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TECHNICAL BULLETIN

Mitochondrial Metabolism Modulators

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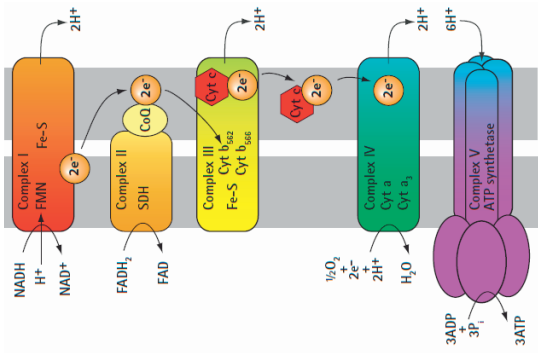
In the evolutionary process, the limited energy production by cells was overcome by a symbiotic relationship between eukaryotic cells and aerobic bacteria that later became mitochondria to produce ATP and perform several other functions in the cell. This evolutionary step demanded the regulation of nuclear replication and gene expression by calorie availability mediated by mitochondrial energetics. It also required a regulatory control of mitochondrial growth and replication in eukaryotic cells. As the mitochondrial role became increasingly specialized, cells developed mechanisms to control the expression of mitochondrial genes.



Within a cell the mitochondria are in constant motion and can move to the areas of high energy demand and may undergo repeated rounds of fission and fusion, which is important in mixing of mitochondrial matrices and redistribution of the mitochondrial DNA.

While mitochondrial DNA encodes only 13 proteins involved in the electron transport system, most of the other mitochondrial proteins are encoded in the nucleus and are assembled in the cytoplasm. Hence, their import into mitochondria and assembly in various mitochondrial compartments are tightly controlled. To overcome incorporation of any abnormal proteins, mitochondria utilize a proteolytic system to selectively remove damaged and misassembled proteins. Cells also utilize the cytosolic ubiquitin-proteasomal system to seek and destroy any misassembled proteins prior to their import into mitochondria. Mitochondria can also fuse with undamaged intact neighboring mitochondria; however, if the damage is severe enough they undergo mitophagy.

The large part of energy (ATP) production is associated with energy-transducing inner mitochondrial membrane. It allows compartmentation to generate a high gradient of protons that forces ATP synthase complex to produce ATP from ADP and Pi. In order to efficiently produce energy for all anabolic and maintenance functions, mitochondria are endowed with five different enzyme complexes. Complexes I, II, III, and IV are the electron transfer complexes, whereas complex V is an energy-conserving complex.

In the Krebs cycle, several reactions generate NADH + H^+. Two electrons (reducing equivalents from hydrogen) are transferred from NADH + H^+ to NADH dehydrogenase (complex I) or from $FADH_2$ containing enzymes to reduce ubiquinone (coenzyme Q10, CoQ) to ubiquinol $CoQH_2$. The electrons from $CoQH_2$ are then transferred successively to complex III, cytochrome c, complex IV, and finally converting $½O_2$ to H_2O. The energy released during the flow of electrons in the electron transport chain pumps protons out across the mitochondrial inner membrane through complexes I, III, and IV creating a proton electrochemical gradient. The energy to convert ADP + Pi to ATP comes from the flow of protons through the ATP synthatase (complex V) back into the matrix. Matrix ATP is then exchanged for cytosolic ADP by the inner membrane adenine nucleotide translocators. For each pair of electrons originating from NADH, 3 equivalents of ATP are synthesized, whereas electrons from succinate generate 2 moles of ATP per mole of succinate oxidized. In order for oxidative phosphorylation to proceed, two conditions are important. First, the inner mitochondrial membrane must be physically intact so that protons can only reenter the mitochondrion by a process coupled to ATP synthesis. Second, a high concentration of protons must be developed on the outside of the inner membrane.

Biologists have studied the movement of protons and the mechanism of ATP production through various complexes in detail over the past fifty years by using a variety of uncoupling agents and inhibitors of electron transport chain, Krebs cycle, and oxidative phosphorylation. The study of mitochondrial metabolism using these compounds has led to the identification of bioenergetic control points for cell replication, differentiation, and death. Use of specific inhibitors has also helped to distinguish the electron transport system from the phosphorylation system and define the sequence of redox carriers along the respiratory chain. Electron transport inhibitors bind to components of the electron transport chain and block the passage of electrons from one carrier to the next. They do exhibit certain degree of specificity and bind only to specific molecules in the electron transport system.

References:

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The irreversible types of inhibitors cause a complete cessation of electron transport. However, reversible inhibitors, such as rotenone, cause only a partial blockage and some electrons might still pass through their site of action.

Another group of compounds that can inhibit ATP production in mitochondria are the uncouplers of oxidative phosphorylation. In the presence of uncouplers the rate of electron transport cannot be regulated by an intact chemiosmotic gradient (concentration difference of protons across the inner membrane). For example, 2,4-dinitrophenol (DNP) acts as a proton ionophore and binds to protons on one side of the membrane. Being lipid-soluble it can easily diffuse to the opposite side of the membrane where it releases bound protons. Hence, it becomes difficult to maintain a proton gradient for proper functioning of mitochondria. Most of the uncouplers are hydrophobic weak acids with protonophoric activities. They inhibit the coupling between the electron transport and phosphorylation reactions without affecting the respiratory chain and ATP synthetase activity. Uncouplers prevent the coupling reaction in a manner that the energy produced by redox reactions cannot be utilized for phosphorylation. The translocated protons do not return to the interior through ATP synthetase. Hence, the uncouplers can cause maximum respiratory rates, but the electron transport fails to generate any ATP. Oligomycin and Aurovertin B, which act as metabolic poisons, can bind to and block ATP synthase activity in complex V.

