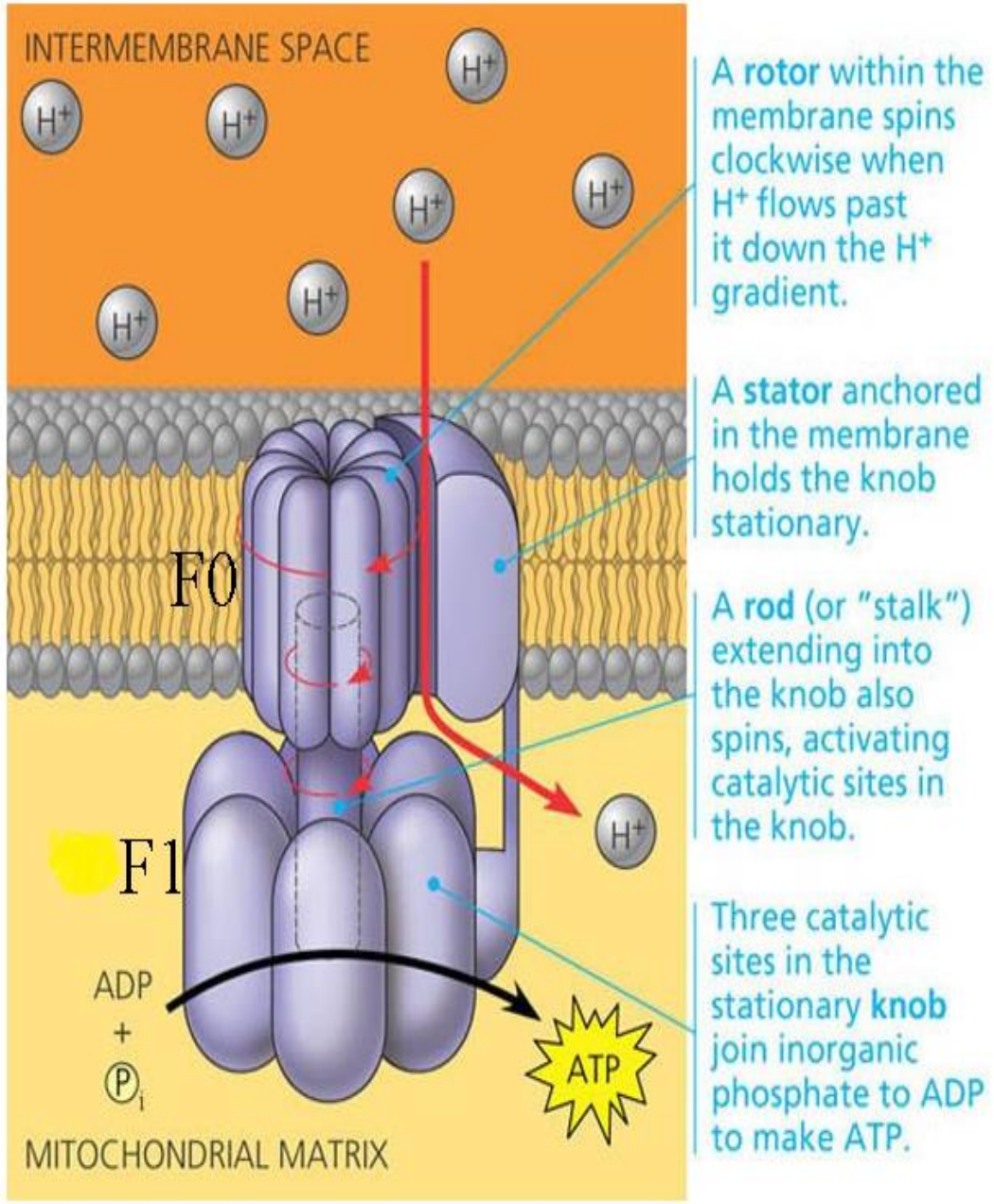
فريق طوفان الأقصى

METABOLISM

Modifide N.

Writer: Suhaib Zaiter
Ahmad Alkashef

Corrector: Alaa Khader



- **NOTE:** **ATP synthase is the enzyme that is responsible for ATP synthesis.**
- **Some people call it complex delpuoc si ti tub ,5 .CTE htiw**

STRUCTURE AND MECHANISM OF ATP SYNTHASE-COMPLEX V

1. F0 is the proton channel of the complex
2. F1 hydrolyzes ATP in the absence of proton gradient
3. The stalk between F1 and F0 contains several proteins, one of which is sensitive to oligomycin. This antibiotic inhibits ATP synthesis by interfering with the utilization of the proton gradient.
4. ATP SYNTHASE catalyzes the reaction:

ADP +Pi---→ ATP +H2O

https://youtu.be/U26Jz3K1w2k (Animation video)

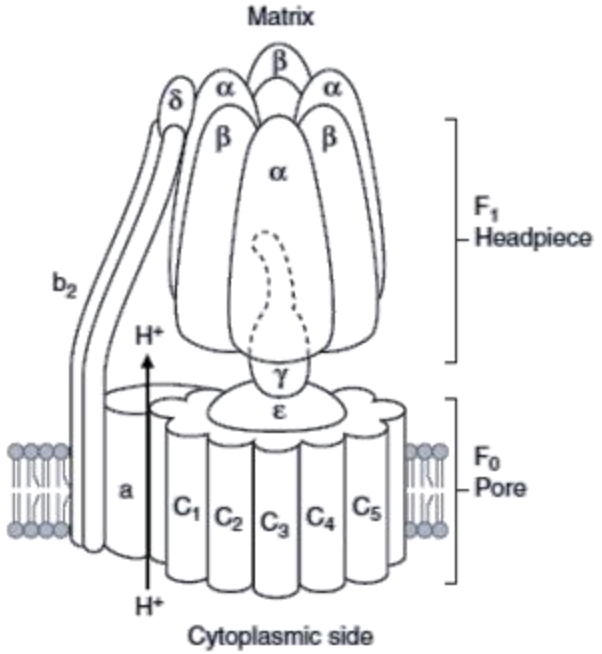
- **The complement in this slide:**

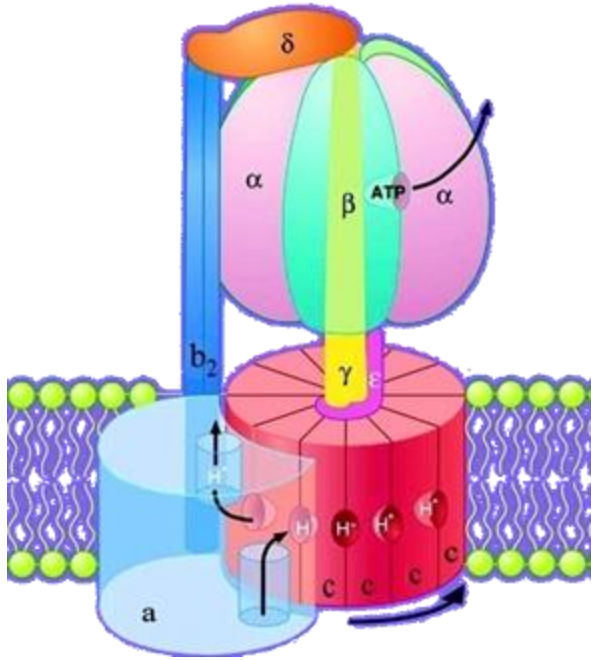
- it is divided into 2 connected parts F0(F zero) and F1.
- The function of each part is different from the other.
- F0(F zero): it is responsible of the passage of protons to reach F1
- F0 is called like this because it is inhibited by **Oligomycin (antibiotic that inhibit the passage of protons).**
- F1:it is the part that will phosphorylate ADP in the presence of Pi to give an ATP

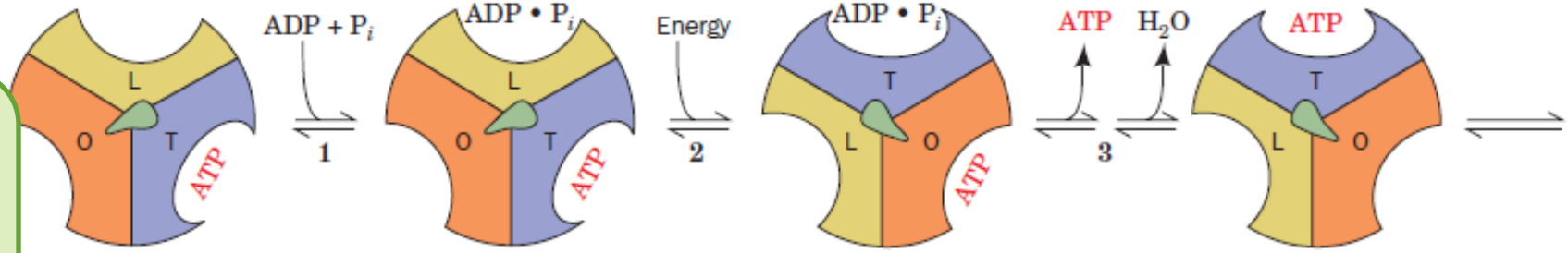
ATP Synthase

- NOTE: I will explain it in the coming slides

- F1:
  - "γ" subunit: rotates angled
  - "β" subunit: binds
  - "α" subunit: structural
  - 3 conformations: tight (T), loose (L), open (O)
- Fo:
  - "a" subunit: point of entry & exit
  - "c" subunit rotates (10 –14)
  - 4H+/ATP







