Mitochondria at Work

Subjects: Immunology
Contributor: Volker Schirrmacher

Mitochondria can adapt to the requirements of different organs. For instance long-life energy supply for the heart or metabolism for function of the liver. Dysregulations are observed in all major chronic diseases. The paper includes interventional strategies for cardiovascular diseases, metabolic syndromes, cancer, cachexia and immune system exhaustion.

Keywords: Chronic diseases ; OXPHOS ; TCA ; cyanobacteria, glycosylation ; cancer ; tumor microenvironment ; cachexia ; hydrogen ; oxygen ; redox enzymes

1. Introduction

Energy and the environment are important and timely issues in present-day discussions. Engineers develop techniques to use hydrogen (H) instead of carbon (C) as fuel, and sunlight (photons) as solar energy to burn H_2, with the help of oxygen (O_2), into water molecules. Nature invented its own technology 1.5 to 3.5 billion years ago. It uses H_2, O_2, and CO_2 to generate glucose for metabolism and energy production. The ins-and-outs of the gases O_2 and CO_2 are in balance with the environment. Details about the evolution of life on earth can be found in an excellent textbook [1].

Cyanobacteria invented photosynthesis, a process to assimilate C from CO_2 for sugar (glucose) synthesis. The energy was taken from photons and electrons (e^-). In those times, without O_2 in the atmosphere, nicotinamide adenine dinucleotide (NAD) was used as the redox system, NAD/NADH. It served to remove H from H_2O molecules and generate protons (H^+), electrons (e^-), and the reduction equivalent NADH, according to the formula: $NAD^+ + 2H = NADH + H^+ + e^-$. Archaebacteria used a process of anaerobic glycolysis to degrade glucose and to produce and store energy in the form of adenosine triphosphate (ATP). Since those times, ATP has served as a main energy source in cells on earth. Once the atmosphere was enriched with O_2 (>1%), eubacteria invented aerobic glycolysis of sugars via oxidative phosphorylation (OXPHOS), resulting in more efficient production of ATP. During further evolution towards eukaryotic cells, nature developed membrane-enclosed nuclei and cellular organelles specialized for energy supply and metabolic processes: mitochondria and chloroplasts. These organelles are equipped with biomembrane-associated or matrix-embedded enzymes to catalyze biochemical redox processes, involving electron transport chains and metabolic processes. Magnesium, containing chlorophyll in chloroplasts, absorbs and transfers energy from photons. Iron, containing cytochromes in mitochondria, with hem as cofactor, transport electrons to generate ATP. The iron of hemoglobin reacts in a reversible manner with molecular oxygen, in which one atom of iron combines with one molecule of oxygen [2]. Multiple lines of recent evidence support the hypothesis that mitochondria and chloroplasts were transferred from bacteria to eukaryotic cells, most likely via endocytosis and symbiosis. The cytoplasm of eukaryotic cells, in contrast to these organelles, exerts anaerobic glycolysis, such as in ancient times. Mitochondria have been (and are) fundamental for the emergence of metazoans [3]. The perpetual work of these cellular organelles over billions of years under changing environmental conditions is of great fascination.

Aerobic and “truncated” aerobic glycolysis are examples of regulated or dysregulated biochemical processes in various types of cells [2]. The role of mitochondria in these processes will be described and discussed. Heart muscle cells will be used as an example of cells degrading pyruvate via OXPHOS, to its full extent, for maximal ATP production. Skeletal muscle cells serve as an example for cells, which, under stress, degrade pyruvate via fermentation to lactate. Liver cells serve as an example of cells busy with mitochondrial metabolic (anabolic and catabolic) processes (e.g., glycogen storage, detoxification).

New insights will be provided into the metabolism of cells from the immune system, in particular T lymphocytes and their various subsets. Upon activation, T cells switch their metabolism from a resting state to a state allowing cell proliferation and defined immune functions. Distinct transcription factors will be mentioned, which have been identified as key regulators of metabolism of T cell subsets, such as naive, Th1, Th2, Th9, Th17, Treg, and memory T cells (MTCs) [4].

