X. ORGANELLES OF THE ENERGOGENESIS. MITOCHONDRIA

Mitochondria are the organelles specialized in generating the energy necessary for carrying out the vital processes of the animal eukaryotic cell. There are non-specific intracytoplasmic organelles, present in all cell types, except for the red cells. In vegetal cells, the organelles where the transformation of the light energy into, chemical energy takes place are the chloroplasts.

X.1. MORPHOLOGY

- ***Form and dimension*** vary from one cellular type to another, in light microscopy, on fixed compositions; there were described three "static" forms:

-granular shape – mitochondria;
-chain/strings of granules – chondriomite;
-long shape (sticks) – chondriocont.

- ***The dimensions*** are variable, with transverse diameter between 0.1-0.5 μ and longitudinal diameter between 1-5 μ.
- ***The number varies*** depending on the cellular type, cell age and metabolic activity. For example, there are 3000 miochondria in hepatocyte, 300 mitochondria in nephrocyte and 24 mitochondria in spermatozoon (around the axoneme of intermediary piece).
- ***The intracellular disposal*** varies depending on the cellular type and on the cell's momentary energetic needs:

-spread out over the whole cellular area (hepatocyte);
-at the metabolic active pole- basal pole (entrecote, nephrocyte) and apical pole (secretory cells);
-around the nucleus - in the S phase of the cellular cycle (DNA synthesis).

X.2. ULTRASTRUCTURE

The electron microscopy has proved that the ultrastructure of the mitochondria is ideally adapted to the function of the ATP production. In EM the mitochondria are composed by outer membrane, intermembrane space, inner membrane and matrix.

1. Outer membrane - is trilaminated, lipoproteic (contains 60% proteins, 40% lipids); it is smooth, with a thickness of 6 nm. The outer membrane contains an increased quantity of cholesterol and a reduced quantity of cardiolipin (diphosphatidyl-glycerol), which grants the membrane a better permeability and realises so the changes between the cytosol and organelle. The major component of the membrane is an integral protein – porin – which has the capacity of forming channels in the external membrane, which are permeable to molecules with molecular weight lower than 10000 daltons. In conclusion, the intermembrane space and the cytoplasm can be considered a continuous compartment for molecules smaller than 10000 daltons.

It contains a great number of enzymes: CoA ligase (facilities the passing of the fatty acids from the cytosol); monoamino oxidase (catalyses the dissemination reactions of mono- and diamines).

2. Intermembrane space is electrono-clear, homogenous in EM, with a diameter between 7-8 nm. It performs an active role in the transportation of substances from the cytosol to matrix and reverse. This space contains several enzymes that use the ATP passing out of the matrix to phosphorylate other nucleotides: adenylate kinase (maintains the balance ATP/ADP/AMP), nucleosidediphospho kinase.

In pathological cases, anorganic material accumulations (calcium salts) can be found, which appear electronodense in EM.

3. Inner membrane is trilaminated, lipoproteic (80% proteins and 20% lipids). The great proteins quantity ensures an intense functional activity at the internal membrane level: the ATP synthesis mechanisms, the respiratory chain, transportation of the underlayers and products originated in the oxidative phosphorylation to the inside and outside of the mitochondrion.

It has a low cholesterol content and an increased cardiolipin content (phospholipid with the function of reducing the lipid bilayer permeability for small ions; so it represents a barrier between the mitochondrial matrix and the rest of he mitochondrion.

The inner membrane presents invaginations "mitochondrial cristae", which lead to an important increase of the surface. The form of cristae differs with the cellular type: lamellar, tubular, vesicular; short or long; rarely a crista crosses the mitochondrion. The position is also different: perpendicular or parallel to the longitudinal axis, spiral and radial. The number of cristae is greater when the cells are in full activity and smaller when the cell is at rest.

The presence of some membranous subunits named submitochondrial particles (elementary particles, electrons transporting particles) has been described by applying negative staining techniques in EM on the matrix side of the inner membrane. Submitochondrial particles are of protein nature (ATP – synthetase) and are composed of:

- F_0 portion / part, which crosses the lipid molecular bilayer. It contains 5-8 subunits classified in subunits "a" and "b" with a role in protons guiding; a subunit "c" of protein-lipid nature ad 2-5 accessory proteins.
- F_1 portion / part, attached to F_0, towards the mitochondrial matrix. It is composed of 9 subunits, noted: 3α, 3β, γ, δ, ε, with oligomeric structure. α subunits have the role of catalyzing the conversion of ADP to ATP; β subunits regulate the ADP/ATP level; γ and δ subunits bind F_1 to F_0 and the role of subunit ε is unknown (Figure 99).

