed from a dimer of two identical subunits. They lie end-to-end in the groove of the actin helix and cover the myosin binding site on the thin filament when intracellular calcium is low. Each tropomyosin molecule has a troponin complex bound to one end, and it covers seven G-actin subunits.

Troponin is a complex of three polypeptides: troponins T, I and C. These polypeptides are named for their apparent functions within the troponin complex: **troponin T** for its tropomyosin binding, **troponin I** for its inhibitory role in calcium regulation of contraction, and **troponin C** for its calcium-binding activity. The troponin complex is elongated, with subunits C and I forming a globular head region and the T polypeptide forming a long tail domain. The tail domain formed from the T- subunit binds with tropomyosin, which is thought to position the complex on the actin thin filament.

α-Actinin binds to the ends of thin filaments. It exists where thin filaments insert into the Z lines of sarcomeres and may help bind microfilaments to the plasma membrane in moving cells.

Actin microfilaments are often grouped as bundles close to the plasma membrane. These membrane-associated microfilaments function in the:

-anchorage and movement of membrane proteins:

-movement of the plasma membrane (as in endocytosis, exocytosis, and cytokinesis):

-formation of the structural core of microvilli on absorbative cells;

-extension of cell processes;

-locomotion of cells (by pseudopodes).

VII.3.2. MYOSIN FILAMENTS

Myosin was also first described in muscle cells, but is now known to be a ubiquitous component of nonmuscle cells. The major form of myosin found in most cells, including skeletal muscle, is referred to as myosin II. Myosin II has a molecular weight of approximately 460 kDa, with two identical heavy chains of 200 kDa, which form a coiled -coil helical tail and two globular heads. The myosin molecule also contains two pairs of light chains with molecular

weight of 20 and 18 kDa. These light chains are found associated with the myosin heads. If purified myosin is proteolytically cleaved with the enzyme papain, the globular heads (called SF1 fragments) can be separated from the myosin tails.

The myosin tails brought to physiologic ionic strength and pH will spontaneously form 15-nm-diameter thick filaments, similar to those found in skeletal muscle. The SF1 heads contain all of myosin ATPase activity required for muscle contraction. If the purified heads are added to preformed F-actin and viewed by electron microscopy, the SF1 fragments look like arrowheads that all face in one direction. The pointed end of the arrowheads face the minus, or slow-growing, end of the filament and the barbed end faces the plus, or fast –growing, end. The polymerization of myosin to form myosin thick filaments is initiated by the end-to-end association of the rodlike tail domains of myosin II molecules. This results in the formation of the bilpolar thick filament, with globular heads at either end separated by a 160-nm central bare zone consisting of myosin II tails domains. At the filament ends the myosin globular head domains protrude from a 10.7-nm-diameter central core at intervals of 14 nm. The succesive myosin heads rotate around the fiber, which forms a filament containing six rows of myosin head domains to contact the adjacent thin filaments of the sarcomere. In muscle, the rodlike fibrous tails of 300 to 400 myosin II dimers pack together to form the bipolar thick filament with 15 nm thick.

Contraction in all cells involves interactions of actin and myosin.

VII.4. MICROVILLI

Epithelial tissue, which lines the surface of the body, the internal organs, body cavities, tubes, and ducts, contains absorptive epithelial cells with numerous fingerlike projections, called microvilli, on their apical surface. These microvilli increase the surface area of the epithelial cell's apical plasma membrane, thereby permitting a greater absorption of important nutrients. The microvilli, which are approximately 80 nm wide and 1µm long, need a stable cytoskeletal scaffolding to maintain their shape and upright position. A very stable and highly structured core of 20 to 30 bundled acting filaments, which run parallel to the microvillus, and attach to the cytoplasmic surface of the plasma membrane serves as this scaffolding.

The actin filaments are bundled by two proteins, named *fimbrin* and *villin*. Actin-bundling proteins are characterized by having two binding sites for F-actin. As they bind to the sides of actin filaments in a helical staircase, they group the filaments into parallel bundles. The actin bundles are attached at their plus end to the tip of the microvilli plasma membrane by undefined proteins. The lateral attachments of actin bundles to the side wall of the microvilli's plasma membrane are through a complex containing calmodulin and myosin I (minimyosin). The core bundles of microvillar actin filaments end just below the apical plasma membrane's surafce in a region of the epithelial cell, called the *terminal web* because it contains a meshwork of actin filaments, actin-binding proteins, and intermediate filaments. The actin-cross-linking protein, spectrin II, and short myosin filaments run perpendicular to and attach adjacent actin core bundles. These attachments of the core bundles to spectrin II and myosin are though to hold the microvilli upright. Spectrin II also cross-links the actin core bundles to intermediate filaments.

