IX. ORGANELLES INVOLVED IN MACROMOLECULAR SYNTHESIS

IX.1. THE RIBOSOMES

Ribosomes are non-membrane organelles found in the cytoplasm of all eukaryotic cells, exception are erythrocytes. The number of ribosomes varies depending on the type of cell and its activities. For example, liver cells which manufacture blood proteins, have much greater number of ribosomes than do fat cells, which synthesize triglycerides. Ribosomes play an important role in protein synthesis.

IX.1.1. STRUCTURE AND ULTRASTRUCTURE

Ribosomes cannot be detected in light microscopy, but cytoplasmic basophilia is associated with cells that are producing large amounts of protein, so those cells contain a large number of ribosomes. The presence of ribosomes was detected by electron microscopy. G.E.Palade in 1956 discovered the ribosomes, realized a structural model of ribosomes and received the Nobel Price.

Ribosomes are ovoid structures, and measure 15-20 nm in diameter. They are composed by two subunits:

-the ribosomal small subunit, which has a 40S sedimentation coefficient in eukaryotes and 30S in prokaryotes;

-the ribosomal large subunit, which has a 60S sedimentation coefficient in eukaryotes and 50S in prokaryotes.

These subunits form an 80S ribosome in eukaryotic cells, which is found either associated with the cytoplasmic surface of the endoplasmic reticulum or free within the cytosol.

Ribosomes can be:

-free, because they are not ER- membrane attached, despite the fact that many are associated with the cytoskeleton;

-associated with the cytoplasmic surface of the endoplasmic reticulum by two glycoproteins called *glycophorines* (type 1 with MW=65ooo Da and type II with MW= 63oooDa) or with the outer membrane of the nuclear envelope;

-groups of ribosomes that form short spiral arrays called **polyribosomes** or **polysomes** (Figure 87), in which many ribosomes are attached to a thread of messenger RNA (mRNA).

Membrane - bound and free ribosomes are structurally and functionally identical. Whether a ribosome becomes bound or free does not depend on the ribosome, per se, but upon the sequence of the protein being synthesized. Proteins synthesized on membrane-associated ribosomes include integral plasma membrane proteins, ER, Golgi complex, lysosomal proteins and proteins to be secreted from the cell. Free ribosomes, or more appropriately membrane-nonassociated ribosomes, are responsible for synthesis of cytosolic proteins, peripheral membrane proteins, or some proteins destined for the nucleus, mitochondria, and peroxisomes.

Ribosomes contain RNA and proteins (1/1), water and ions like -Mg^{2+} and Ca^{2+}.

IX.1.2. MOLECULAR MECHANISM OF THE PROTEIN'S BIOSYNTHESIS

The biosynthesis of proteins is realized by following rules:

- unfolds always from the amino end to the carboxyl end;

- the type of the synthesized chain is determined exclusively by the mRNA and not by the ribosomes type; the same ribosomes can synthesize various polypeptide chains, depending on the matrix they are bound on;
- the biosynthesis was studied at PK and take places in 3 stages: a) initiation phase, elongation phase and finishing phase.

a) Initiation phase:

- methionine is bound to a specific tRNA, after which the amino group is blocked by formylation;
- formylmethionyl – tRNA binds to the small subunit (30S) and together with the mRNA forms an initiation complex;
- proteins that are named initiating factors (IF_1, IF_2, IF_3) and GTP take part in the formation of the initiation complex;
- formylmethionyl – tRNA recognises he AUG codon from the mRNA;
- the initiation complex binds to the 50S subunit and forms 70S complex, the process being accompanied by the GTP hydrolysis;
- the initiation complex has, at the level of the big subunit, 2 locuses: peptidyl locus (P), where the formylmethionyl – tRNA binds and aminoacyl locus (A) which is empty in the initiation complex.

b) Elongation phase consists of three steps:

1. Insertion of another amino acid – tRNA into the A locus. The new inserted species is determined by the free codon from the A locus of the mRNA (the anticodon brought by the tRNA corresponds to the codon from the mRNA). The insertion of the new amino acid is made with the participation of a protein (elongation factor) and a GTP.
2. Formation of a peptide bound by transferring the formylmethionyl from P locus to A locus (to the amino group from the aminoacyl – tRNA). The reaction is catalysed by the peptidyl transferase. Consequently, on the P locus remains a discharged tRNA.
3. Translocation consists in: tRNA leaves the P locus, peptidyl – tRNA moves from A locus to P locus, mRNA moves with 3 nucleotides from the 5' end to the 3' end. The translocation needs the hydrolysis of the third GTP molecule and of an elongation fact named translocase.