Each of the membranous subunits contains the enzymes of a respiratory chain, and the submitochondrial particles number represents the number of respiratory chains /mitochondrion. This number differs from one cellular type to another; hepatic cells – 15000 submitochondrial particles/ mitochondrion, and cardiac cells – 40000 submitochondrial particles/ mitochondrion.

The following groups of enzymes are situated on the inner membrane:

a) enzymes of the electrons-transporting chain: cytochrome oxidase (the marker enzyme of the inner membrane), NADH coenzyme C reductase (complex I), succinate coenzyme C reductase (complex II), CH_2 cytochrome C reductase (complex III), cytochrome C reductase (complex IV);
b) enzymes of the Krebs cycle: succinate dehydrogenase (SDH) which represents the marker enzyme, β hydroxybutirate dehydrogenase;
c) enzymes of the oxidative phosphorylation ADP$\rightarrow$ATP;
d) enzymes from the elongation system of fatty acids;
e) cytochrome c, F_1, a, ATPase.

4. The mitochondrial matrix contains mitochondrial DNA, ribosomes (ribosomal RNA and ribosomal proteins), about 200 enzymes and contractile proteins. Te mitochondrial DNA is a circular molecule resembling the one in the prokaryote. The human mitochondrial genome has a complete sequence and contains 16569 nucleotides (less than 10^{-5} times the size of the nuclear genome). Each mitochondrion contains 5 – 10 genomes and mitochondrial DNA represents less than 1% from the total cellular DNA. The enzymes are represented by the enzymes of the Krebs cycle (malate dehydrogenase, isocitrate dehydrogenase, fumarase), enzymes involved in the metabolism of the amino acids, enzymes of the fatty acids oxidation. The proteins realize a gel consistency of the matrix, with viscous aspect.

X.3. MITOCHONDRION'S FUNCTION

The metabolic processes located in the mitochondrion can be classified as follows: I) processes of the energetic metabolism; II) biosynthetic processes.

I. Processes of the energetic metabolism

Mitochondria are real "cellular thermal power-plants" which convert and set free the energy stored in simple organic substances (carbohydrates, fatty acids, amino acids) into a macroergic compound (ATP). This compound, on the action of ATPase, is split and liberates the energy it contains. By complete oxidation at CO_2 and H_2O, each glucose molecule produces 36 ATP molecules (compared with the anaerob glycolisis, where 1 glucose molecule produces 2 ATP molecules). The cells have fat deposits (triglycerides) and sugars (glycogen) in the adipose tissue, liver and muscles in order to ensure the continuous supply of fatty acids and pyruvate. In case of absence of exogen contribution, the lipid deposits are sufficient for one month and the glycogen for one day.

In 1961 Mitchell assumed that the mitochondrial production of ATP is realized through a mechanism which he named "chemiosmotic coupling". The chemiosmotic couplimg takes place in three stages: a) cytoplasmic stage, b) matrix stage and c) membranous stage (inner membrane stage).

a) Cytoplasmic stage

- At the level of adipose cells, the free fatty acids are set free into the blood, cross the plasma membrane and once they are in the cytoplasm, they are converted to acyl CoA which can passes the mitochondrial membranes.
- Glucose is converted into pyruvate by the means of the glycolysis Embden-Meyerhoff path:

$$Glucose + 2NAD^{+} + 2ADP^{2-} + 2Pi \rightarrow 2pyruvate + 2NADH + 2ATP^{4-} + 2H^{+}$$

The pyruvate passes the mitochondrial membranes to the matrix.

b) Matrix stage – sublayer oxidation

- Acyl CoA originated in the oxidation of fatty acids undergoes a cycle of reactions that determine the shortening of the molecule with 2 carbon atoms and the producing of an acetyl CoA molecule / cycle.
- The pyruvate originated in the glycolisis process is also converted to acetyl CoA.