- Can run backwards: means that we can see hydrolysis of ATP because ATP-synthase also can act as ATPase.
  ATP --> ADP + Pi

- **F1 structure:** composed of many different subunits **α, β, γ, δ, and ε.**
- These alpha and beta subunits they will make hexamer (6 subunits aggregated with each other, 3 α and 3 β) الترتيب: (**alpha** ,beta, **alpha**, beta ,**alpha**, beta)
- Gamma ,γ, is a single subunit as well as the Epsilon ,**ε,** single subunit.
- the Gamma subunit which is part of the F1 is extended(attached) within the F0.
- There is a structure which is the "a" subunit in F0 and a delta subunit that connects the "a" unit of F0 with the hexamers of the F1 part.so this arm is composed of this delta subunit that connects F0 with F1.
- **F0 structure:** it's composed of 2 different subunits. One is called "**a**" subunit and the other is called "**C**" subunit. The clusters of **C** subunits --> **C** ring
  - The "a" subunit of the F0 has 2 important structures to remember **2 half channels.**
  - One extended from the inter mitochondrial space(opens in the inter membrane space) and the other half channel open in the mitochondrial matrix.
  - You would see the importance of these 2 half channels and C ring in **passing the protons** from the inter membrane space to the F1 which is found in the matrix.

- **The complement in this slide:** now remember that this part of the enzyme (Betaβ) is a catalytic site (means that the site where oxidative phosphorylation could occur)
- Alpha is a non-catalytic, it's structural to help beta.
- The G amma subunit in F1 is important because since it is connected to the C ring and as you will see C ring will rotate so Gamma subunit also will rotate, rotation of the Gamma subunit with the proton transfer from F0 to F1 will activate Beta subunit to do the catalysis.
- Delta subunit is important because it makes things in their places(fix them in their places).

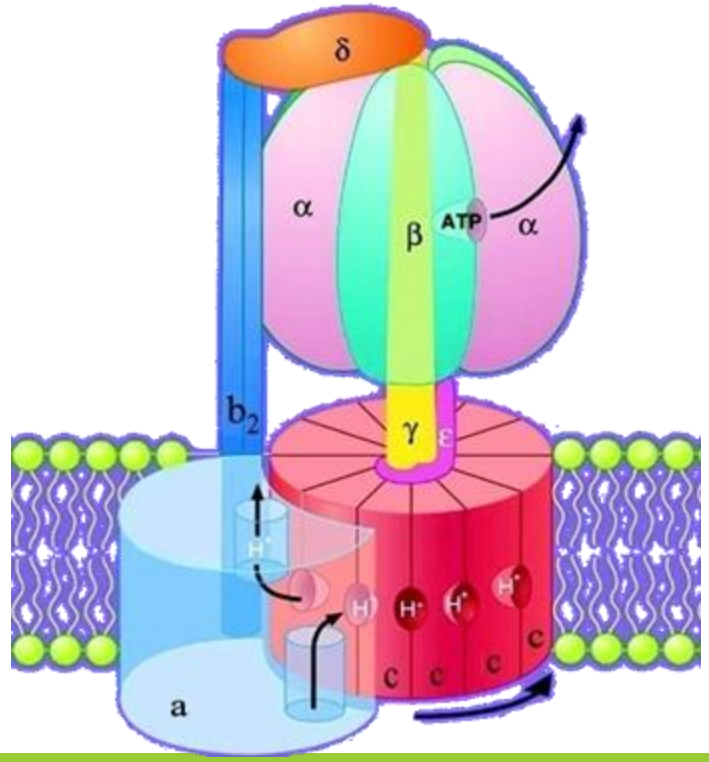
Beta : Catalytic

Alpha: Structural

Gamma: connect F0 with F1 and it will rotate with the C ring (Alpha and Beta will not rotate)

