METABOLISM

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ARKAN ACADEMY

Mitochondria

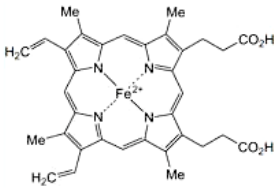
- Most of the components of the mitochondria are present in the *matrix*, which is a gel-like solution consisting of 50% proteins such as pyruvate dehydrogenase complex, TCA, FA breaking, amino acid oxidation enzymes, mtDNA, mtRNA and mt-ribosomes
  - It contains *most the fuel oxidation* reaction except the glycolysis (in the cytosol)

- Mitochondria have 2 membranes:
  - *Outer Mitochondrial Membrane:* ***Permeable*** to small molecules and ions (MW<5000) due to the presence of porins (transmembrane channels)
    - ✓ Similar to the cell membrane (less than 3% cardiolipin, 45% cholesterol)
  - *Inner Mitochondrial Membrane:* ***Impermeable*** even to very small ions such as H^+, but it has transporter to some specific materials
    - ✓ Consists of 22% of cardiolipin ***without cholesterol***
    - ✓ It contains the Electron transport chain (*ETC*) and *ATP synthase*

Cytochromes

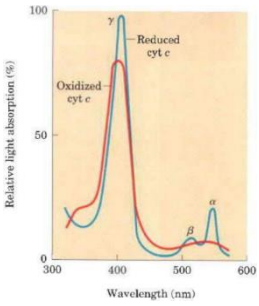
- *Cytochromes:* They are Hemoproteins (contain **heme** and **iron** as prosthetic groups)

- *Heme:* It is a large organic molecule consisting of a ***porphyrin*** molecule with iron in the center and many side chains
  - Porphyrin consists of ***4 fused pyrrole*** rings with a Nitrogen in each ring which binds the central iron
  - Heme in the hemoglobin must be ferrous (Fe^{+2}) but in cytochromes it alternates between *ferrous* (Fe^{+2}, reduced) and *ferric* (Fe^{+3}, oxidized) because it is important in the ***transfer of electrons*** to release energy
  - Heme can carry **<u>one electron</u>**, but its ΔE^o differ between different cytochromes



- The mitochondria have 3 subtypes of cytochromes including a, b and c differing in the side chains that are attached to the pyrrole rings

- Each type of cytochromes differs in its light absorption in the reduced state having 3 absorption bands (α, β, γ) in the visible range
  - **α band** is the **<u>most differentiative</u>** between different types of cytochromes
  - Some cytochromes are named by the exact α band wavelength such as Cytochrome b_{562}, Cytochrome c_{550}, Cytochrome c_{551}



- Cytochromes **a and b** are statice transmembrane proteins but cytochrome **c** is a mobile peripheral protein on the outer leaflet of the inner mitochondrial membrane

Oxidative Phosphorylation (OxPhos)

- It is the process of producing ATP by the utilization of the energy released during the ***transfer of electrons*** form an electron donor (NADH, $FADH_2$) to the electron acceptor (O_2)
  - Electron donors will be oxidized and electron acceptor will be reduced
  - The electron transfer occurs in the Electron transport Chain (ETC)

The production of ATP in other process is called substrate level phosphorylation

- **ETC:** It is a series of protein complexes in the inner mitochondrial membrane
  - It consists of 4 protein complexes, cytochrome c and ubiquinone
  - Each element in the chain accept electrons form the upstream element and transfer it to the downstream one until reaching O_2 (the final electron acceptor)

Complex I

- *NADH Dehydrogenase* or *NADH Oxidase* or *NADH-Q oxidoreductase*
- A huge ***flavoprotein*** membrane-spanning complex, consist of more than 25 polypeptide chain and have FMN (Flavin Mono Nucleotide) tightly bound to it
- Contains 7 proteins called **Fe.S** centers of 2 types at least
- Binds and interacts with NADH and Coenzyme Q
  - Transfer 1 electron by oxidizing **NADH**, and reducing **Coenzyme Q**

Complex II

- Succinate dehydrogenase complex or Electron Transfer Flavoprotein ETF-CoQ oxidoreductase
- It is a ***flavoprotein***, contains FAD (Flavin Adenosine Dinucleotide) bounds tightly to it
- Contains some **Fe.S** centers
- Substrates oxidized by FAD-linked enzymes **bypass complex-I**, such as Succinate dehydrogenase (6^{th} step of TCA), Fatty acyl CoA dehydrogenase and Mitochondrial glycerol phosphate dehydrogenase