- Acetyl CoA represents the base sublayer of the citric cycle (Krebs). Acetyl CoA oxidation, at each round of the citric cycle, leads to obtaining of $2CO_2$ (which will be eliminated from the cell), 3NADH (nicotinamide adenine dinucleotide) and $1FADH_2$ (flavin adenine dinucleotide).

c) Membranous stage (inner membrane stage)

The three NADH molecules and the $FADH_2$ molecule transfer two electrons to the acceptor molecules situated at the level of the mitochondrial inner membrane. This fact will determine the reducing of oxygen and producing of water (within the respiratory chain) and simultaneously the producing of ATP (phosphorylation).

The respiratory chain (the chain of transfer oxidation) represents an electrons transporting chain, situated at the level of inner membrane on its outer part. The electrons transfer from NADH and $FADH_2$ to the oxygen is catalysed by a series of electrons "carriers" associate with 4 complex proteins:

- NADH dehydrogenase complex is the largest of the respiratory enzyme complexes, with a mass of about 8000000 daltons and more than 22 polypeptide chain;
- ubiquinone (coenzyme Q);
- b-c_1 complex;
- cytochrome oxidase complex is the best characterized complex with a mass of about 300000 daltons.

The reaction chain unfolds in 3 stages (Figure 100):

- the NADH dehydrogenase complex takes over electrons fro NADH and transfer these to the ubiquinone;
- b-c_1 complex takes over electrons from ubiquinone and transfer them to the cytochrome c;
- the cytochrome oxidase complex accepts $4e^-$ from the cytochrome c and delivers to it O_2 like in the following reaction: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

At the same time with the electrons transfer from one complex to another, there also takes place the protons' movement from the matrix side to the outer side of the inner membrane. The protons pass to the mitochondrial space through the channels made by porins. The protons (H^+) movement from the matrix to the intermembrane space has two effects:

- the potential of the inner membrane is of 160 mV (negative on the matrix side and positive on the outer side);
- the pH – value of the intermembrane and cytoplasmic space is about 7, compared to the matrix space which is 8.

The coupling of oxidation with phosphorylation – The protons resulted from the respiratory chain, pumped towards the matrix, return in the inner membrane at the level of ATP – synthetase complex, where they take part in the ATP synthesis. The newly synthesized ATP moves from the mitochondrial matrix in the intermembrane space and towards the cytoplasm through an antiport system with the ADP.

In conclusion, the processes of the energetic metabolism at mitochondrial level are realized in three stages:

1. The sublayer oxidation within the Krebs cycle, which represents either the final path for the oxidation of glucose, lipids, and proteins; and the main source of electrons for the respiratory chain. The sublayer oxidation is realized in the mitochondrial matrix.
2. The respiratory chain (transfer oxidation chain), which determines the water formation and the pumping of the protons towards the intermembrane space. The process takes place on the outer side f the inner membrane.

3.Oxidative phosphorylation (ADP + P_i → ATP) takes place on the matrix side of the inner membrane. Oxidation of a NADH molecule produces 3 ATP molecules, and of a $FADH_2$ molecule produces 2 ATP molecules.

II. Biosynthetic processes

1.Extranuclear heredity - The mitochondrial matrix contains DNA, RNA, ribosomes and the enzymes necessary for the expression of the mitochondrial genes. The human mitochondrial genome, with circular organisation, was arranged sequentially. Almost the whole genome is made of encoded sequences. The 16569 nucleotides form genes for the codification of 13 proteins (subunits of the complex proteins involved in electrons transport and the oxidative phosphorylation); 2 genes for rRNA and 22 genes for tRNA. The mitochondrial genes originate only from the mother.
2.Realize stages from the hem synthesis.
3.Mitochondria represent a reservoir for calcium, accumulated in the matrix as calcium phosphate or in the intermembrane space.

X.4. THE ORIGIN OF MITOCHONDRIA

The endosymbiont hypothesis has a historical value. Prokaryotes penetrated into the eukaryotic cell, which lost their virulence, surrounded themselves by a second membrane and adapted to the host's metabolism.

Mitochondria renew continuously by autophagy, "de nuovo" by the components' biosynthesis, by chondrodieresis (splitting process of the functional mitochondria). Usually, they double their mass and divide themselves into two parts at each cellular cycle. There are also mitochondria, which divide more rapidly and others which don't divide at all over the whole period of a cellular cycle.