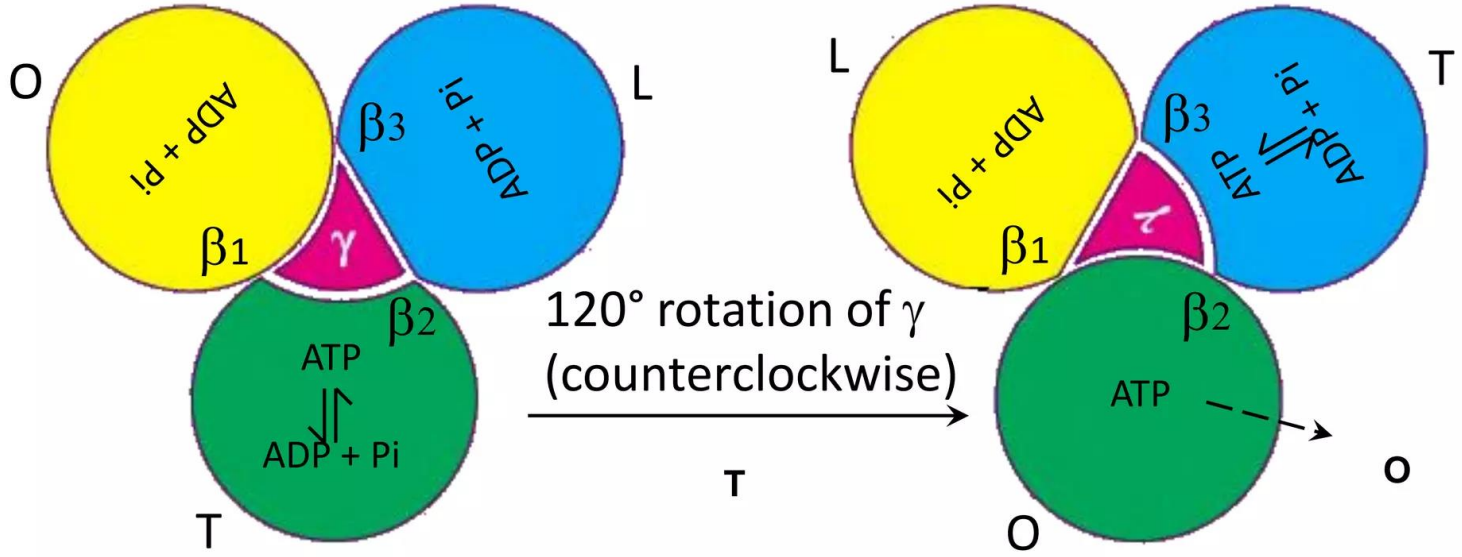
Epsilon: will help gamma

Delta: also connect F0 with F1



Binding-change mechanism of ATP synthesis

- Rotation of gama subunit drives release of tightly bound ATP
- 3 active sites cycle through 3 structural states: O, open; L, loose-binding; T, tight-binding
- At T site, ADP + Pi → ATP, but ATP can't dissociate
- G rotation causes T → O, L → T, O → L
- As a result of the T→O structural change, ATP can now dissociate from what is now an O site.



- **The complement in this slide:** when the protons comes from the inter membrane space via the C ring, C ring will rotate and as a result Gamma subunit will rotate, when it rotates it will stimulate Beta subunit for action.
- Beta subunits have 3 active sites or 3 conformation states: T state (tight), L state (Loose) and O state (Open).
- L state is the place that binds ADP and Pi but still they are far from each other on the L state **(no ATP formation or release happen here).**
- In the T state ADP will be phosphorylated and forms the ATP **(ATP formation but no ATP release)**
- In O state, T ATP will be transferred to O site of the Beta subunit and then it will be released in the matrix.
- Once the proton pass the C ring, the C ring will rotate with a part of F1 which is Gamma subunit
- once Gamma rotate, it will lead to change the conformation of the Beta subunits. e.g T----->O , O----->L , L----->T **while rotation**
- (بشكل معين وبس يصير في دوران بتغيروا لشكل آخر يعني قاعد بقول انه بس ما يكون في دوران بكونوا)
- **REMEMBER**: nor T nor L nor Alpha subunits will release ATP, only the site in which ATP is released is the O site of the Beta subunit.
- Alpha subunit will bind/have ATP but it will not be released.