Mitochondria also play important roles in pathophysiological processes of diseases of major importance, such as cardiovascular disease (CVD) and cancer. Recent research has identified a variety of features of metabolic dysregulation, such as “truncated” aerobic glycolysis in cancer that could become targets of new therapeutics. The Warburg effect of aerobic glycolysis [5] fulfills the needs, not only of cancer cells, but also of an activated immune system. This appears of particular significance in the tumor microenvironment (TME) where cancer cells and immune cells compete for supply of energy and nutrients.

2. Structure, Function, and Potential Origin of Mitochondria

The first observation of a metabolic alteration in cancer cells was made about a century ago [2][5]. This Warburg effect of aerobic glycolysis, with excess lactate production, became, only recently, an active research field, termed metabolic reprogramming. Metabolism refers to all biochemical processes of a cell, and of a multicellular organism, to maintain steady-state homeostasis and survival. What will be summarized first is a prerequisite to understand the newest discoveries of mitochondrial research. The basis is described in biochemistry, cell biology, and physiology textbooks.

Phosphate metabolism, for example, includes, among others, biochemical energy transfer via ATP, maintenance of genetic information with DNA and RNA nucleotides and membrane structural integrity via glycerophospholipids [6].

Table 1 presents an overview of energy production by bacteria. During anaerobic times, the energy source for electrons was, for example, sulfur (H_2S), oxygen (H_2O), or methane (CH_4). As prokaryotic cells, archaebacteria (methanogenic, sulfurogenic, and thermoacidophilic) started to sequester their genome, which later led to development of a membrane-enclosed nucleus. Archaebacteria and early eubacteria had already developed the processes of glycolysis (catabolism of glucose to pyruvate) and fermentation (e.g., catabolism of pyruvate to lactate), with all their necessary enzymes and co-enzymes. These types of anaerobic metabolism have survived times, and are still at work in the cytoplasm of plant and animal cells, including Homo sapiens. Important coenzymes are NAD^+ and its phosphate ($NADP^+$), coenzyme A (CoA), and flavin adenine dinucleotide (FAD). Glycolysis is a term for degradation of glucose to pyruvate with the help of adenosine diphosphate (ADP) and inorganic phosphate (Pi). It involves 10 enzymatic steps and works according to the formula: 1 glucose + 2 ADP + 2 Pi + 2 NAD = 2 pyruvate + 2 ATP + 2 NADH2 [1].

OXPHOS in mitochondria starts from pyruvate, which enters the mitochondrial matrix (MM) to become decarboxylated and converted into acetyl-CoA. This 2-carbon molecule then condenses with a 4-carbon molecule to produce the 6-carbon molecule citrate. The core of cellular metabolism is found in the citrate or tricarboxylic acid cycle (TCA or citrate cycle). Citrate is decarboxylated successively to generate 5-carbon and 4-carbon molecules. The released CO_2 diffuses to the outside. After conversion to the 4-carbon molecule oxaloacetate, the TCA cycle can start again being driven by acetyl-CoA. The four NADH molecules that are generated per cycle diffuse to the inner mitochondrial membrane (IMM) and transfer their hydrogen reduction equivalents into the electron transport chain (ETC) for generation of energy. This ETC respiration chain, catalyzed by iron ($Fe^{3+} + e^- = Fe^{2+}$), serves to transfer electrons from a high level of energy to a level of low energy. It is made up of five enzyme complexes: NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), cytochrome-c reductase (complex IV), cytochrome-c oxidase (complex IV), and ATP synthase (complex V). An electrochemical proton gradient (ΔH^+) between the peri-mitochondrial cleft and the MM drives the production of ATP from ADP and Pi, catalyzed by the ATP synthase. The energy of this gradient also drives specific inner mitochondrial membrane (IMM) transporters for Pi, pyruvate and Ca^{2+}. The mitochondrial production of ATP is done by oxidizing the major products of glycolysis from the cytosol: pyruvate and NADH.

The TCA cycle involves five enzymatic steps to generate energy and to convert small metabolites into precursors for biosynthetic pathways. It finally produces from protons, electrons, and oxygen water, according to the formula: $4H^+ + 4e^- + O_2 = 2H_2O$. This burning of hydrogen is facilitated by cytochrome enzymes in the IMM. Mitochondrial OXPHOS and ETC respiration produces approximately 13-times more ATP per molecule glucose than glycolysis. The ATP produced in mitochondria crosses out through the IMM, with the help of a specific protein carrier, and across the outer mitochondrial membrane (OMM) via porins into the cytoplasm. ADP returns via the same route.

