D. Mitochondria and the oxidative phosphorylation chain

One of the fundamental differences between eukaryotic cells and prokaryotic cells is the presence in eukaryotic cells of organelles that divide the cell space into specialized compartments. Mitochondria are one such organelle and play a key role in cell function. In particular, they are responsible for producing ATP, a high-energy molecule involved in most cellular metabolic pathways. Mitochondria provide 90% of the energy used by our cells and are considered the "powerhouses" of the cell.

I. Theory of the prokaryotic origin of mitochondria

Mitochondria probably evolved from aerobic prokaryotic bacteria that were internalized by primitive eukaryotic cells.

Arguments in favor of the endosymbiotic theory:

-No nucleus but 2 to 10 circular DNA molecules.

-Sequence homology with the DNA of certain bacteria.

-Relationship between mitoribosomes and bacterial ribosomes: sensitivity to certain antibiotics (chloramphenicol)

-Biochemical composition of the inner membrane: absence of cholesterol and presence of cardiolipin.

II. Morphological appearance of mitochondria under an optical microscope

These are the oldest known organelles. They are large (1-2 to 10 μm long and 0.5 to 1 μm large), so they can be seen under an optical microscope. In fixed cells, three different appearances can be distinguished (this structural polymorphism does not alter their function):

- In the form of granules = these are mitochondria
- In the form of filaments or bacilli = chondrioconts
- In strings of granules = chondriomites.

The collection of mitochondria in a cell is called a chondriome. Mitochondria are always present in very large numbers, proportional to the cell's energy requirement. They are located in the most active area of the cell. They are found in high quantities in cells with high energy requirements, such as muscle cells or the flagellum of a sperm cell. Conversely, they are found in limited numbers in plant cells.

III. Structure and composition

III.1. Global distribution

- H_2O à 65%
- Proteins 20%
- Lipids 10%

- Nucleotides 1%
- Cations
- Vitamins A and C

III.2. Organization of the mitochondria

It is bounded by two membranes: an outer membrane (relatively flat and smooth) and an inner membrane (highly folded). They are formed of a lipid bilayer and proteins. Between the two is the intermembrane space (outer chamber), which is 6 to 8 nm thick. The space enclosed by the inner membrane is the matrix space (inner chamber) containing the matrix.

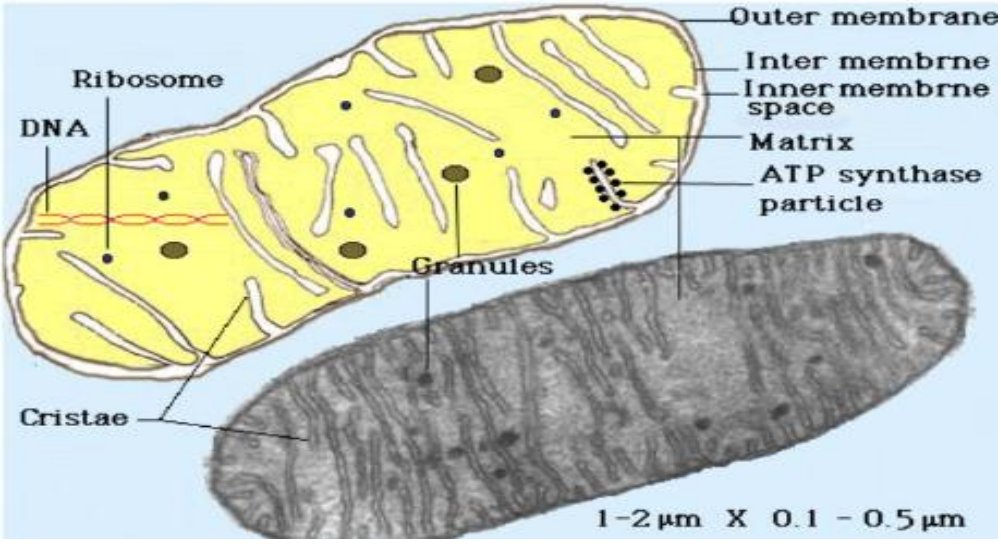

Figure III. Mitochondrial ultrastructure **[29].**

• **The outer membrane:** is permeable to all molecules of 5 kDa or less through the presence of porins. It also contains translocases, protein transporters involved in protein import.

