

DOCTOR 2020 | JU



# METABOLISM

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## ATP synthase:

\* As the name implies, ATP synthase is the enzyme that is responsible for the synthesis of ATP.

\* It is composed of **two major parts: F<sub>0</sub> & F<sub>1</sub>**

### **F<sub>0</sub> (membrane domain) :**

- **“a” subunit** (C shaped subunit that can't rotate, point of entry and exit of protons)
- **12 “C” subunits** ( can rotate )

### **F<sub>1</sub> (Headpiece matrix domain):**

- **“γ” subunit** ( angled, rotates )
- **3 “β” subunits** (bind, catalytic activity)
- **3 “α” subunits** ( structural function, holding the enzyme in place )
- **“δ” subunit**

**Note:** more details for each subunit will explained in a while

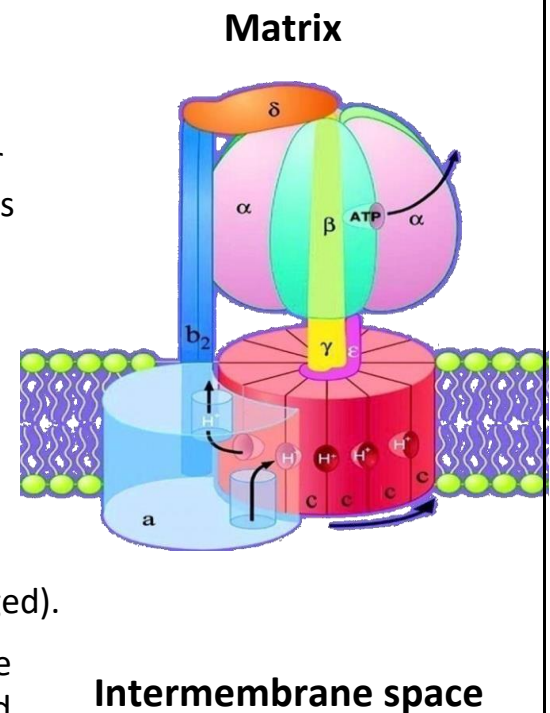
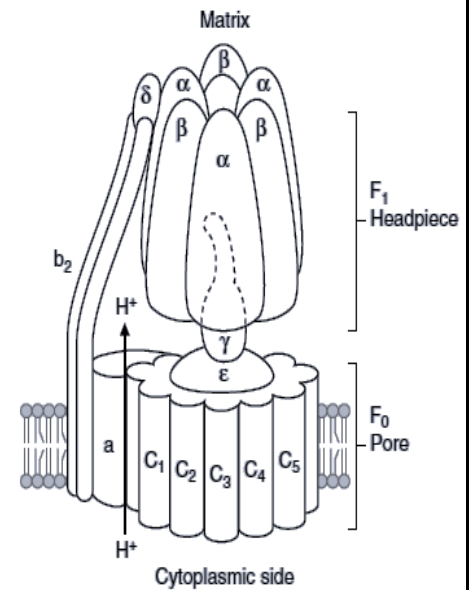
## Now, what exactly happens ?!

- Protons concentration are larger outside (in the intermembrane space), trying to enter according to their **electrochemical gradient** which is the motive force in this process.

- The only way for protons to enter is the ATP synthase, specially the **“a” subunit**, which contains a proton channel. However, this channel is not a direct channel.

- In other words, protons that passes through the channel will not move directly to the matrix. What happens actually is that protons will face a **“C” subunit** that has a **glutamic acid** in its structure (negatively charged).

- The proton will attach to the Glutamate, thus it will lose its negative charge and becomes protonated. This will lead to a conformational change in the hole “C” subunit leading to the movement of the “C” subunit from its location and exposing a new “C” subunit to the “a” subunit channel, which also contains a **glutamic acid** that will become deprotonated... etc → **until full rotation occur (12 H<sup>+</sup> for full rotation, because there are 12 “C” subunits, each one needs 1 H<sup>+</sup> to rotate)**



**Intermembrane space**

- After full rotation: there is another opening in the **(a)** subunit (totally different opening) designed to lose protons from “C” subunits to the matrix ( glutamic acids are able to loss protons due to **different ph values** and **H<sup>+</sup> concentration** in the matrix side)

- Notice that “C” subunits are connected to a “**γ**” subunit and the Rotation of “C” subunits will cause the rotation of “γ” subunit.