Apart from supplying energy, mitochondria are involved in other cellular processes: signaling, differentiation, maintenance of cell cycle and cell growth, and cell death (intrinsic apoptosis following cytochrome-c release). They are also involved in catabolic and anabolic biochemical processes, often upon interaction with the cell’s cytoplasm. Examples of such catabolic processes are ketone body degradation (e.g., via acetyl-CoA-acetyl-transferase) and the urea cycle for elimination of NH_3. Examples for anabolic processes are gluconeogenesis and the pentose phosphate pathway, leading to de novo purine biosynthesis. Such metabolites are used for synthesis of macromolecules, such as hem and porphyrins, steroid hormones, RNA, and DNA.

All of this textbook knowledge is important to understand how mitochondria work in different cell types under physiological or pathophysiological conditions. This has consequences for diagnosis and therapy of mitochondria-associated symptoms [7].

Table 1. Energy production by bacteria.

Feature	Bacterium	Energy Source	Metabolism	Enzyme	Co-Enzyme	End Product
Glycolysis	Archaebacteria (e.g., Chromatiaceae)	H_2S e^-	Hexose to pyruvate	Ferredoxin-NADP-Reductase	Ferredoxin 2Fe-2S	S or S^- NADPH ATP
Glycolysis	Early Eubacteria	H_2O e^-	Hexose to pyruvate	GAPDH [1]	NAD [2]	NADH ATP
Fermentation	Early Eubacteria	H_2O e^-	Pyruvate to lactate	LDH [3]	NAD	CO_2 NADH ATP
Oxidative Phosphorylation, Cellular Respiration	Aerobic Eubacteria	H^+ O_2 e^-	Pyruvate to TCA, Respiration chain	Cytochrome-c Oxidase	Cytochrome-c (Fe^{3+} to Fe^{2+})	CO_2 H_2O ATP
Photosynthesis	Cyanobacteria	H^+ O_2 Photons	Photosystem I Photosystem II	Plastocyanin: Ferredoxin-Oxidoreductase H_2O: Plastoquinone Oxidoreductase	Chlorophyll P700 Chlorophyll P680	O_2 NADPH ATP

[1] GAPDH = glyceraldehyde-3-phosphate dehydrogenase; [2] NAD = nicotinamide adenine dinucleotide; [3] LDH = lactate dehydrogenase.

Interestingly, cyanobacteria and plant chloroplasts perform a biochemical reaction, which is in the opposite direction compared to mitochondria. They photolyse water ($2H_2O = 4H^+ + 4e^- + O_2$) with the help of sun light to generate energy. For comparison: mankind tries to generate energy from water by electrolysis. Nature invented for enzymatic catalysis of this reaction intracytoplasmic sophisticated thylakoid membrane systems containing pigments, such as chlorophyll, carotenoids, and phycoerythrin. These are able to absorb light, of wavelengths 560 to 670 nm. In the light reaction, chloroplasts transfer energy to electrons at a high orbital and produce NADPH and ATP. This allows, in the dark reaction, to assimilate CO_2 to produce glucose (Calvin cycle). The photosystem I, with its ferredoxin-$NADP^+$-reductase produces NADPH + H^+, and the photosystem II, with its ATP synthase (dark reaction) produces, finally, the energy “currency” ATP, in which phosphate metabolism plays an important role [6]. Conservation and conversion of energy in plant cells involves 1) chloroplasts (generation of NADPH, ATP, glucose, and starch); 2) cytosol (degradation of glucose or starch to pyruvate involving glyceraldehyde-3-phosphate dehydrogenase (GAPDH); and 3) mitochondria (degradation of pyruvate via TCA and ETC to ATP).

Without the synthesis of glucose and starch by plant cells, the energy metabolism of animal cells would not be possible. Plant products, serving as nutrients, become degraded in the intestine before their metabolites (e.g., glucose, fatty acids) are permitted to enter the blood stream. Chloroplasts take up three molecules of CO_2 and release three molecules of O_2 per molecule glucose. Mitochondria take up three molecules of O_2 and release three molecules of CO_2 per molecule glucose. There is, thus, a net balance between consumption and waste production executed, and finely tuned by the cellular organelles mitochondria and chloroplasts.