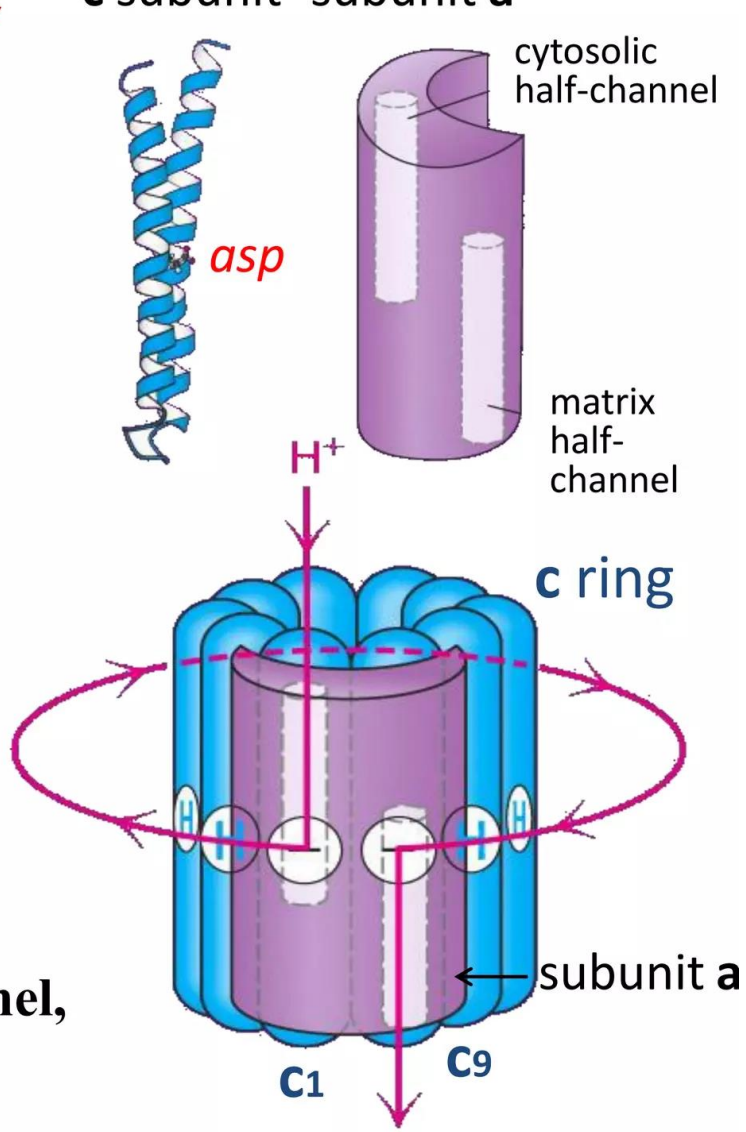
H^+ path through membrane

c ring & a subunit structure

- **each c subunit has 2 membrane-spanning a helices**
  - **midway along 1 helix: *asp***
  - **COOH↔COO^-**
- **a subunit has 2 half-channels**

H^+ path

- **H^+ from cytosol diffuses via half-channel to *asp* on c ring subunit (c1)**
- **this subunit can now move to interface membrane, allowing c ring to rotate**
- **c9 now interfaces matrix half-channel, allowing H^+ to diffuse into matrix**



NOTE: protons will pass through there

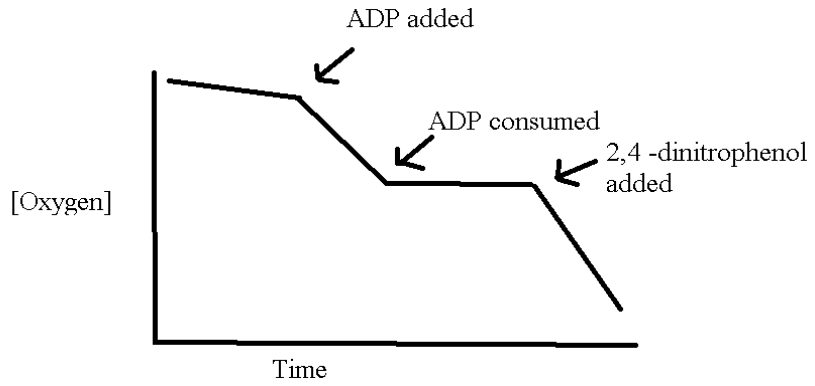
- **NOTE:** proton will enter cytosolic half channel and because of specific mechanism the C ring will rotate 360º and the proton will exit from the matrix half channel.
- What is the importance of C ring? C ring rotation and in the C ring there in the first half way النقطة البيضة القصد there is an amino acid called aspartate, which negatively charged that binds with protons.
- It will rotate until it reaches proton release site in second half cycle. And
- Because of the hydrophobicity and hydrophilicity changes, proton will be released into F1 region .

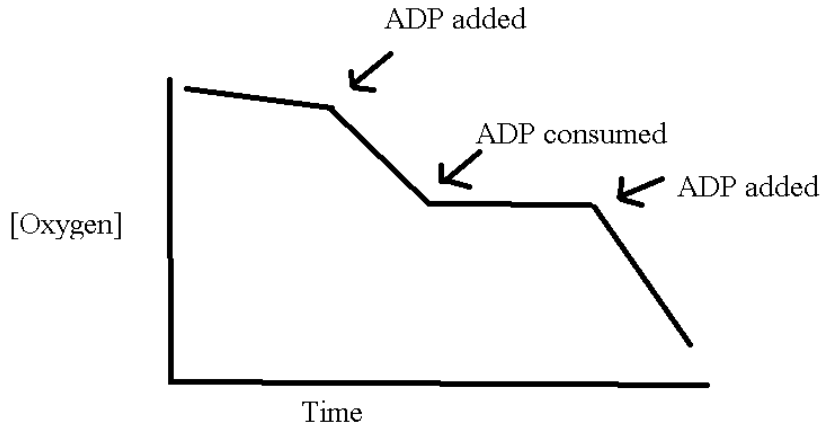
واال : كلمات صاحب تسجيل الصوت
سلايد كنت ناوي اخلص الميتا بس 50
شكلها هتخلص علي 😅

- **NOTE: C ring, without the space, is what meta is :)**

- We are talking about the control on the ADP effect on the oxidative phosphorylation and electron flow.