• **The inner membrane:** folds to form numerous cristae, which increases its total surface area. The cristae come in different shapes: tubular, saccular, laminar, and triangular, which can co-exist in the same mitochondrion and evolve over time. The lipid composition of the inner membrane is unique: it contains a majority of phosphatidylcholine and cardiolipin. This membrane contains the electron transport chain, ATP synthase, and numerous transporters that facilitate the passage of elements such as pyruvate, fatty acids, ATP, ADP, and $H_2PO_4^-$, which are compounds necessary for ATP production. The inner membrane also contains translocases, which are involved in protein import.

• **The internal chamber (matrix space):** dense fluid containing polynucleotides (DNA and RNA) and phosphate nucleotides (ADP, ATP). There are also small ribosomes called mitoribosomes. Finally,

there are reserves in the form of granules (calcium phosphates). There are also numerous proteins (the protein concentration is 500 mg/ml in the matrix), including around a hundred enzymes and coenzymes:

- Oxidation enzymes;
- Enzymes involved in the replication, transcription, and translation of genetic material;
- Coenzyme A, a small molecule (derived from a B vitamin) involved in the enzymatic transfer of acyl groups. Finally, Mg^{+2} and Ca^{+2} ions (20 to 50% of cellular calcium is stored in the mitochondria).

• **The outer chamber (intermembrane space):** kinase-type enzymes are found here, which catalyze the phosphorylation of various molecules. Of particular note is the presence of adenyl kinases, which catalyze the phosphorylation of AMP to ADP according to the reaction: AMP + ATP ==> 2 ADP

IV. Glycolysis, β-oxidation, and the citric acid cycle

In the cytosol, glucose enters the metabolic pathway known as glycolysis, which consists of a series of reactions that convert one molecule of glucose into two molecules of pyruvate, with the associated production of 2 ATP and 2 NADH, H+. Pyruvate is transported into the mitochondria, where it is then converted into acetyl-CoA by pyruvate dehydrogenase. Fatty acids are imported into the mitochondria and also converted into acetyl-CoA. Acetyl-CoA then enters a third metabolic pathway, the citric acid cycle, in which it is dehydrogenated, producing $FADH_2$, NADH, H^+, and CO_2.