Coenzyme Q (Ubiquinone)

- It is also called as coenzyme Q10
- A **non-protein** electron carrier consisting of a Lipid-soluble benzoquinone with an isoprenoid side chain
  - It is small and hydrophobic (*freely mobile* and diffusible)
- It can carry and donate up to **2 electrons**
  - Coenzyme Q (Ubiquinone) is the *fully oxidized form*
  - When it accepts 1 electron, it becomes semiquinone anion ($\cdot QH$)
  - When it accepts another electron, it becomes ubiquinol (QH_2), the *fully reduced form*
- It transfers electrons from complexes I and II (2 electron donors) into complex III (1 electron acceptor)

Sometimes prescribed for recovering **MI patients**

Complex III

- Q-cytochrome-C-Oxidoreductase
- It is a hemoprotein that consists of 11 subunits, 2 of them are cytochrome subunits (***Cytochrome bc1***)
  - Contain 3 heme groups in two cytochrome subunits (b_L and b_H-type heme and c-type heme)
- Contains iron sulfur (***Fe.S***) center
- Contain two <u>CoQ binding sites</u>

Cytochrome C

- Transfers electrons from complex III into complex IV

Complex IV

- Cytochrome c oxidase
- Passes electrons from Cytochrome c to O_2
- Contains ***cytochrome a*** and ***a3***
- Contains two ***copper*** sites
- Contains oxygen binding sites where O_2 must accept **<u>4 electrons</u>** to be reduced to **<u>2 H_2O</u>**
  - It has a very lower K_m (higher affinity) than myoglobin and hemoglobin

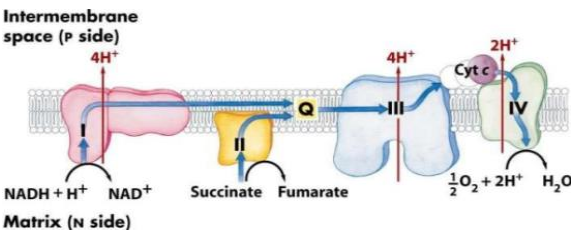
Notes:

- Along the ETC, electrons are transferred from the least redox potential (most negative, oxidation) which is **NADH** into the highest redox potential (most positive, reduction) which is **O_2**

- Electron transfer inhibitors include:
  - *Rotenone (insecticide) and amytal (sedative):* Inhibits NADH-Q Oxidoreductase (complex I)
  - *Antimycin A (antibiotic):* Inhibits electron flow (in complex III) between <u>cyt b and c1</u>
  - *Cyanides (CN^-), Azide (N_3^-) and CO:* inhibit cytochrome c oxidase (complex IV)
    - ✓ Cyanides involve the cyanoglycosides such as *amygdalin* which is a misnomer B17 present in edible plants pits

- In the presence of O_2, the ETC works normally and all the components are oxidized
- But in the absence of O_2, ETC components will become reduced which impedes the ATP production
- In the presence of inhibitor, all the upstream components are reduced and all the downstream components become oxidized

- Complexes I, III and IV can act as **proton pumps** that pump protons from the mitochondrial *matrix* to the *intermembranous* space which drives ATP synthesis



- For each 2 electrons, complex I and III, pumps **4 H^+**
- For each 2 electrons, complex IV pumps **2 H^+**
- Complex II can't pump protons

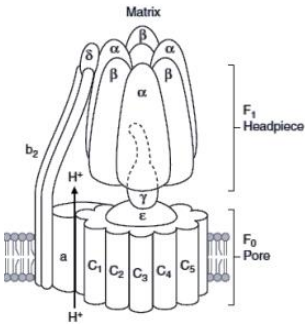
Electrons transferred from:
- NADH: pump 10 H^+
- $FADH_2$: pump 6 H^+

- The transfer of electrons can be in 3 types:
  - Direct transfer (like the reduction of Fe^{+3} to Fe^{+2})
  - Transfer as a hydrogen atom
  - Transfer as a hydride ion

- ***Chemiosmotic hypothesis:*** It is the utilization of the *proton gradient* across the Inner Mitochondrial membrane in the *production of ATP*
  - Proton gradient is generated by the proton pumps (Complex I, III, IV) which move proton from the mitochondrial matrix into the intermembranous space by utilizing the energy released from the El00ectron transfer through the ETC
  - Then protons can pass through Complex V (ATP synthase) to couple the movement of protons with the phosphorylation of ADP into ATP
  - The founder of this theory is Peter Mitchell (1961)