The apical surface of cells from intestine and proximal convoluted tubule has a distinctive border of microvilli. This surface structure is called striated border -for the intestinal absorptive cell; and brush border -for the kidney tubule cell.

VII.5. INTERMEDIATE FILAMENTS

Intermediate filaments are 10 nm diameters and, therefore, intermediate in thickness between actin filaments and microtubules.

Intermediate filaments perform a structural and supporting role. Examples: intermediate filaments within muscle cells link together the Z-disks of a adjacent myofibrils; neurofilaments within the axon serve as a structural support to resist breakage of these long slender processes; intermediate filaments of epithelial cells interconnect spot desmosomes, stabilizing epithelial sheets. Intermediate filaments exhibit the following particularities:

- The intermediate filaments in various human and animal cells are composed of a heterogeneous group of proteins.
- The intermediate filaments subunits are fibrous proteins.
- Almost all of the intermediate filament subunits are incorporated into stable intermediate filaments within various cells.
- No energy in the form of ATP or GTP hydrolysis is required for intermediate filament polymerization.
- Intermediate filaments have no polarity.
- Intermediate filaments are composed of a heterogeneous class of subunits (Table XII).

Table XII – Types of Intermediate Filament Proteins

Intermediate Filament	**Subunits**	**Cell Type**
Keratin filaments	Type I acidic keratins Type II neutral/basic keratins (40-65 kDa)	Epithelial cells
Neurofilaments	NF_L (70 kDa) NF_M (140 kDa) NF_H (210 kDa)	Neurons
Vimentin-containing filaments	Vimentin (55 kDa) Vimentin + glial fibrilary Acidic protein (50 kDa) Vimentin + desmin (51 kDa)	Fibroblasts Glial cells Muscle cells
Nuclear lamina	Lamins A, B, and C (65-75 kDa)	All nucleated cells

NF = Neurofilament

The cell type-specify of intermediate filaments proteins has been useful to pathologists, who use fluorescent intermediate filaments type- specific monoclonal antibodies to identify the tissue of origin of metastatic cancer cells. Ex: antikeratin antibodies are used to identify tonofilaments in epithelial (particularly skin) tumors, and antivimentin antibodies are used to identify tumor cells of mesenchymal origin.

VII.6. MICROTUBULES

Microtubules are the third type of cytoskeletal filament. They are found in the **axoneme** of **cilia** and **flagella**, **basal bodies** of cilia, **mitotic spindle** "fibers", **centrioles** from which the spindle fibers radiate, elongated cell processes (such as growing axons), cytoplasm, generally. Microtubules may be seen with the light microscope by using special stains, polarization, or phase contrast optics. Microtubules may be distinguished from filamentous and fibrilar

cytoplasmic components at the light microscopic level by using antibodies to tubulin, the primary protein component of microtubules, conjugated with fluorescent dyes.

Microtubules are involved in numerous essential cellular activities that relate to cytoskeletal functions. These activities include:

-cell elongation and movement (migration);

-intracellular transport of secretory granules;

-movement of chromosomes during mitosis and meiosis;

-maintenance of cell shape, particularly asymmetric shape;

-beating of cilia and flagella.

In the activities listed above that involve movement of cells or their organelles, microtubules serve as guides for molecular motors, molecules that are attached to the moving structures and that ratchet along a tubular or filamentous track. Their principal component is the protein tubulin, a heterodimer composed of nonidentical α-and β-subunits, with each subunit having a molecular weight of near 50 kDa. Through the electron microscope, microtubules are hollow cylinders with a diameter of 24 nm. When viewed in cross section, the wall of each microtubule is seen to be composed of 13 tubulin dimers, which represent 13 protofilaments composed of tubulin subunits. As microtubules assemble, the tubulin molecules are added to the growing microtubule to form the 13 protofilaments. The individual protofilaments are organized such that alpha- and beta-tubulin subunits alternate along the length of the protofilament, which provide the microtubule with an inherent polarity. Tubulin is one of the most highly conserved proteins known. The significance of this is as yet unclear, but it is presumed that this results from the many essential functional subdomains within the tubulin molecule. Tubulin contains regions for GTP binding, for interacting with microtubule-associated proteins, and for several different drug-binding sites.