- The Rotation of **C** subunits induces the angled gamma subunit to hit **B-subunit** (catalytic ones)>> inducing conformational change for protein.

Animation for ATP synthase mechanism.

[https://www.youtube.com/watch?v=k\\_DQ1FjFuYM](https://www.youtube.com/watch?v=k_DQ1FjFuYM)

**NOTE:** If the gamma subunit is straight, it won't do any action.

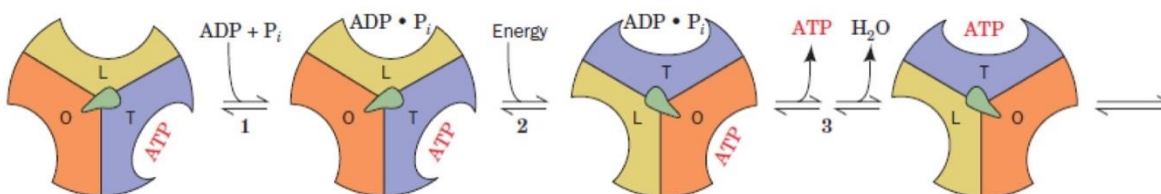
- Beta subunit can undergo **3 conformational changes**:

1) **Open**

2) **loose**

3) **tight**

At first it is open, Open has **high affinity for ADP** with an inorganic phosphate, when a conformational change occurs, it turns into loose, which makes ADP and Pi close together, then another change takes place that makes the subunit tight and generate ATP, and it comes back to open conformation, which has low affinity for ATP, so **ATP is released**.



As any other enzyme, ATP synthase can catalyze the backward reaction (reactions in which forward reaction is catalyzed by different enzyme from the backward reactions are few), thus **ATP synthase can act as an ATPase** that hydrolyzes **ATP** to produce **ADP** and **inorganic phosphate**.

### ➤ **When ATP synthase acts as an ATPase?**

When the electrochemical gradient is flipped back ( concentration of H<sup>+</sup> in the matrix is higher than that in the intermembrane space) → rotation of “C” subunits if flipped, thus gamma subunit will hit beta subunits differently, leading to hydrolyze ATP rather than forming it.

## Energy yield, efficiency and efficacy of ETC:

In order to know the efficiency of the ETC, you need to calculate the theoretical yield which is **the difference of potential energy between the NADH or FADH<sub>2</sub> and O<sub>2</sub>**.

As you know the capacity of energy for **NADH is -53Kcal** and **-41Kcal for FADH<sub>2</sub>**. On the other hand, the actual yield is 2.5 ATP (-18 kcal) For NADH, and 1.5 ATP for FADH<sub>2</sub> (-11 kcal).

- $18/53 = 33\%$  efficiency of NADH (low in OxPhos, not as krebs cycle)
- $11/41 = 26\%$  efficiency of FADH<sub>2</sub>

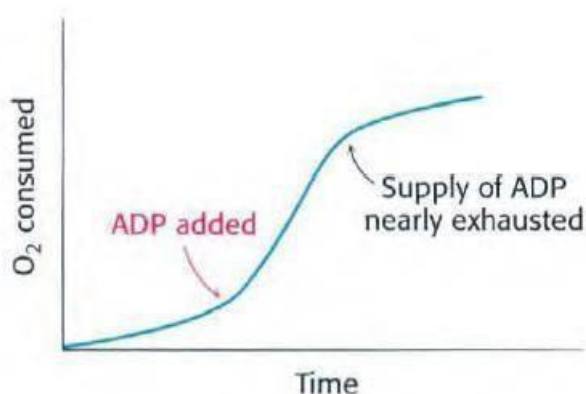
## **Notice that energy yield is low compared to citric acid cycle, why is that?**

As we know, inner mitochondrial membrane is impermeable to any molecule, thus energy is needed for any pumping process (**Anions, Ca<sup>+2</sup>phosphate, ATP, substrates**) either from outside to inside or from inside to outside. Moreover, **25% of energy** provided by ETC is required to move synthesized ATP from mitochondria to cytosol.

## Regulation for oxidative phosphorylation process:

### physiological regulation:

- The concentration of ADP is the most important factor in controlling the whole respiratory process as it also the only allosteric activator for isocitrate dehydrogenase. (**Respiratory control**)
- calcium also activates the ADP enzymes.
- The activity of the process is reflected by O<sub>2</sub> usage as it is the final oxygen acceptor, the **more O<sub>2</sub> is consumed, the more efficient and active ETC is.**



When you add ADP there's a sharp increase in O<sub>2</sub> consumption and once the ADP supply ends the rate decreases.