The elongation cycle repeats itself; each time, at the end of three timing periods, the A locus becomes vacant. Each time, right in front of it, there will be a codon, which can be recognized by the tRNA- aminoacyl with complementary anticodon.

c) Finishing / ending phase takes place, when at the end of an elongation cycle, in front of the A locus from the ribosome gets one of the three nonsense codons (UAA, UAG or UGA). In the cytoplasm does not exist tRNA with anticodons complementary to these codons. The nonsense codons are recognized by a protein called "release factor", which produces the hydrolysis polypeptide – tRNA on the P locus. Thus the polypeptide chain is free into the cytoplasm, the ribosome dissociates into the two subunits, mRNA and tRNA are set free. The obtained polypeptidic chain is not the final product.

Medical implications:

- streptomycin acts on the small subunit producing the mistaken reading of the mRNA;
- tetracycline blocks the tRNA binding on the A locus;

- cloramphenicol and cycloheximide act on the big subunit, inhibiting the action of the peptidyl transferase;
- erythromycin hiders the translocation;
- puromycin determines the premature ending of the polypeptide chain.
- diphtheria toxin blocks the elongation phase at PK, inactivating the translocase.

IX.2. THE ENDOPLASMIC RETICULUM

All eukaryotic cells have an **endoplasmic reticulum (ER**), exception are erythrocytes. Its membrane typically constitutes more than half of the total membrane of an average animal cell. It is organized into a netlike labyrinth of branching tubules and flattened sacks extending throughout the cytosol. The tubules and sacks are all thought to interconnect, so that the ER membrane forms a continuous sheet enclosing a single internal space. This highly convoluted space is called **ER lumen** or the **ER cisternal space**, and it often occupies more than 10% of the total cell volume. The ER membrane separates the ER lumen from the cytosol, and it mediates the selective transfer of molecules between these two compartments.

The ER plays a central part in lipid and protein biosynthesis. Its membrane is the site of production of all the transmembrane proteins and lipids for most of the cell's organelles, including the ER itself, the Golgi apparatus, lysosomes, endosomes, secretory vesicles, and the plasma membrane. The ER membrane also makes a major contribution to mitochondrial and peroxisomal membranes by producing most of their lipids. In addition, almost all the proteins that will be secreted to the cell exterior -as well as those destined for the lumen of the ER, Golgi apparatus, or lysosomes are initially delivered to the ER lumen.

IX.2.1. STRUCTURE AND ULTRASTRUCTURE

In light microscopy, certain areas of eukaryotic cytoplasm stain intensely with basic dyes. These regions, termed ***ergastoplasm***, were found to be especially prominent in cells involved in secretion. The basophilic staining is due to the presence of RNA. The ergastoplasm in secretory cells is the light microscopic image of the organelle called the ***rough-surfaced endoplasmic reticulum*** or ***RER.***

In TEM, endoplasmic reticulum appears as a heterogeneous collection of membranous tubules, vesicles and sacks.

The relationship between the endoplasmic reticulum and the ergastoplasm of classical light microscopy first became apparent when George Palade discovered that the endoplasmic reticulum exists in two forms: -a "granular" or rough endoplasmic reticulum (RER) containing attached ribosomes;

-an "agranular" or smooth endoplasmic reticulum (SER) lacking attached ribosomes.

Ribosomes contain large amounts of RNA, an acidic molecule exhibiting a strong affinity for basic dyes. It is therefore the presence of attached ribosomes in the rough endoplasmic reticulum that causes the cytoplasm to stain with basic dyes.

The rough and smooth endoplasmic reticulum differs in ways other than the presence or absence of attached ribosomes. Rough ER is generally arranged as large flattened sheets of membrane, while smooth ER more typically consists of an interconnected series of convoluted tubules. The relative abundance of smooth and rough ER varies among cell types, with rough ER predominating in cells actively synthesizing protein for export, and an extensive smooth ER

associated with cells involved in the metabolism of steroid hormones, drugs, and toxic substances.