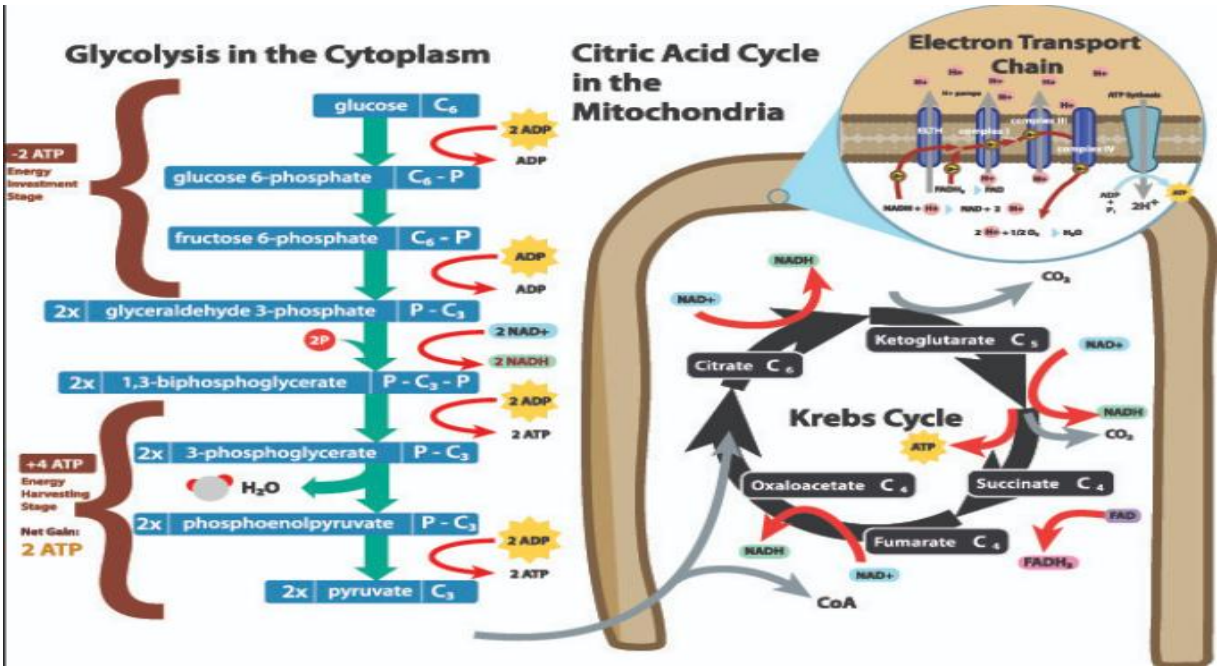

Figure III. Localization of metabolic processes **[30].**

V. Fonctions of mitochondria

V.1. Oxidative phosphorylation

V.1.1. The respiratory chain

The respiratory chain is located in the inner mitochondrial membrane. This electron transport chain consists of four protein complexes:

- Complex I: NADH-coenzyme Q oxidoreductase;
- Complex II: succinate-coenzyme Q oxidoreductase;
- Complex III: coenzyme Q-cytochrome c oxidoreductase;
- Complex IV: cytochrome c oxidase;
- Coenzyme Q (ubiquinone) and cytochrome C: are mobile carriers in the respiratory chain.

Much of the energy produced in catabolic pathways is contained in NADH and $FADH_2$; it will be converted into ATP in the mitochondria: reduced mitochondrial coenzymes transfer their two electrons to a system of transporters which, through a cascade of redox reactions, carries these electrons to the final acceptor, molecular oxygen. The inner membrane is impermeable to H^+ ions, but during this electron transfer, a proton gradient forms on either side of the membrane, enabling ATP synthesis in a reaction catalyzed by mitochondrial ATP synthase. Respiration and ADP phosphorylation are therefore coupled via this proton gradient.

NADH, formed in the matrix, transfers its reducing equivalents to NADH dehydrogenase (complex I), accessible via the internal surface of the inner membrane. These electrons travel from complex I to molecular oxygen via coenzyme Q, complex III, cytochrome C, and complex IV. Molecular oxygen, the final electron acceptor, is reduced to H_2O.

The FADH2 produced in the mitochondrial matrix transfers its reducing equivalents to complex II. The electron pathway is then identical to that followed by the electrons supplied by NADH (coenzyme Q - complex III - cytochrome C - complex IV).

During this transfer, protons are expelled from the matrix into the intermembrane space (at complexes I, III, and IV), creating an electrochemical gradient of H+ ions on either side of the inner membrane that contains the energy of oxidation. It consists of a pH gradient (the matrix becomes more basic) and a charge gradient (the matrix side of the inner membrane is negatively charged).

Protons can only return to the matrix through specific passages formed by ATP synthase. The electrochemical gradient of protons thus provides the energy necessary for ATP synthesis; it is said to be discharged.

