Metabolic Minimap

Mitochondrial ATP Formation

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This minimap is perhaps the second most vital of them all (the first, of course, being photosynthesis), but its fairly obvious title has been the subject of much thought. The object has been to interrelate three pathways in which energy generated by respiration is coupled to the synthesis of ATP from ADP and phosphate. The formation of ATP is the end point, the consummation, of catabolism and is the major purpose of what in our metabolic pathways chart we designate and identify as the backbone of metabolism. This starts with glycolysis, which takes place in the cytoplasm, and leads on to pyruvate dehydrogenase, the citric acid cycle, and oxidative phosphorylation, all of which take place in the mitochondria. Oxidative phosphorylation, however, is no longer an appropriate description of the two interrelated but separated sequences that lead to ATP formation. The phosphorylation is not itself oxidative but depends on the product of the preceding oxidative sequence *i.e.* translocated protons. The (oxidative) electromotive proton translocation provides the proton motive force that then drives ATP synthesis. Mitochondrial ATP formation seemed to be a more accurate description for the whole process than ATP synthesis, because there are 14 prior reactions in the synthesis of ADP. Three pathways are shown in this minimap (Fig. 1), the citric acid cycle, including pyruvate oxidation, aerobic oxidation of NADH and succinate, and ATP synthesis. Some relevant, and hopefully controversial, points about each are now considered.

THE CITRIC ACID CYCLE, INCLUDING PYRUVATE OXIDATION

This starts with pyruvate derived mainly as the product of glycolysis of carbohydrates in the cytoplasm. This is oxidatively decarboxylated to acetyl-CoA, which is also the product of lipid catabolism within the mitochondrial matrix. Acetyl-CoA enters the citric acid cycle where it undergoes four oxidation reactions resulting in the formation of three NADH and one succinate, together with CO_2 and water.

The vital importance of NADH formation is accentuated in the map by red arrows, and this led to a search for the origin of its hydride ions, also intended to be shown in red. In the case of isocitrate and malate the answer seemed clear; straightforward dehydrogenation! However, in malate both hydrogens come from water in the previous reaction. I never anticipated any problems with the pyruvate and oxoglutarate dehydrogenase reactions. I had been happy to live with the citric acid cycle for 60 years but had never really thought about this part of it before. Where do the hydride ions in the NADH come from? There is no structural hydrogen that can be utilized, and whereas coenzymes may be a transient source they cannot (by definition) be the ultimate. Although the electrons of the hydride ion come from the carbon skeletons of the substrates being oxidized, the protons must come from water. I consulted my text books, but none asked, let alone answered the question, so I consulted colleagues. They thought about it and then (nearly, but not all) acquiesced, sometimes reluctantly, and some thought it of little importance. However, most of the textbooks do discuss the more academic question of the origin of the CO_2 evolved in the citric acid cycle but ignore what seems to me to be much more important reactions involving the fundamental purpose of the cycle, the provision of NADH. The significance of water in biochemistry can often fail to be appreciated.

All the reactions of the citric acid cycle take place in the mitochondrial matrix with the exception of succinic dehydrogenase, which is part of Complex II of the inner membrane. It is important not to regard $FADH_2$ as the product of this reaction, which is still often done. FAD is the first, but only a transient, carrier of electrons from succinate to ubiquinone. Indeed the official name of the enzyme is succinate dehydrogenase (ubiquinone).

AEROBIC OXIDATION

The mitochondrion is often regarded as the powerhouse of the cell, and this designation becomes much more meaningful if we remember that a flow of electrons is an electric current, and NADH and succinate provide the fuel for an electricity generator. The pathway is often called the electron transport chain, but its function is to create a flow of electrons (shown in Fig. 1 as heavy red arrows) to provide the energy needed to translocate protons from the mitochondrial matrix to the intermembrane space (shown in Fig. 1 as heavy blue arrows).

Four complexes are involved. Complexes I and II are linked to Complex III via the Q-cycle and Complexes III or IV via cytochrome *c*. Proton translocation takes place in Complexes I, III, and IV (approximately) as shown.