IX.2.2. BIOCHEMICAL COMPOSITION OF ENDOPLASMIC RETICULAR MEMBRANES:

Endoplasmic reticular membranes have a higher protein concentration (60%-70%protein by weight) than plasma membranes. Many of these proteins have important enzymatic functions. Endoplasmic reticular membranes have a lower phospholipid concentration (30%-40% by weight) than the plasma membrane.

The phospholipids of endoplasmic reticular membranes are mostly phosphatidylcholine (55%) and phosphatidylethanolamine (25%), and they have a very low concentration of sphingomyelin (5%) compared to plasma membrane.

Enzyme constituents include:

- glucose-6-phosphatase which is an ER enzyme involved in the regulation of blood glucose levels. This enzyme cleaves phosphate from glucose-6-phosphate, thus liberating glucose into the bloodstream. This enzyme is particularly abundant in the liver, which stores and metabolizes glycogen.
- cytocrome P450 is involved in detoxification.

IX.2.3. THE ROUGH ENDOPLASMIC RETICULUM - RER

Structure. RER is generally arranged as large flattened sheets of membrane. RER membranes are studded with electron-dense particles called ***ribosomes.*** Ribosomes are bound to RER membranes by ribosome-binding proteins called ***ribophorins***, which have molecular weights of 63 and 65kD. Ribophorins add some structural rigidity to membranes. In many instances, the RER is continuous with the outer membrane of the nuclear envelope.

Functions. The main function of RER is the synthesis of proteins that are eventually secreted from the cells. Ribosomes are protein synthesis sites. Nascent polypeptide chains grow on ribosomes. Copying the genetic message from DNA to messenger RNA is called transcription; reading the message in messenger RNA to synthesize polypeptides is called translation.

mRNA is synthesized (transcribed) in the nucleolus and travels from the nucleus to the cytoplasm through nuclear pores. The mRNA attaches to the polysomes that then translate the genetic message carried in the mRNA into a sequence of amino acids that are linked to form a polypeptide, the basic building block of proteins. All mRNA initially attaches to free ribosomes in the cytoplasm to form polysomes.

If the protein to be synthesized is one that will be exported or will become part of the plasma membrane, the first group of amino acids that are linked to one another form a signal peptide (signal sequence) that will bind to a receptor on the membrane of the RER. When the ribosomes (polysomes) binds to the RER membrane, the signal peptide or a subsequent sequence instructs the newly formed peptide to pass through the membrane into the lumen of the RER cistern. For simple secretory proteins, sequences along the polypeptide may instruct the forming protein to pass back and forth through the membrane, creating the functional domains that the protein will exhibit at its final membrane site.

Polysomes of the RER synthesize proteins for export from the cell and integral proteins of the plasma membrane.

As polypeptide chains are synthesized by the membrane-bound polysomes, the protein is injected into the lumen of the cistern, where it may be further modified, concentrated, or carried

to another pert of the cell in the continuous channels of the RER. The RER is particularly well developed in those cells that are making proteins destined to leave the cell as well as in cells with very large amounts of plasma membrane, such as nerve cells. The secretory cells include glandular cells, fibroblasts, plasma cells, odontoblasts, osteoblasts. The RER is not limited, however, to secretory cells and neurons. Virtually every cell of the body contains profiles of RER, but they may be few in number, a reflection of the degree of protein secretion, and dispersed so that with the light microscope they are not evident as areas of basophilia.

In agreement with the observation that the RER is most highly developed in active secretory cells, secretory proteins are exclusively synthesized by the ribosomes of the RER. In all cells, proteins that are to become permanent components of the lysosome, Golgi apparatus, RER, or nuclear envelope or integral components of the plasma membrane are also synthesized on the ribosomes of the RER.

Secretory proteins pass through the membrane of the RER to its lumen, where they may be modified and stored.

Proteins destined for secretion are unique in that they have a hydrophobic signal domain or region of the molecule at their initial forming end. The signal domain of the forming protein induces its receptor-mediated attachment to the membrane of the RER and then the insertion of the protein into and through the membrane as it is being synthesized. This is referred to as contranslational insertion of protein into the RER cisternae. If the forming protein is not to be threaded in its entirety through the membrane, a new hydrophobic domain will stop the threading process and cause the protein to be anchored permanently in the membrane at this site.