Of particular importance in both organelles are the electron transport chains mediated by redox enzymes. Models of quantum theory locate electrons on orbitals surrounding the atom at defined distances. The higher the main quantum number, the further away the electron and the more reactive it can be in redox systems. Electrons at δ-orbitals of enzyme A, with high electron density, transfer electrons to enzyme B with lower electron density. In this way, the reducing enzyme A becomes oxidized and the oxidized enzyme form B becomes reduced. Many biological redox systems contain ions from metals (e.g., Fe, Cu, and Mn); others contain disulfide bridges (e.g., glutathione, thioredoxin). Important coenzymes are based on quinone (e.g., ubiquinone (Q10), plastoquinone, vitamin E, and K), nicotinamide (e.g., NAD, NADP), or flavin (e.g., FAD). Q10 is a vitamin-like compound. It is expressed in humans as ubiquinol (reduced form) or ubiquinone (oxidized form). It plays a key role in electron transport in OXPHOS. It acts as a potent antioxidant, membrane stabilizer, and cofactor in the production of ATP.

Table 2 provides an overview of the structure of mitochondria and compares them with chloroplasts. Both eukaryotic organelles have the size of bacteria and are surrounded within the eukaryotic cell by a double membrane. The IMM consists of lipids characteristic for bacteria (e.g., cardiolipin (CL)), while the outer membrane consists of lipids and porin molecules characteristic for eubacteria. Between the two membranes exists a space, which is used to establish the above-mentioned proton gradient for the ATP synthase pump.

The OMM contains integral membrane proteins called porins. One of these trafficking molecules is the voltage-dependent anion channel (VDAC), a transporter of nucleotides, ions, and metabolites between the cytosol and the intermembrane space. The inner membrane contains proteins of the electron transport chain redox reactions, ATP synthase, and specific transport proteins that regulate metabolite passage into and out of the mitochondrial matrix. Depending on the mechanism and direction of transported molecules, one differentiates between antiporter, symporter, and uniporter. The driving force comes either from the membrane potential (e.g., Ca^{2+} influx and ATP/ADP exchange) or from proton gradients (e.g., import of pyruvate and Pi).

The inner membrane is compartmentalized into cristae, which expand the surface area. For typical liver mitochondria, the area of the inner membrane is about five times the size of the outer membrane. Cells that have a high demand for ATP, such as muscle cells, contain even more cristae. The IMM contains more than 151 different polypeptides. One of these is the phospholipid CL, which is coded by mitochondrial DNA (mtDNA). It contains four fatty acids rather than two. These many fatty acids apparently help to make the inner membrane impermeable.

Table 2. Structural features of mitochondria and chloroplasts.

Feature	Mitochondrion	Chloroplast
Size and form	Like bacterium (2 mm)	Like bacterium (2 mm)
Inner membrane	Without 3-OH steroids	Without 3-OH steroids
Outer membrane	With 3-OH steroids With porin molecules Like eukaryotic cell	With 3-OH steroids With porin molecules Like eukaryotic cell
DNA	mtDNA ring without histone	ptDNA ring without histone
Replication	One start site	One start site
Copy number	About 10	About 100
RNA	rRNAs tRNAs mRNAs	rRNAs tRNAs mRNAs
Ribosomes	70S	70S

Start of protein	N-formyl-methionine	N-formyl-methionine
Inner membrane proteins Respiration chain	NADH dehydrogenase (p [1]) Cytochrome c oxidase (p) ATP synthase (p)	Photosystem I (p) Photosystem II (p) ATP synthase (2 + 4)
Inner membrane lipid	Cardiolipin	Cardiolipin
Import from cell cytoplasm	Yes (proteins, sugar, fatty acids)	Yes (proteins, sugar, fatty acids)
Export to cell cytoplasm	No	No

[1] p = partial; only a part of the enzyme polypeptide chains are encoded by mtDNA or ptDNA.