ATP synthase (Complex V, ATPase)

- ATP synthase catalyzes the reaction: ADP +P_i → ATP + H_2O
- **F_0** (rotator) consisting of:
  - a subunit which represents a *point of entry and exit* of protons
  - C ring which *rotates* as the protons cross the membrane



- **F_1** catalyze the synthesis or hydrolyzes ATP in the absence of proton gradient
  - α is a structural subunit
  - β is the catalytic subunit that produce ATP
  - γ connect the F_1 to the F_0 (C ring), and rotates with it

Production of *1 ATP*, requires pumping *4 protons*
- 1 NADH : **3 ATP**
- 1 $FADH_2$: **2 ATP**

- During the synthesis of ATP, the subunits rotate causing conformational changes

- F_1 has 3 conformations:
  - Loose-binding (L): relatively *inactive*, ***held*** ADP and P_i in position
  - Tight-binding (T): *active* form, ***catalyze*** the formation of ATP
  - Open (O): ***release*** produced ATP (dissociation)

- ***Respiratory control or acceptor control:*** It is the regulation if the rate of OxPhos and ETC by the presence of **ADP** which indicates low energy state
  - ADP is the most important regulatory factor, which activates the process of ATP synthesis
  - ETC is ***tightly coupled*** to phosphorylation (simultaneously)
  - The rate of oxygen consumption by mitochondria increases markedly when ADP is added and then returns to its initial value when the added ADP has been converted into ATP

- *Oligomycin* prevents the influx of H^+ through ATP synthase (*inhibit ATP synthase*)

- Regulated uncoupling proteins (UCPs): They are proteins that collapse the coupling between ETC and ATP synthase, causing the release of energy in the form of heat instead of producing ATP
  - *UCP1 (Thermogenin):* Present in the brown *adipose tissue* (non-shivering thermogenesis)
    - ✓ In <u>infants</u> it presents in the neck, breast and around the kidney
  - *UCP2* in *most cells*
  - *UCP3* in the *skeletal* muscle
  - *UCP4*, *UCP5* in the *brain*

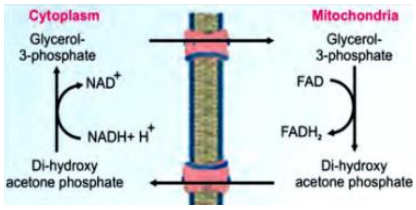
ETC is the major source of heat

- Mutations in UCPs can affect the tendency of <u>obesity</u> and cause <u>cardiometabolic diseases</u>
- Unregulated (non-physiological) chemical uncouplers involve *2,4- dinitrophenol (DNP)* and other acidic aromatic compounds
  - It disrupts the coupling by carrying the proton across the inner mitochondrial membrane which dissipate the PMF (proton motive force, proton gradient)
  - It causes the <u>increase in O_2</u> consumption with <u>no ATP</u> production

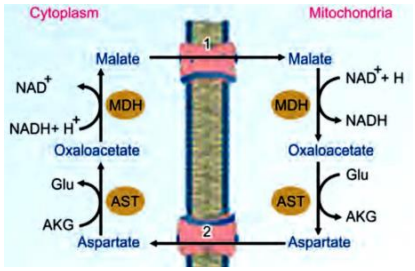
- Genetic diseases in the OxPhos:
  - *mtDNA mutations:* which has a 10 times higher rate than nuclear DNA mutations
    - ✓ mtDNA is a small double strands circular DNA
    - ✓ Encode 13 protein subunits (7 of complex I, 1 of complex III, 3 of complex IV, 2 of F_0)
    - ✓ Encodes components for translation of its own mRNA: a large and small rRNA and tRNAs
    - ✓ ***Maternal*** inheritance
    - ✓ ***Replicative segregation*** (due to random splitting of mitochondria) causing ***heteroplasmy*** (accumulated mutation in some mitochondria more than other ones)
    - ✓ Affecting more the highest ATP demand organs (CNS, heart, skeletal muscle and kidney, liver)
  - *Nuclear DNA mutations*
    - ✓ 1,000 proteins
    - ✓ Usually, autosomal recessive inheritance
    - ✓ Expressed in all tissues
    - ✓ Phenotypic expression with high ATP demand