RECEPTOR OR ACCEPTOR CONTROL





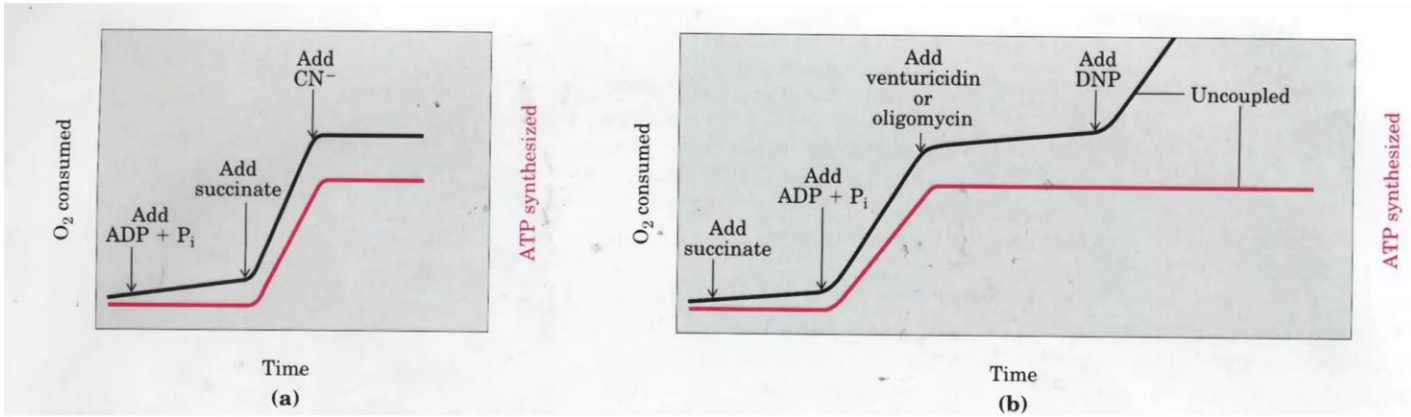
Electron transport is normally tightly coupled to oxidative phosphorylation so that electrons do not flow through the respiratory chain unless ADP is simultaneously phosphorylated to ATP.

Uncoupling agents, such as 2,4- dinitrophenol, collapse the proton gradient as they can channel protons across the membrane. Under this condition, electrons transport runs unchecked at its maximal rate in the absence of the acceptor ADP.

Electron transfer to O_2 was found to be coupled to ATP synthesis from ADP + P_i in isolated mitochondria

- ATP would not be synthesized when only ADP and P_i are added in isolated mitochondria suspensions.
- O_2 consumption, an indication of electron flow, was detected when a reductant (e.g., succinate) is added, accompanied by an increase of ATP synthesis.
- Both O_2 consumption and ATP synthesis were suppressed when inhibitors of respiratory chain (e.g., cyanide, CO, or antimycin A) was added.
- **ATP synthesis depends on the occurrence of electron flow in mitochondria**.

- O_2 consumption (thus electron flow) was neither observed if ADP was not added to the suspension, although a reductant is provided!
- The O_2 consumption was also not observed in the presence of inhibitors of ATP synthase (e.g., oligomycin or venturicidin).
- **Electron flow also depends on ATP synthesis!**



Electron transfer was found to be obligatorily coupled to ATP Synthesis in isolated mitochondria suspensions:
neither occurs without the other.

NOTE:

- This access is the oxygen consumption or electron flow in the electron transport chain, and this represent the time.
- The red curve represent the ATP profile and the black curve represent the oxygen consumption.
- Here we have ADP and inorganic phosphate but still we didn't add the reductant or the succinate that will be oxidized.

The complement in this slide:

- At this point, there is as you see this gradual increase in the oxygen consumption but there is no ATP synthesis till this region or this amount of time.
- What do you conclude?... that when you add ADP the oxygen consumption is increased and because the electron flow was increasing.
- Now if you add succinate at this point and when you look at the oxygen consumption at ATP production there is a big shift in increase of oxygen consumption that reflects in the increase of electron flow at the same time there is an increasing in ATP production
- It means that there is a coupling. Once you have electron flow you have oxygen consumption and thus you have ATP production
- If you add at this point CN-, (is an inhibitor of cytochrome c oxidase), there will be no more oxygen consumption because there was the electron flow was locked and thus no more ATP production.
- This indicates that synthesis of ATP is coupled with the oxygen consumption and the electron flow in the electron transport chain.