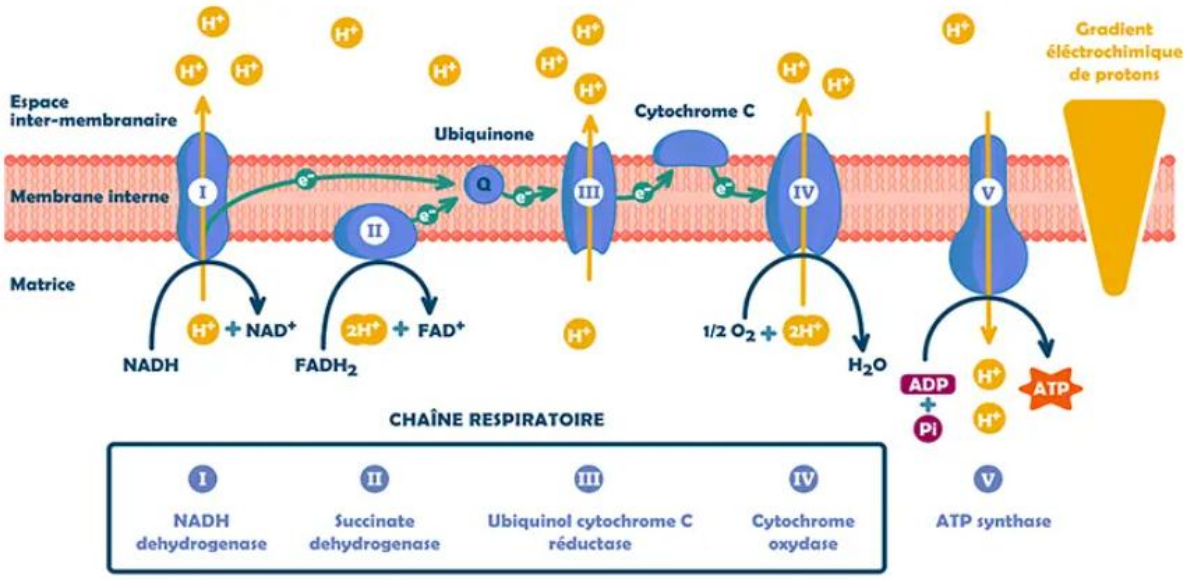

Figure III. Functioning of oxidative phosphorylation complexes **[31].**

V.1.2. ATP synthase

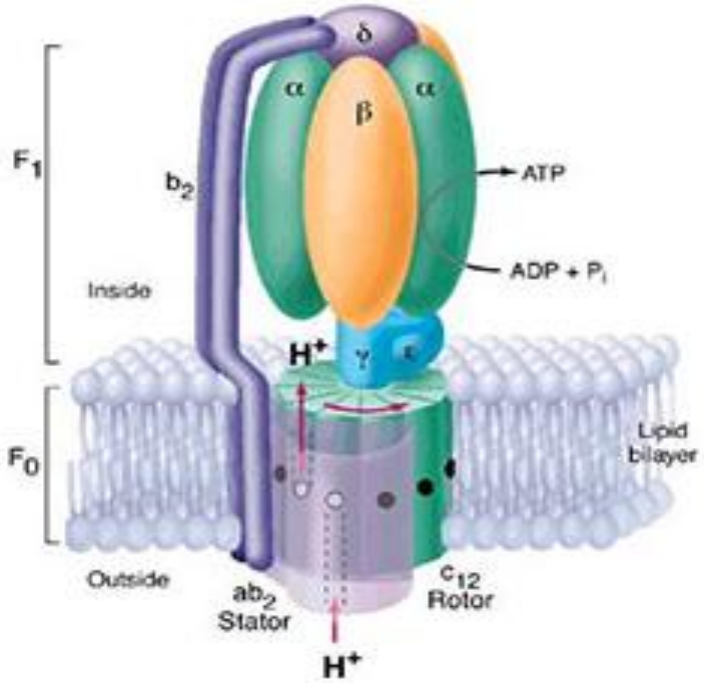

Figure III. Structure of ATP synthase **[32].**

Cellular respiration provides the energy needed to produce ATP. Cellular respiration creates a gradient of protons (H+), which accumulate on one side of the membrane. In the presence of this proton gradient (proton motive force), the protons pass to the other side of the membrane through the ATP synthase protein complex and release energy that is reused for ATP synthesis (attaching the phosphate group to ADP to form ATP). It consists of an intramembrane F0 subunit that acts as a proton channel, an F1 subunit bathed in the mitochondrial matrix that has ATP synthase activity, and a static part that stabilizes the structure.

V.2. Role of mitochondria in steroid hormone synthesis

Mitochondria are involved in the biosynthesis of steroid hormones, of which cholesterol is the precursor. Cytochromes P450scc and P450aldo, in the mitochondrial matrix, are responsible for introducing cholesterol into the steroid biosynthesis pathway. Depending on the endocrine gland in which it occurs, this biosynthesis can lead to the formation of:

- testosterone, in the testicles,
- progesterone and estradiol, in the ovaries,
- glucocorticoids such as cortisol
- mineralocorticoids such as aldosterone (role in ion balance), in the adrenal gland.

V.3. Role of mitochondria in Ca^{2+} homeostasis

Mitochondria (along with the endoplasmic reticulum) also participate in regulating intracellular calcium concentration. However, the membrane pump that ensures the passage of Ca^{2+} into the matrix has not yet been identified.

V.4. Role of mitochondria in programmed cell death (apoptosis)

In this process, the leakage of cytochrome c through a newly created pore in the outer mitochondrial membrane, PTPC (permeability transition pore complex), which includes, among other proteins, the VDAC anion channel (mentioned above), plays a key role. Cytochrome c, thus released into the cytoplasm, participates in the assembly of a protein complex (apoptosome), responsible for the activation of proteases, proteolytic enzymes called caspases. Caspases destroy important molecular components of the nucleus and cytoplasm, leading to cell death. This cell will be phagocytosed by macrophages or neighboring cells, leaving no trace, which prevents the triggering of an inflammatory response.