Shuttling systems

- They involve the process of moving ATP and NADH between the mitochondria and the cytosol
- **NADH Shuttling** by transferring the energy of NADH produced in the cytosol (by glycolysis) into the mitochondria, by interconversion between metabolic intermediates between the cytosol and mitochondria (each *cytosolic NADH* can yield of *2 ATP* molecules)
- NADH shuttling has 2 ways:
  - *Glycerol 3-Phosphate shuttle*
    - ✓ By *glycerolphosphate dehydrogenase*
    - ✓ NADH in the cytosol passes its electrons to the mitochondria in the form of glycerol 3-phosphate as $FADH_2$
    - ✓ In *skeletal muscles* and *brain*



  - *Malate-Aspartate shuttle*
    - ✓ By *malate dehydrogenase*
    - ✓ It involves 2 membrane carriers and 4 enzymes
    - ✓ More readily ***reversible*** than the first system
    - ✓ Mainly in the *liver*, *kidney* and *heart*
    - ✓ Works when the $NADH/NAD^+$ ratio in the cytosol is higher than the mitochondrial matrix



Box 37.3: NAD^+ dependent enzymes

1. Lactate dehydrogenase (lactate → pyruvate) (see Fig. 9.14)
2. Glyceraldehyde-3-phosphate dehydrogenase (glyceraldehyde-3-phosphate → 1,3-bisphosphoglycerate) (see Fig.9.10)
3. Pyruvate dehydrogenase (pyruvate → acetyl CoA) (see Fig.9.22)
4. Alpha ketoglutarate dehydrogenase (alpha ketoglutarate → succinyl CoA) (see Fig.19.2)
5. Beta hydroxyacyl CoA dehydrogenase (beta hydroxyacyl CoA → beta ketoacyl CoA (see Step 3, Fig.12.9)
6. Glutamate dehydrogenase (Glutamate → alpha ketoglutarate

- **ATP/ADP shuttling**, to transport ADP into the mitochondria and ATP out of it
  - It is done by *ATP-ADP translocase* (adenine nucleotide translocase, *ANT*) which is highly abundant in the inner mitochondrial membrane (14% of its proteins)
  - ATP and ADP flow is **coupled**, having the same affinity for both molecules
  - ANT has only single nucleotide-binding site alternating between ATP and ADP
  - Inhibition of ANT causes inhibition of cellular respiration and OxPhos

- Inorganic phosphate (Pi) is transported from the cytosol to the mitochondria by a phosphate carrier

Past Papers

1. In a patient with cyanide poisoning, The expected effect on cytochrome C oxidase:
   A. More oxidized
   B. More reduced
   C. No effect
   D. All of the above can be correct

2. What subunit is the proton path in ATPase?
   A. α subunit
   B. β subunit
   C. C subunit
   D. A subunit

3. What is the role of γ subunit in ATPase?
   A. Binding
   B. Rotation
   C. Structural
   D. Catalysis

4. How do uncoupling proteins influence ATP synthesis and oxygen consumption in mitochondria?
   A. Decrease respiration & decreases ATP formation
   B. Decrease respiration & increase ATP formation
   C. Increase respiration & decrease crease ATP formation
   D. Increase respiration & Increase ATP formation

5. Most O_2 in your body consumed during breathing is converted into?
   A. CO and CO_2
   B. CO_2
   C. H_2O
   D. CO

6. Which of the following is True about Ubiquinone – Cytochrome C:
   A. Ubiquinone is a 2 electron donor – Cytochrome C is a 1 electron acceptor
   B. Ubiquinone is a 1 electron donor – Cytochrome C is a 2 electron acceptor
   C. Ubiquinone is a 2 electron donor – Cytochrome C is a 2 electron acceptor.
   D. Ubiquinone is a 1 electron donor – Cytochrome C is a 1 electron acceptor.

7. The main regulator of the respiratory chain reaction is the level of:
   A. Oxygen
   B. ATP
   C. ADP
   D. Calcium ions
   E. Electron carriers