Growing microtubules have an inherent structural polarity. This polarity occurs because of the orientation of the tubulin subunits in the microtubule polymer. When growing microtubules are analyzed in vitro, subunits add to one end of the elongating polymer faster (the plus end) than to the other end (the minus end). Inside cells, the minus end of the microtubule is capped owing its association with the centrosome complex; therefore, only the events that occur at the plus end will be considered. Current ideas concerning microtubule dynamics focus on the binding of GTP by tubulin subunits during microtubule assembly and the subsequent hydrolysis of the bound GTP to GDP.

It is an equilibrium between the tubule dimmers from the cytoplasm and the polymerized tubulin in the microtubules. This equilibrium can be shifted in the direction of the unpolymerized dimer by exposure of cells or isolated microtubules to low temperature or high pressure. Alkaloids, such as colchicine, vincristine and vinblastine, bind tubulin and prevent polymerization into protofilaments and microtubules. This effect is the basis of the experimental inhibition of mitosis by colchicine and for the use of vincristine and vinblastine as chemotherapeutic agents in cancer treatment. Calcium also inhibits assembly of microtubules from tubulin.

VII.7. CELLULAR MOVEMENTS THAT ARE BASED ON INTERACTION BETWEEN MICROTUBULE – DYNEIN

Cilia, flagella, centriols and basal bodies are organells, which are composed by microtubules.

VII.7.1. CILIA

Cilia and flagella are specialized cellular appendages that extend from the surfaces of several different cell types. Cilia and flagella are very similar ultrastructurally.

Cilia are prominent in the respiratory tract and on the apical surface of the epithelial cells that line the oviduct. In the respiratory tract, cilia are involved in clearing mucus from the respiratory and nasal passages, whereas those that line the oviduct are involved in transporting ova toward the uterus. In the **light microscope**, cilia appear as short, fine, hair-like structures emanating from the free surface of the cell. A thin- dark-staining band is usually seen extending across the cell at the base of the cilia. This dark staining band is due to structures, known as ***basal bodies*** that take up stain and, when viewed with the light microscope, collectively appear as a continuous band (Figure 63). However, each cilium is associated with a single basal body that is separated and distinct from those of adjacent cilia.

At the core of the organelle is the axoneme, a complex structure composed of microtubules and various other proteins that allows ciliary bending. Each cilium, when examined by **electron microscopy** and viewed in longitudinal profile, reveals an internal content of microtubules. When the cilium is viewed in cross-sectional profile, the apogeal microtubules are arranged in a distinctive nine-plus-two array. The term ***nine-plus-two*** refers to the orientation of the microtubules that make up the axoneme. In axonemes, there are two complete central microtubules (the central pair) that are surrounded by a circumferential ring of nine doublet microtubules (Figure 64). The outer doublets are arranged so that each doublet pair is composed of one complete microtubule (the ***A microtubule***) which consists of 13 protofilaments, and an incomplete microtubule (the ***B microtubule***), which is composed of only 11 protofilaments. The B microtubule shares a portion of the A microtubule wall. The "9+2 "array of microtubules courses from the tip of the cilium to its base, where the outer paired microtubules join the basal body. The basal body is a modified centriole consisting of nine short microtubule triplets arranged in a ring. Each of the paired microtubules of the cilium is continuous with two of the triplet microtubules of the basal body. The central two microtubules of the cilium end at the level of the top of the basal body. Therefore, a cross section of the basal body would reveal nine circularly arranged microtubule triplets but not the two central single microtubules of the cilium.

In addition to the microtubules, several other important proteins can be found in axonemes. These accessory proteins are absolutely essential for normal ciliary function. Extending from the A microtubule of each doublet toward the B microtubule of the neighboring doublet are two proteinaceous arms. These arms are actually the enzyme ***dynein***, the ATPase that is responsible for ciliary motility. Also extending between the A and B of the neighboring doublet is a protein called ***nexin***. This attaches neighboring doublets to each other. Finally, a ***radial spoke*** extends off each doublet and makes contact with an electron-dense sheath surrounding the central pair of microtubules, thereby connecting the doublet microtubules to the central pair. The dynein arms, nexin links, and spoke proteins exhibit a periodicity along the entire length of the axoneme. In addition to these prominent proteins, numerous other minor proteins are present in the axoneme.