## 1- Regulation by inhibition:

The mechanism of this regulation is by **inhibiting the enzymes**, so all of the complexes can be inhibited.

By inhibiting enzymes, we stop the electron transfer and pumping of protons will be stopped. Which will result in decreasing the electrochemical gradient across the membrane. This is fatal because we need high electrochemical gradient for the ATP synthesis process to work effectively.

Notice that inhibition is done on a **coupled reaction**; in other words, ETC is still coupled to phosphorylation process.

- **Examples of inhibitors:**

| Specific inhibitor   | Target                        |
|--|-------------------------------|
| Rotenone (insecticide) & Amytal (sedative)                               | NADH-Q oxidoreductase         |
| Antimycin A (antibiotic)   | Q-cytochrome c oxidoreductase |
| Cyanide (CN <sup>-</sup> ), Azide (N <sub>3</sub> <sup>-</sup> ), & (CO) | Cytochrome c oxidase          |
| Oligomycin (antibiotic)  | ATP synthase                  |

The ATP synthase “membrane domain” is named as Fo because it is inhibited by **Oligomycin**

For a full ETC inhibition, Complex 3, 4 must be inhibited, because inhibiting complex 1 **or** 2 will leave an uninhibited entry point (either for **NADH** or **FADH<sub>2</sub>**). The inhibition of any complex will lead to stabilize the previous complexes in a **reduced form** and the upcoming complexes in an oxidized form.

### The toxic effect of CO & Cyanide:

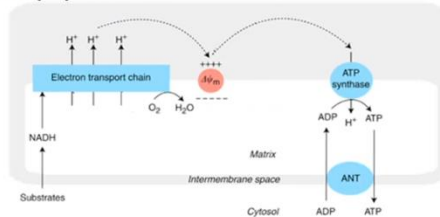
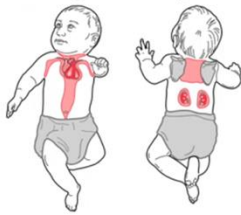
CO and Cyanide mimic O<sub>2</sub>, they go parallel with it, they can bind to where oxygen binds (where you find heme) >> myoglobin/hemoglobin/complex 4 in ETC, So when cyanide or CO bind to complex **4**, they will inhibit the movement of electrons >>So, no proton will be pumped >> No ATP will be synthesized.

Cyanide is found in some fruits' seeds; however, large number of these seeds is needed to achieve cyanide toxicity.

## 2- uncoupling regulation:

### Regulation – Uncoupling Regulated - Uncoupling proteins (UCPs)

- Short-circuiting ATP synthase
- UCP1 (thermogenin):
  - ✓ Brown adipose tissue, non-shivering thermogenesis
  - ✓ Infants: neck, breast, around kidneys
  - ✓ Fatty acids directly activates UCP1
- UCP2 (most cells); UCP3 (skeletal muscle); {UCP4, UCP5} (brain)
- Obesity tendency in some populations



Recall that uncoupling proteins described in the previous lecture, which are used to regulate the process by changing the H<sup>+</sup> concentration.

Scientists have worked on producing a chemical that is able to bind with protons and transport them across the inner mitochondrial membrane, thus no ATP will be produced → treat obesity.