The complement in this slide:

- Now, look at this this is oxygen consumption, this is time, this is the substrate that is going to be oxidized and produced the reducing bond, the same thing there is little increase in electron flow and oxygen consumption in low ATP because there was no ADP and in organic phosphate, and at this point you add the ADP in the presence of succinate … look what happened 😳 !!! An increase in oxygen consumption because of an increase of electron trans flow of electrons, and there is an increase in ATP production or synthesis

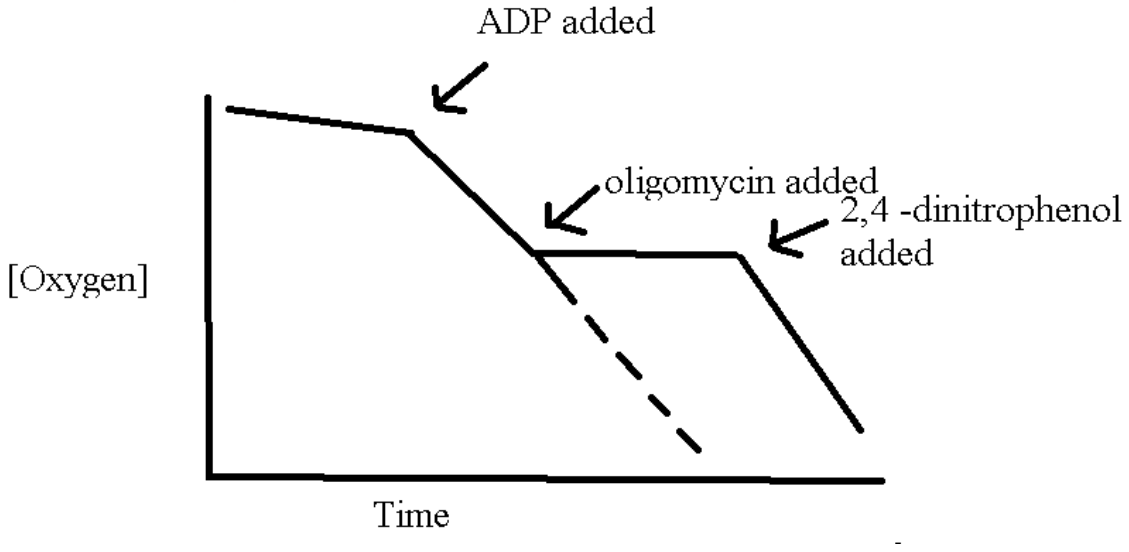
The complement in this slide:

- Now if you add oligomycin… what is the oligomycin 😳??? Inhibitor ATP synthase enzyme F0 part
- Inhibition of ATP synthase compare this rate of oxygen consumption vs adding ADP, it is suppressed because ATP synthesis stops, so electron flow and oxygen consumption will be suppressed, so ATP synthesis and oxygen consumption as will electron flow, they are coupled if one stops other will stop if both works both will work.
- Now if you add at this point, you put oligomycin and make an inhibition electron flow and make inhibition for oxygen consumption and inhibition for ATP synthesis, if you add here compound called DNP or dinitrophenol this compound is uncoupler it will separate (uncouple) ATP synthesis from electron flow and oxygen consumption, the presence of uncoupler oxygen consumption and electron flow increased comparable of this and the same as this but there is **no ATP synthesis** , what is happening here? 🤴.... This DNP is considered as uncoupler it will take the protons out from the intermembrane space not letting them to go through ATP-ase but will **collect** them and break the gradient take them inside the matrix, because it is hydrophobic by any needs it will take these protons and pass them in the matrix of the mitochondria because of this the electron flow **continues oxygen consumption continues but no ATP.**
- The energy was dissipated(اطلقت) instead of synthesizing ATP, it was dissipated as heat, and this is why's in some periods of times before thirty to forty years people were using DNP to lose weight because there will be no ATP production but all the energy that coming from food is dissipated as heat, but nowadays they stop it because they found there is side effects

NOTE:

- **Why we called it the F 0 part?**
- **Because it is inhibited by Oligomycin**

RECEPTOR OR ACCEPTOR CONTROL....



Oligomycin inhibits the increased oxygen consumption stimulated by the addition of ADP: phosphorylation of ADP to ATP is also inhibited under these conditions.

Oligomycin prevents the utilization of the proton gradient.

Uncouplers relieve the inhibition of oxygen consumption.

Brown fat cell contain endogenous uncouplers that enhance metabolism and produce heat. This mechanism is important to protect sensitive areas of humans newborn from cold.

