CHAPTER 8

Cell Biological Consequences of OXPHOS Disorders

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Abstract

During the past century mitochondria have been recognized to play a central role in many cellular functions. Apart from producing cellular energy in the form of ATP (adenosine 5'-triphosphate) this organelle harbors essential parts of the urea cycle and is crucial for the breakdown of fatty acids, heat generation and the biosynthesis of heme, pyrimidines, amino acids, phospholipids and nucleotides. In addition to these 'classical' functions, mitochondria are also key players in cellular signaling through their involvement in apoptosis, generation of reactive nitrogen- and oxygen species (ROS/RNS), transduction of electrical signals and calcium homeostasis. This chapter summarizes current insights concerning the consequences of oxidative phosphorylation (OXPHOS) dysfunction at the cellular level. We will start with illustrating how mitochondrial and cellular metabolism is intertwined during ATP generation, calcium transport and ROS production. Moreover, the relation between mitochondrial morphology and function will be addressed. Next, we will summarize how OXPHOS deficiency and cellular functioning have been analyzed using pharmacological model systems and patient-derived cell lines. Also results of mathematical modeling, applied to integrate and understand the complex experimental data, will be treated. Finally, we will discuss possible adaptive mechanisms that counterbalance OXPHOS deficiency in the living cell.

Mitochondrial Function in the Living Cell

Production of ATP

Mitochondria are present in virtually all eukaryotic cells and in higher animals they produce ~95% of the principal carrier of chemical energy, ATP. These cellular power plants are fueled not only by the pyruvate produced from sugars by glycolysis in the cytosol but also by fatty acids. Pyruvate and fatty acids are transported from the cytosol into the mitochondrial matrix, where they are used as carbon sources for the tricarboxylic acid cycle (TCA) and fatty acid oxidation (β-oxidation), respectively. The products of these pathways, NADH and FADH₂, subsequently feed reducing equivalents into the electron transport chain (ETC) embedded within the mitochondrial inner membrane (MIM; Fig. 1). The ETC consists of four respiratory chain enzyme complexes (complex I to complex IV) and a transport system (ubiquinone and cytochrome c). Together they transfer electrons from the hydrogens on NADH and FADH₂ to oxygen. The electrons start with very high energy and lose it in small steps as they pass along the ETC. At three locations within the chain, complex I, complex III and complex IV, these quanta of energy are used to expel 4, 4 and 2 protons per electron respectively from the matrix.
Figure 1. Central role of oxidative phosphorylation in mitochondrial metabolism. By expelling $H^+$ from the mitochondrial matrix across the mitochondrial inner membrane (MIM), Complex I, III and IV generate a proton motive force (pmf) that is used for the production of ATP and as an energy source by several mitochondrial transporters. The latter are driven by the pH gradient ($\Delta pH_m$) or mitochondrial membrane potential ($\Delta \psi_m$) across the mitochondrial inner membrane (MIM). In this way the pmf is used to supply pyruvate to the tricarboxylic acid (TCA) cycle, to transport ions and other small solutes, and to import nuclear-encoded mitochondrial proteins via the TIM/TOM import machinery. Moreover, the export of ATP and inhibition of the permeability transition pore (PTP) require $\Delta \psi_m$. Ionic calcium ($Ca^{2+}$), once taken up, increases the production of ATP by stimulating several key-enzymes of the TCA cycle and complex V. Ca$^{2+}$ overload will eventually lead to opening of the PTP. If the duration of this opening is above a certain threshold, apoptosis will be triggered. Abbreviations: IMS= inter membrane space, TIM= translocase of the inner membrane; TOM= translocase of the outer membrane.

The $H^+$ ejection results in the establishment of an $H^+$ electrochemical gradient ($\Delta \mu_H$ or pmf) which can be written as:

$$\Delta \mu_H = z \cdot F \cdot \Delta \psi + R \cdot T \cdot \ln \left( \frac{[H^+]_{\text{matrix}}}{[H^+]_{\text{cytosol}}} \right)$$

where $\Delta \psi$ denotes the membrane potential difference ($\psi_{\text{in}} - \psi_{\text{out}}$) in millivolts; $z$ is the charge of the ion, $F$ is the Faraday constant, $R$ is the gas constant and $T$ is the temperature in Kelvin. Because the first term is about -160 mV and the second equals -60 mV, the total pmf value is -220 mV (inside of mitochondrial inner membrane negative). At complex V ($F_o/F_1$-ATPase), protons are allowed to flow back into the mitochondrial matrix to drive the synthesis of ATP from ADP and inorganic phosphate ($P_i$). In intact mitochondria, the ETC and ATP synthesis are effectively coupled and together constitute the oxidative phosphorylation (OXPHOS) system. It is important to realize that the primary event is not the generation of ATP but proton pumping and maintenance of $\Delta \mu_H$. This is illustrated by the fact that complex V, rapidly turns from the main producer into main consumer of ATP during anoxic conditions in an effort to preserve $\Delta \mu_H$.4