On completion of protein synthesis, the ribosome detaches from the RER membrane and is again free in the cytoplasm. The region of the newly formed protein that extends into the lumen of the RER is exposed to modification by enzymes present there. For example, most proteins receive an oligosaccharide transferred from a lipid donor to the amide N of certain asparagine residues (thus referred to as N-linked oligosaccharide). The initial hydrophobic domain is usually cleaved by a protease. Disulfide bonds and internal hydrogen bonds are established to achieve the correct three-dimensional conformation of the molecule.

Except for those few proteins that remain permanent residents of the RER membranes and those proteins secreted by the constitutive pathway, the newly synthesized proteins are normally delivered to the Golgi apparatus within minutes. In some cells in which the constitutive pathway is dominant, namely plasma cells and developing fibroblasts, newly synthesized protein may accumulate in the RER cisternae, causing their engorgement and distension.

IX.2.4. SMOOTH ENDOPLASMIC RETICULUM - SER

Regions of ER that lack bound ribosomes are called smooth endoplasmic reticulum, or smooth ER. In the great majority of cells such regions are scanty, and there is only a small region of the ER that is partly smooth and partly rough. This region is said to consist of transitional elements because it is from here that transport vesicles carrying newly synthesized proteins and lipids bud off for transport to the Golgi apparatus. In certain specialized cells, the smooth ER is abundant and has additional functions. In particular, it is usually prominent in cells that specialize in ***lipid metabolism***: cells that synthesize steroid hormones from cholesterol, for example, have an expanded smooth ER compartment to accommodate the enzymes needed to make cholesterol and to modify it to form the hormones.

The main cell type in the liver, the hepatocyte, provides another example. It is the principal site of the production of lipoprotein particles; these particles carry lipids via the bloodstream to other sites in the body. The enzymes that synthesize the lipid components of lipoproteins are located in the membrane of the smooth ER, which also contains enzymes that

catalyze a series of reactions to detoxify both lipid-soluble drugs and various harmful compounds produced by metabolism. The most extensively studied of the ***detoxification reactions*** are catalyzed by the ***cytochrome P450 family*** of enzymes, which catalyze a series of reactions whereby water-insoluble drugs or metabolites that would otherwise accumulate to toxic levels in cell membranes are rendered sufficiently water-soluble to leave the cell and be excreted in the urine. Because the rough ER alone cannot house enough of these and other necessary enzymes, a major portion of the membrane in a hepatocyte normally consists of smooth ER.

When large quantities of certain compounds, such as the drug phenobarbital, enter the circulation, detoxification enzymes are synthesized in the liver in unusually large amounts, and the smooth ER doubles in surface area within a few days. Once the drug disappears, the excess smooth ER membrane is specifically and rapidly removed by lysosome-dependent process called autophagocytosis.

Another function of the ER in most eukaryotic cells is ***to sequester Ca^{2+}*** into the cytosol from the ER, and its subsequent reuptake, mediate many rapid responses to extracellular signals. The storage of Ca^{2+} in the ER lumen is facilitated by the high concentrations of Ca^{2+}-binding proteins there. In some cell types, and perhaps in most, specific regions of the ER are specialized for Ca^{2+} storage. Muscle cells, for example, have an abundant specialized smooth ER, called the ***sarcoplasmatic reticulum***, which sequesters Ca^{2+} from the cytosol by means of a Ca^{2+}-ATPase that pumps in Ca^{2+}; the release and reuptake of Ca^{2+} by the sarcoplasmatic reticulum mediates the contraction and relaxation of the myofibrils during each round of muscle contraction.

IX.3. THE GOLGI APPARATUS

The Golgi apparatus was described more than 100 years ago by Camillo Golgi. In studies of osmium-impregnated nerve cells, he discovered an organelle that formed net-works around the nucleus. It was also described as well developed in secretory cells. Changes in the shape and location of the Golgi complex relative to secretory state were described even before it was viewed with the electron microscope and its functional relationship to the RER was established. It is very active both in cells that secrete protein by exocytosis and in cells, such as nerve cells that synthesize large amounts of plasma membrane and membrane-associated proteins. The Golgi apparatus does not stain with hematoxylin or eosin.