Cilia undergo a regular and synchronous undulating movement. In the living state, each cilium exhibits a rapid forward movement in a rigid state, the ***effective stroke*** but becomes flexible and bends on the slower return movement, the ***recovery stroke***. The plane of movement of a cilium is perpendicular to a line joining the central pair of microtubules. Through sequential timing, the cilia in successive rows start their beat so that each row is slightly more advanced in

its cycle than the following row, thus creating a wave that sweeps across the epithelium. This ***metachronal rhytm*** is responsible for moving mucus over epithelial surfaces or facilitating the flow of fluid and other substances through tubular organs and ducts.

Ciliary activity is based on the movement of the doublet microtubules in relation to one another. The dynein arms extend from the A microtubule to form temporary cross-bridges with the B microtubule of the adjacent doublet. Addition of ATP produces a sliding movement of the bridge along the B microtubule, and as a result the cilium bends.

A structural abnormality involving absence of dynein arms has been found in some individuals with ***Kartagener's syndrome***, a hereditary disease associated with chronic respiratory difficulty (including bronchitis and sinusitis) and visceral asymmetry (they can have a complete situs inversus/ transposition of the viscera; inversion of the viscera may be related to the lack of ciliary activity during the developmental process). Inversion of the viscera may occur as a result of abnormal microtubular structure. Males with Kartagener's syndrome are sterile. The flagellum of the sperm, which is similar in structure to the cilium, is immotile. In contrast, some females afflicted with the syndrome may be fertile. In such individuals, the ciliary movement may be sufficient, though impaired, to permit transport of the ovum through the oviduct.

VII.7.2. FLAGELLA

The major type of flagellated cell in humans is the spermatozoon. For the mature sperm cell, the beating flagellum provides the force that allows the sperm to swim. The mature human sperm is about 60 µm long.

The spearm head is flattened and pointed and measures 4.5 µm long/ 3µm wide/ 1µm thick. The acrosomal cap that covers the anterior two-thirds of the nucleus contains hyaluronidase, neuroaminidase, acid phosphatase, and a trypsin-like protease called acrosin. These acrosomal enzymes are essntial for the penetration of the zona pellucida of the ovum. The release of acrosomal enzymes as the sperm touches the egg is the first step in the acrosome reaction. This is a complex process that facilitates sperm penetration and subsequent fertilization and prevents the entry of additional sperm into the ovum.

The sperm tail is subdivided into the neck, the middle piece, the principal piece, and the end piece. The short neck contains the centrioles and the origin of the coarse fibers. The middle piece, about 7 µm long, contains the mitochondria, helically wrapped around the coarse fibers and the axonemal complex. These mitochondria provide the energy for movement of the tail and, thus, the motility of the sperm. The principal piece measures about 40 µm long and contains the fibrous sheath external to the coarse fibers and the axonemal complex. The end piece, approximately the last 5 µm of the flagellum in the mature sperm, contains only the axonemal complex. Flagella has a very similar ultrastructure like the cilia, and has a helicoidal movement.

VII.7.2. CENTRIOLES AND BASAL BODIES

Centrioles are paired, short, rod-like cytoplasmic bodies visible with the light microscope. Early cytologists recognized centrioles as forming the ends of the mitotic spindle. In nondivinding cells, the centrioles are usually found close to the nucleus, often partially surrounded by the Golgi apparatus. This part of the cell was called the ***cell center, centrosome***, or ***centrosphere.***

The TEM reveals that each rod-shaped centriole is about 0.2 µm long and consists of nine triplets of microtubules that are oriented parallel to the long axis of the organelle. The three

microtubules are fused to one another, with adjacent microtubules sharing a common wall. The innermost or A microtubule is a complete ring of 13 protofilaments; the middle and outer microtubules B and C, respectively, appear C-shaped because of the protofilaments they share with each other and with the A microtubule.

The paired centrioles in a resting cell are arranged at right angles to each other but they are not connected.

Basal bodies -each cilium must have a basal body. Basal bodies are produced by the repeated replication of centrioles and the migration of the newly formed organelles to the apical surface of the cell. Each centriole-derived basal body serves as the organizer for the assembly of the microtubules of a cilium. The organizing role of the basal body is: the axonemal microtubule doublets are continuous with the A and B microtubules of the basal body from which they develop by addition of tubulin dimers at the growing end.

Basal bodies have nine peripheral triplets of microtubules.