The matrix, the space enclosed by the IMM, is the site where ATP is being produced. It contains a concentrated mixture of enzymes, special mitochondrial ribosomes, tRNA, and several copies of the mitochondrial DNA genome. The enzymes facilitate oxidation of pyruvate and fatty acids, and running of the TCA cycle. The reduction equivalents NADH and FADH2 are produced within the matrix via the TCA cycle but are also produced in the cytoplasm by glycolysis. The electrons from NADH and FADH2 are transferred to oxygen (O_2), an energy-rich molecule, and hydrogen (protons) in several steps via the ETC.

Mitochondria have their own genetic material in the form of circular DNA. Moreover, they have the machinery to produce their own RNAs and proteins. A human mitochondrial DNA sequence revealed 16,569 base pairs encoding 37 genes: 22 tRNAs, 2 rRNAs, and 13 peptide genes [7]. Like in bacteria, the DNA has a ring structure without histone and one start site of replication. The start of protein synthesis via bacteria-like ribosomes occurs, such as in bacteria, via N-formyl-methionine. Human mitochondrial DNA is double-stranded and is passed from the mother to her offspring during reproduction. Such DNA probes are being used in forensic medicine and in paleo-anthropology. Similarities between human mitochondria and bacteria have recently been described also with respect to the DNA base excision repair (BER) system [8].

Mitochondrial nuclear genes POLG, POLG2, TWNK, and SSBP1 encode the core mtDNA replisome. The mtDNA is replicated by a core set of proteins: polymerase γ, Twinkle, and the single-stranded DNA binding protein [9]. Polymerase γ efficiently replicates through many natural template barriers (e.g., double stranded DNA, structured genes, G-quadruplexes) [10].

The molecular machineries for mitochondrial fusion and fission are essential for mitochondrial homeostasis in health and disease. The fusion of the OMM and the IMM is mediated by dynamin-like proteins (DLPs) [11]. Mitochondrial fission is likely due, among others, to increasing FIS1 and decreasing Mfn2 [12]. An example of the consequence of too much fission is cigarette smoke-induced pulmonary endothelial injury [12]. An example of the consequence of too much fusion is COVID-19. Based on new and compelling evidence, it is proposed that fusion is promoted, causing mitochondrial elongation, and providing a receptive intracellular environment for viral replication in infected cells [13].

The hypothesis of an endosymbiotic relationship of mitochondria with their host cells was popularized by Lynn Margulis in 1986 [14]. An alternative hypothesis, the autogenous hypothesis, is nowadays less widely accepted. The symbiotic relationship was probably developed 1.7 to 2 billion years ago. It appears likely that mitochondria developed earlier than chloroplasts. This might explain that the copy number of ptDNA is about tenfold higher than that of mtDNA (Table 1). It might also explain that animal and plant cells have mitochondria, and that only plant cells have, in addition, chloroplasts. The reduction equivalents, NADH of animal cells and NADPH of plant cells, contain high-energy electrons, and are, thus, being used as fuel to produce ATP.

In terms of evolution, the incorporation of mitochondria into the eukaryotic cell facilitated the use of oxygen (for OXPHOS and respiration) and its detoxification (to reduce oxidative stress induced by reactive-oxygen-species (ROS)). The higher the mitochondrial metabolism, the higher the production of ROS. For protection against their toxic effects, mitochondria developed enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), glutathione transferase, and cytochrome-c-peroxidase. These enzymes neutralize ROS by finally producing water molecules [15].

Symbiosis between bacterial organelles and eukaryotic cells over time of evolution has likely optimized the interaction between the two types of worlds. Import of metabolites from the cell's cytoplasm to the organelles occurs freely or via porin molecules. Export from the organelles to the cytoplasm, however, does not occur. Some of the inner membrane proteins are only partially encoded by organelle-specific DNA. Other parts of these enzyme complexes are derived from the eukaryotic cell. The majority of mitochondrial proteins are cell-nucleus encoded. Mitochondria, the powerhouse of the cell, are semi-autonomous cell organelles.

These insights into the origin (evolution), structure (molecular and cell biology), and function (biochemistry, physiology) of mitochondria are considered important to understand the pathophysiology of major chronic human diseases, such as CVD, metabolic syndrome (MetS), neurodegenerative diseases, immune system disorders, and cancer.

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