In the ***light microscope***, secretary cells that have a large Googly apparatus, plasma cells, osteoblasts, or cells of the epididymis, typically exhibit a clear area partially surrounded by the ergastoplasm.

In ***electron microscopy*** we see that the Golgi apparatus is usually located near the cell nucleus, and in animal cells it is often close to the centrosome, or cell center. We also can describe the ultrastructure.

IX.3.1. ULTRASTRUCTURE. It consists of a collection of flattened, membrane-bounded ***cisternae*** and thus resembles a stack of plates. Each of these ***Golgi stacks*** usually consists of four to six cisternae. The number of Golgi stacks per cell varies greatly depending on the cell type, but there are many in secretory cells. Swarms of small vesicles are associated with the Golgi stacks, clustered on the side adjoining the ER and along the dilated rims of each cisternae. These ***Golgi vesicles*** are though to transport proteins and lipids both to and from the Golgi apparatus and between the Golgi cistarnae. During their passage through the Golgi apparatus, the transported molecules undergo an ordered series of covalent modifications.

Each Golgi stack has two distinct faces: ***a cis face (or the forming face* or entry face)** and a ***trans face (or the maturing face or exit face).*** Both the ***cis*** and ***trans faces*** are closely connected to special compartments, which are composed of a network of interconnected tubular and cisternal structures. These are the cis Golgi network (also called the intermediary or salvage compartment) and the trans Golgi network, respectively (Figure 93). Proteins and lipids enter the cis Golgi network in transport vesicles from the ER and exit from the trans Golgi network in transport vesicles destined for the cell surface or another compartment. Both networks are thought to be important for protein sorting: proteins entering the cis Golgi network can either move onward in the Golgi apparatus or be returned to the ER; proteins exiting the trans Golgi network are sorted according to whether they are destined for lysosomes, secretory vesicles, or cell surface.

The Golgi apparatus is especially prominent in cells that are specialized for secretion, such as the goblet cells of the intestinal epithelium, which secrete large amounts of polysaccharide-rich mucus into the gut. In such cells unusually large vesicles are found on the trans side of the Golgi apparatus, which faces the plasma membrane domain where secretion occurs.

IX.3.2. FUNCTIONS

1. Protein synthesis. As we discussed, proteins initially synthesized in the endoplasmic reticulum pass to the Golgi apparatus through cis face. It is in the Golgi that oligosaccharides are added to proteins in the synthesis of most glycoproteins, due to the action of Golgi membrane enzymes called glycosyl transferase; and that sulfate groups are added to proteoglycans.
2. Membrane synthesis begins in the endoplasmatic reticulum and continues in the Golgi apparatus. New membranes move to, and fuse with, the plasma membrane. Also Golgi apparatus has a major role in membrane recycling.
3. The Golgi complex may have a dehydrating function relative to the packaging of secretory material. A proton pump has been identified in the Golgi of some cells and is believed to be involved in the concentration (dehydration) of secretory granules.
4. The production of lysosomes -is possible because the lysosomal hydrolases carry a unique marker in the form of the mannose 6 phosphate (M6P) groups, which are added exclusively to the soluble lysosomal enzymes. The M6P groups are recognized by M6P receptor proteins, which are transmembrane proteins present in the trans Golgi network. These receptor proteins bind the lysosomal hydrolases and help package (segregate) them into specific transport vesicles called clathrin -coated vesicles that bud from the trans Golgi network and form the primary lysosomes.

Stages of the secretory cycle:

1. Synthesis and segregation of export proteins at RER level (1min.). By means of channels mediated by ribophorins, the polypeptide chain gets into the RER lumen.
2. Transport through the RER channels to the Golgi apparatus (20-24 min.)
3. Protein condensation and maturation in the Golgi apparatus (1 hour).
4. Intracellular storage as secretory vesicles.
5. Exocytosis depends on the intracellular Ca^{++} concentration and the presence of ATP and cAMP. There are exocytated hormones, enzymes, immunoglobulines, neurotransmitters, serum constituents, components of the glycocalyx, terminal metabolic products.