Site of Action of Select Mitochondrial Metabolism Modulators

Compound	Site of Action
Rotenone	Complex I
Malonate 3-Nitropropionic acid	Complex II
Antimycin A	Complex III
Azide	Complex IV
Carbon monoxide	Complex IV
Cyanide	Complex IV
Oligomycin	Complex V
2,4-Dinitrophenol	Transmembrane H^+ carrier

As a by-product of oxidative phosphorylation, mitochondria produce significant quantities of endogenous reactive oxygen species (ROS), which can damage cellular proteins, lipids, nuclear and mitochondrial DNA. Excessive mitochondrial ROS production can overwhelm the antioxidant defenses and can cause serious damage to mitochondria and the cell. Cumulative oxidative damage and mitochondrial DNA mutations can lead to mitochondrial abnormalities that affect the health of the organism. Mitochondrial cytopathies may result from either mutations in mitochondrial DNA or mutations in nuclear DNA encoding for several mitochondrial proteins. Over forty different mitochondrial cytopathies have been reported in humans that lead to defective or insufficient energy (ATP) production. Hence, mitochondrial medicine is becoming an area of greater interest to physicians, biochemists, and cell biologists. More common symptoms of mitochondrial abnormalities include poor growth, ataxia, mental retardation, type II diabetes, muscular dystrophies, developmental delays, and dementia. Unfortunately, the diagnosis and treatment of mitochondrial diseases poses a great challenge and no universal treatment yet exist.

Mitochondrial Metabolism Modulators

Product	Cat. No.	Description	Size
Atractyloside, Dipotassium Salt, *Atractylis gummifera*	189300	Acts as an ADP/ATP translocase (AAT) inhibitor. Also causes the release of cytochrome c from mitochondria.	50 mg
Bongkrekic Acid, Triammonium Salt	203671	Acts as a ligand of the adenine nucleotide translocator. A potent inhibitor of mitochondrial megachannel. Prevents the apoptotic breakdown of the inner mitochondrial transmembrane potential ($\Delta\Psi m$).	500 µg
Carbonyl Cyanide *m*-Chlorophenylhydrazone (CCCP)	215911	Protonophore. Uncoupling agent for oxidative phosphorylation that inhibits mitochondrial function. Approximately 100 times more effective than 2,4-dinitrophenol. Binds with cytochrome *c* oxidase with high affinity (K_d = 270 nM).	250 mg
CGP-37157	220005	A cell-permeable, specific, and potent inhibitor of the mitochondrial Na^+/Ca^{2+} exchanger (IC_{50} = 360 nM). Enhances the export of Ca^{2+} from isolated mitochondria.	5 mg
(-)-Deguelin, *Mundulea sericea*	252740	A cell-permeable, potent inhibitor of mitochondrial bioenergetics (IC_{50} = 6.9 nM for NADH:ubiquinone oxidoreductase activity in bovine heart ETP) and induces apoptosis and cell cycle arrest	5 mg
Erastin	329600	A cell-permeable piperazinyl-quinazolinone compound that binds to mitochondrial voltage-dependent anion channels (VDAC) and alter its gating; rapidly induce an oxidative, non-apoptotic cell death in several human.	5 mg
F16	341246	A cell-permeable mitochondrial toxin with dual ability to induce apoptosis as well as necrosis in tumor cells. Preferentially accumulates in mitochondria, inhibits oxidative phosphorylation and causes mitochondrial transmembrane depolarization.	25 mg
Hexokinase II VDAC Binding Domain Peptide, Cell-Permeable	376816	A cell-permeable peptide analog of Hexokinase II VDAC binding domain peptide that completely detaches and translocates HXK2 from mitochondria to the cytosol. Does not induce Bax translocation or cytochrome c release when used alone.	1 mg
Oligomycin	495455	A mixture of A, B, and C isomers. A macrolide antibiotic that inhibits membranebound mitochondrial ATPase (F1), preventing phosphoryl group transfer.	10 mg
Rotenone	557368	A mitochondrial toxin and a potent, reversible, and competitive inhibitor of complex I (NADH-CoQ reductase) of the respiratory chain.	1 g
Ru360	557440	A cell-permeable ruthenium amine complex that binds to mitochondria with high affinity (K_d = 340 pM). Specifically blocks Ca^{2+} uptake into mitochondria *in vitro* (IC_{50} = 184 pM) and *in situ* in intact myocytes. (1 set = 10 × 100 µg)	500 µg 1 mg 1 set
Valinomycin, *Streptomyces fulvissimus*	676377	A potassium ionophore of the mobile ion-carrier type uncouples oxidative phosphorylation by binding to sites on membranes rich in sulfhydryl groups.	25 mg 100 mg

For more information, visit www.emdbiosciences.com/MitoBulletin

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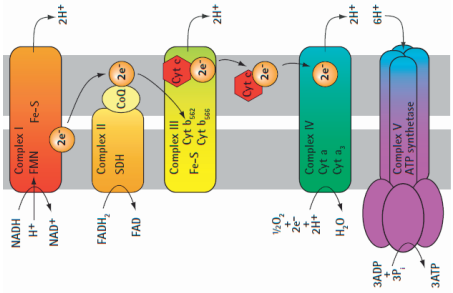

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