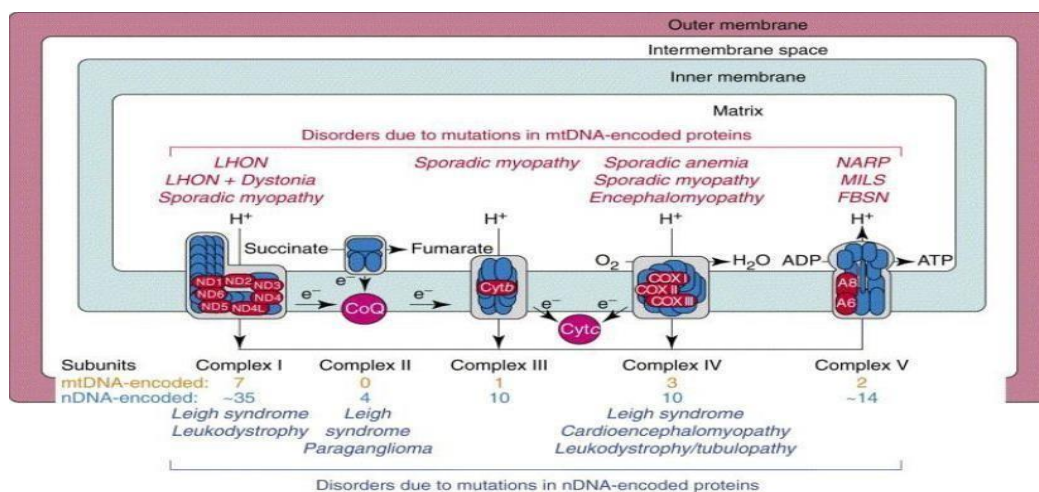
**DNP:** 2,4-dinitrophenol is a drug designed with a benzene ring structure and hydroxyl group, thus it's a **lipophilic molecule** (can cross the membrane) that can bind and release H<sup>+</sup> depending on the pH. In the intermembrane space, pH is low, thus DNP accepts a proton and become protonated then it will cross the inner membrane towards the matrix. In the matrix, H<sup>+</sup> concentration is low, thus the DNP releases a proton and become deprotonated >> so it picks up the proton from the outside and release it toward the matrix.

This drug returns the protons back to the matrix >> generating more heat and less ATP>> less anabolism and building up process. But many problems have been associated by using this chemical inhibitor like: eyes bleeding, blindness and some death cases.



## Oxidative phosphorylation diseases:

- **The 5 complexes in ETC are enzymes (proteins), consist of large number of polypeptides:**
- Mitochondrial DNA works to synthesize part of the complexes' polypeptides:
  - 7 polypeptides out of 25 for complex1
  - 1 subunit from complex 3
  - 3 subunits from complex 4
  - 2 subunits from F0 portion in ATP Synthase.
- **Most of proteins are synthesized by nuclear DNA, then translocated to mitochondria.**
  - So mitochondrial proteins have mixed origin, the mitochondrial DNA makes the lowest percentage of them compared to the nuclear DNA.
  - The genetic diseases that affect the mitochondria, might be from mitochondrial origin (from the mother only as it shows heteroplasmy )or nuclear origin (it will present in all cells of the body).
  - Inner mitochondrial membrane is filled with proteins so that's why nucleus synthesizes higher than 1000 protein.



## Mitochondrial Shuttling Systems

### { Cytosolic NADH }:

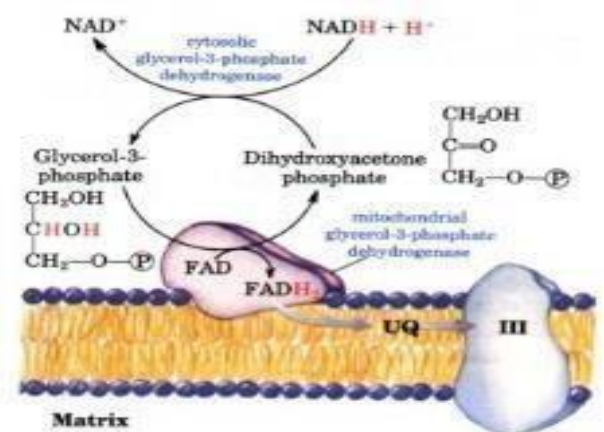
- Glycolysis is one of the sources of NADH from outside the mitochondria.
- There should be a way to get the NADH from outside to inside, because it couldn't enter freely, there must be a specific transporter for it, but there is not. So the electrons from the NADH must be used in another form to enter the mitochondria.

➤ How to translocate electrons from NADH in the cytosol to the mitochondria, to be used as an energy source?

### glycerol- 3 phosphate shuttle:

by Glycerol 3-phosphate dehydrogenase:

- highly distributed in many tissues (muscles & brain)
- Has **mitochondrial** and **cytosolic** copies.
- This enzyme converts dihydroxyacetone phosphate to glycerol 3- phosphate >> this process ( converting ketone to alcohol) uses NADH (take its electrons).
- On the outer surface of the inner membrane, there is the mitochondrial copy of this enzyme that switches the reactions and converts alcohol into ketone extracting the electrons by the coenzyme FADH<sub>2</sub> that is found inside the enzyme.
- Generation of FADH<sub>2</sub> >> coenzyme Q take the electron>> complex 3 (4 protons pumped) >>complex 4 (2 protons pumped) >> **generation of 6 protons** ( less ATP yield than mitochondrial NADH)