8. Which of the following is correct about oligomycin, cyanide, Dinitrophenol:

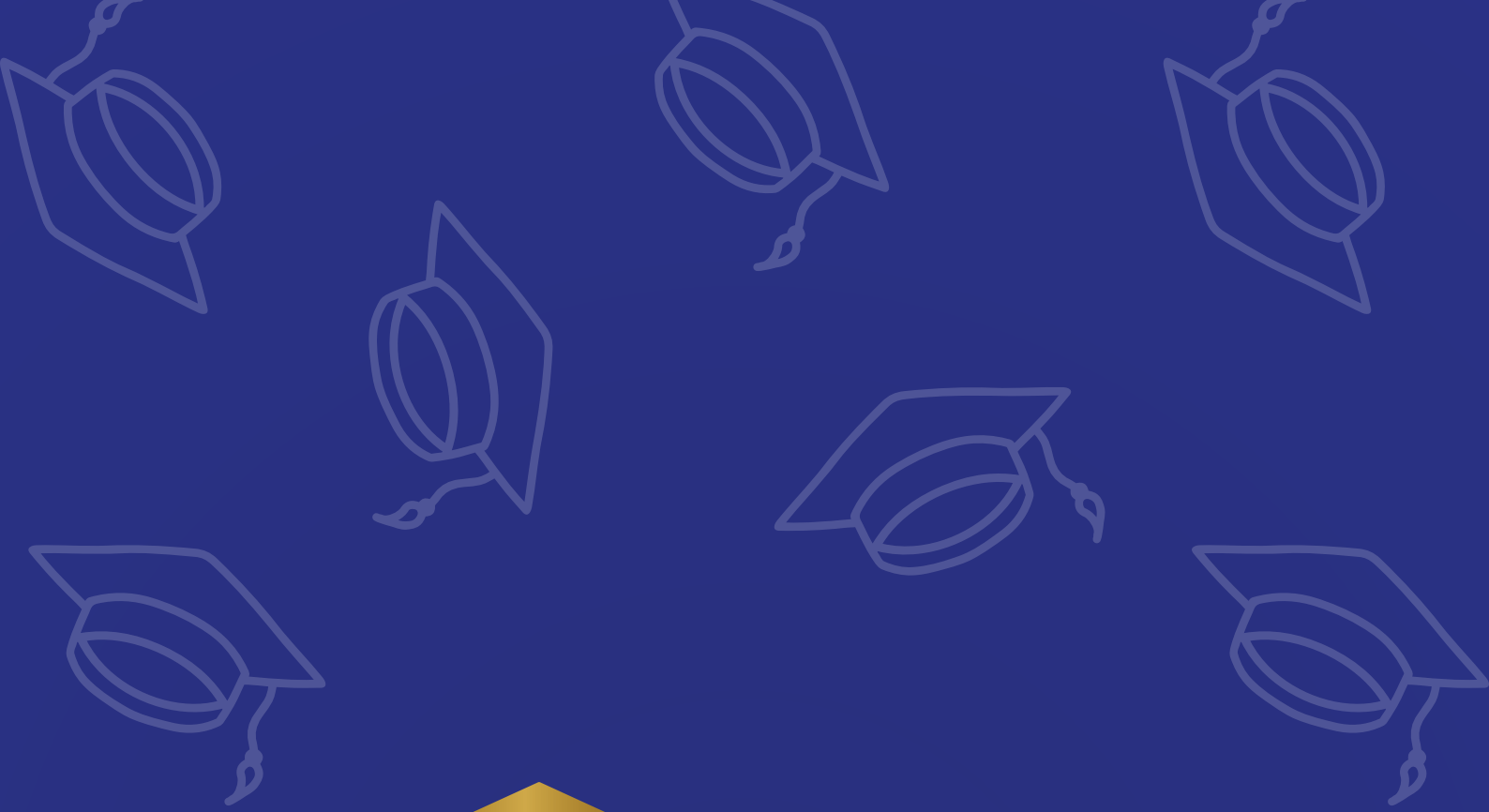
   A. DNP and oligomycin inhibit ATP synthesis, cyanide affects the respiratory chain.
   B. DNP and cyanide inhibit ATP synthesis, oligomycin affects the respiratory chain.
   C. DNP affects the respiratory chain, cyanide and oligomycin inhibit ATP synthesis
   D. All of them inhibit ATP synthesis and the respiratory chain

9. ATP synthase can produce ATP using this mechanism as a direct source of energy:

   A. The oxidation of pyruvate producing CO, and HO
   B. The conversion of glucose to pyruvate
   C. The breakdown of NADH and $FADH_2$
   D. A proton gradient established in the mitochondria
   E. The metabolism of amino acids

10. Ubiquinone is one of the following:

   A. It is a small protein with an iron-sulfur center and can carry one or two electrons between complexes II and III
   B. It is a small organic molecule that can carry one electron between complexes I and III in the mitochondrial inner membrane
   C. It is a large protein embedded in the inner membrane of the mitochondria and can transfer two electrons between complexes I and III
   D. It is a small organic molecule that is free in the mitochondrial inter-membranous space
   E. It is a small organic molecule in the mitochondrial inner membrane that can transfer 1 of 2 electrons to complex III



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