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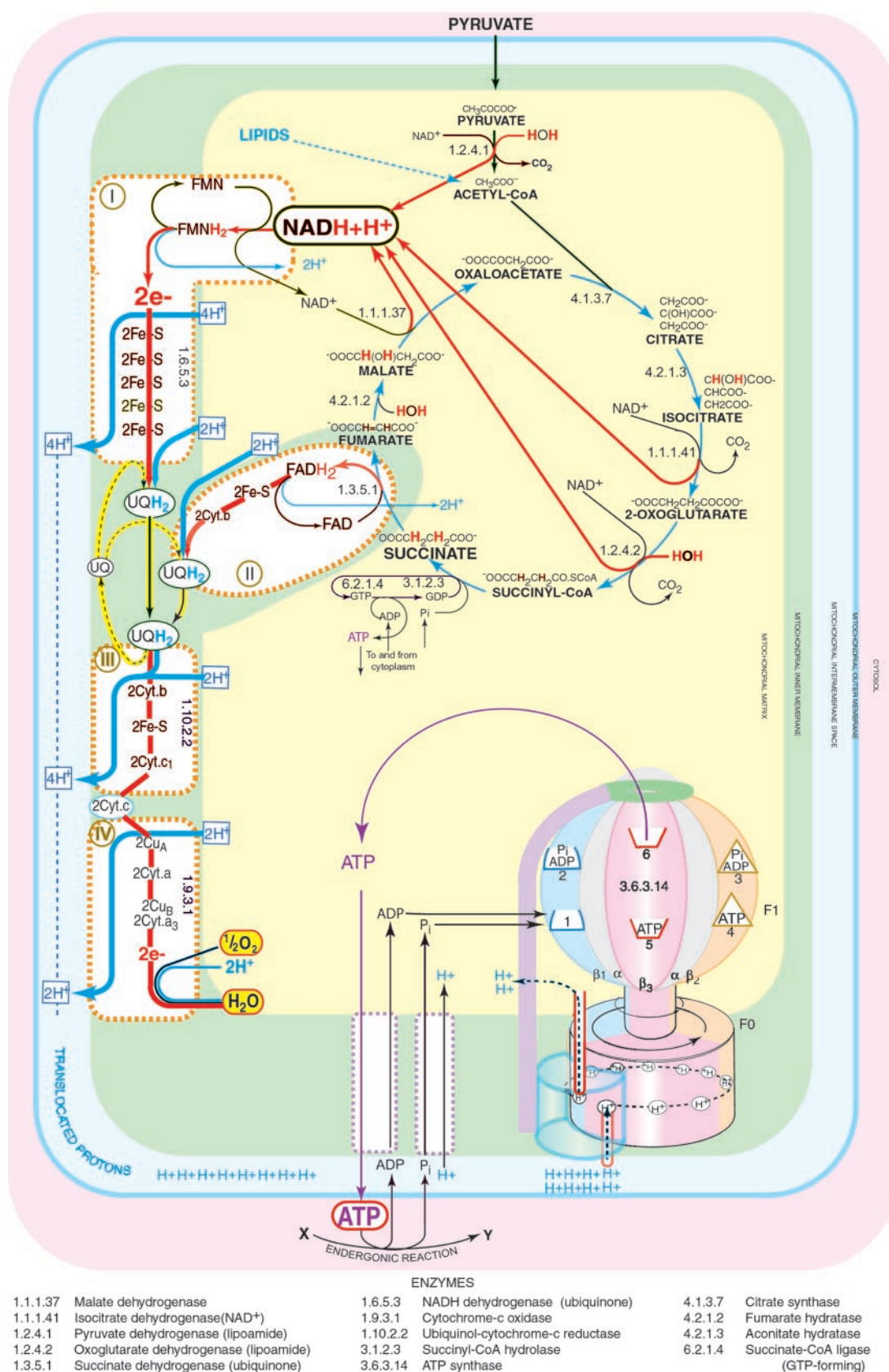# MITOCHONDRIAL ATP FORMATION

FIG. 1. **A metabolic minimap of mitochondrial ATP formation.**

ATP SYNTHASE

This is the all-important reaction in which the proton motive force produced by proton translocation is coupled to the synthesis of ATP from ADP and phosphate. ATP synthase is a complex structure consisting of two domains, F_0 and F_1. F_1 is a spherical structure, which in the case of mitochondria, sticks out into the matrix and is anchored to the membrane by a stator to prevent rotation. It consists of three α- and three β-subunits, all of which can bind nucleotides, but only the β-subunits take part in the synthetic reactions. F_0 is a cylindrical structure capable of rotation when driven by translocated protons and which is linked to a central stalk that can revolve inside F_1. The mechanism that drives ATP synthesis seems to depend on a binding charge concept in which catalytic sites on the β-subunits have different affinities for nucleotides and are designated loose (L), tight (T), and open (O). These are pictured in Fig. 1 on the minimap on the β-subunits colored blue, brown, and pink respectively. The loose (L) sites bind the substrates (ADP and phosphate) reversibly. The T sites then bind the reactants so tightly that ATP is formed. The O sites, which have a very low affinity for substrates, then release the ATP already formed in the T state. The central stalk is driven by the passage of protons through F_0 (counter-clockwise as seen from above), and rotates in 120° stages. At each stage each of the β-subunits in turn change conformation; L changes to T (after binding ADP and phosphate), T to O, and O to L (after releasing ATP). The new L site then binds new ADP and phosphate and begins a new reaction sequence.

One complete revolution of F_0 therefore results in the formation of 3 ATP, one from each of the β-subunits. In this example about 10 protons need to be translocated for each complete revolution of F_0, which means that the formation of 1 ATP requires about 3.3, though other species may be different. ATP synthase is thought to revolve at more than 100 Hz (revolutions/s), which is sufficient to produce a turnover of the weight of our body of ATP each day. This is what makes biochemistry meaningful, wonderful, and fun!

The mechanism of ATP synthase is difficult to describe in words, but an animated picture has been created in PowerPoint (and real animation is proceeding), which makes it much easier to understand. Details of this will appear in later issues of BAMBED.