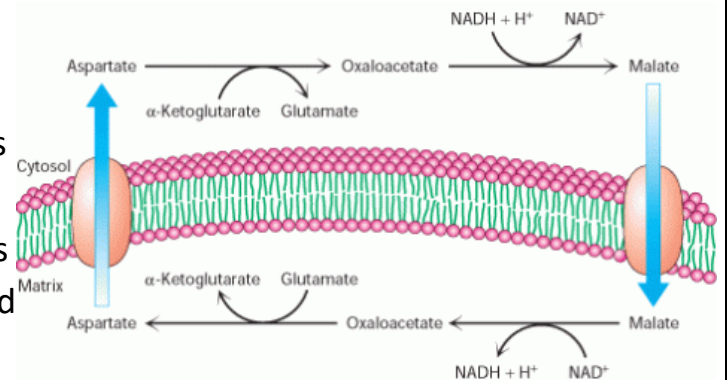
For more clarifications:

<https://www.youtube.com/watch?v=sglxi7l21-M>



## Aspartate-Malate shuttle:

- Aspartate can be converted to oxaloacetate by transaminases.
- Oxaloacetate can be converted to malate (it's the reversible reaction that found in citric acid cycle, where we form NADH) but in this REVERSIBLE reaction, **cytosolic NADH** is used by malate dehydrogenase enzyme.



- malate has a transporter (because of gluconeogenesis) so it can cross through the membrane >> malate is inside the matrix >> converted to oxaloacetate by malate dehydrogenase enzyme and regenerate the NADH, so it can go to complex 1 >> **generating 10 protons.**

**Note:** malate dehydrogenase enzyme has 2 copies:

- **mitochondrial copy** >> convert malate to oxaloacetate.
- **cytosolic copy** >> convert oxaloacetate to malate.

For more clarification:

<https://www.youtube.com/watch?v=nEj8b-sg4ps>

## ATP –ADP Translocase Enzyme:

- Also known as **Adenine Nucleotide Translocase (ANT)**, is a transporter protein that enables the exchange of cytosolic ADP and mitochondrial ATP across the inner mitochondrial membrane.
- Free ADP is transported from the cytoplasm to the mitochondrial matrix, while ATP produced from oxidative phosphorylation is transported from the mitochondrial matrix to the cytoplasm, thus providing the cells with the energy need.
- This enzyme can undergo conformational changes; inside the mitochondria it has high affinity for ATP, so when it binds to ATP it will undergo conformational changes and it opens up out, where it will have low affinity for ATP to release it, and high affinity for ADP so it binds to it and ADP will move inside.

- **Notice that**, the entry of ADP into the matrix is coupled to the exit of ATP (**The ratio between ATP and ADP 1:1**) and this doesn't mean that they have equal concentration.
- This protein forms 14% of the proteins found on the inner membrane of mitochondria.
- The Reaction is endergonic; with 25% of energy spent on this enzyme, that's why the efficiency of ATP synthase is not as much as TCA cycle.
- For more clarification:  
[www.youtube.com/watch?v=bWATYDuUnLE](http://www.youtube.com/watch?v=bWATYDuUnLE)

### **Extra note about cytochrome C & complex 3 :**

A cycle known as a Q cycle occurs in Complex 3. This complex contains heme C (which has high affinity to reduced CoQ) towards the intermembrane space and a Heme B ( which has a high affinity to oxidized or partially oxidized CoQ) towards the matrix.

Reduced CoQ will binds to heme C, giving it **an electron**, which will pass to a cytochrome C and the second electron will pass to Heme B. Accordingly, CoQ become oxidized, thus it will leave heme C and binds to Heme B.

At the level of heme B, electrons will be passed from heme B towards the oxidized CoQ changing it to a partially oxidized.

When another Reduced CoQ binds heme C, the same process will occur and an electron will pass to heme C then to cytochrome C and the second electron will be pass to heme B which is still connected to the first partially oxidized, thus the electron will pass to it.

Accordingly, partially oxidized CoQ ( radicle ) is kept bounded and not freely.

In conclusion, for each 2 CoQ ( carrying 4 electrons) , 2 electrons are regenerated and 2 electrons are passed ( two cytochrome C form ).

**The End**