Oxidative Phosphorylation
P:O ratio

Definition: the number of molecules of inorganic phosphate incorporated into ATP per atom of oxygen used.

P:0 ratio varies with the substrate being oxidized:

With NADH it is 3

With succinate it is 2

With ascorbate it is 1

The overall equation for respiratory chain phosphorylation:

$NADH + H^+ + 3ADP + 3Pi \rightarrow NAD + H_2O + 3ATP$

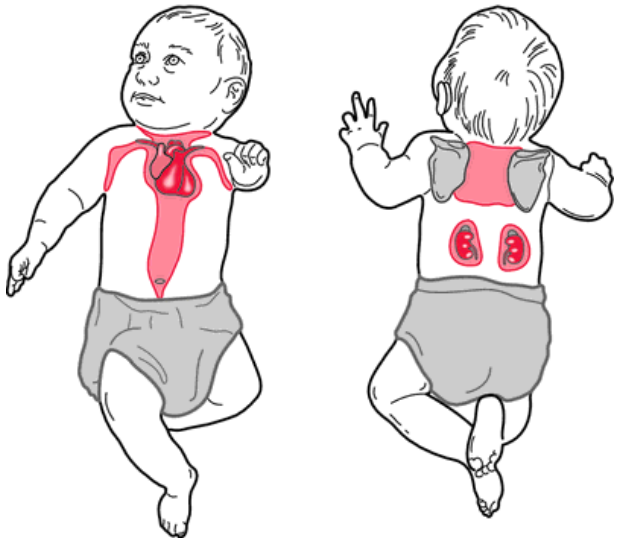
The complement in this slide:

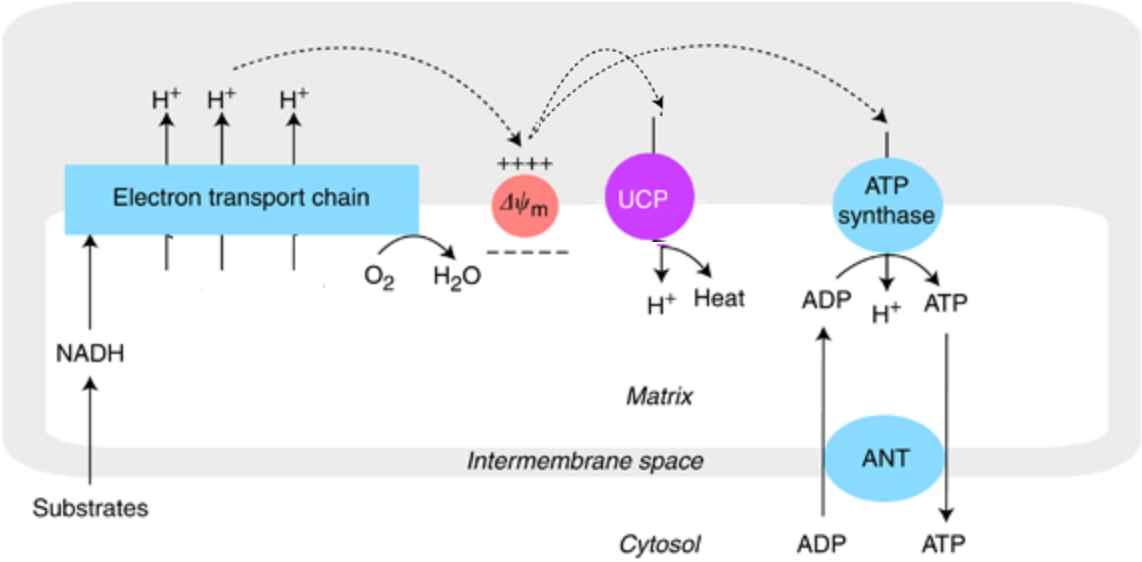
- There is some terminology to indicate about the energy weight that it is produced from oxidation of different metabolize that terminology is called P:O ratio which is defined as how many ATP molecules will be produced per oxygen atom and the P:O ratio is different one metabolite subjected to oxidation to another metabolite so for example metabolite that produce NADH will have more P:O ratio that metabolite FADH2 so if you look or measure or calculate the P:O ratio for succinate will be different from the P:O ratio of malate, malate oxidation will produce NADH, succinate oxidation will produce FADH2 and you saw from electron transport chain FADH2 will produce less ATP than NADH so this P:O ratio for succinate is less than the P:O ratio for malate or pyruvate
- Complete oxidation for example of pyruvate what will be is P:O ratio.
- Complete oxidation of pyruvate to CO2 what will be the P:O ratio of complete oxidation of pyruvate?

The answer is: 14: 5 v2

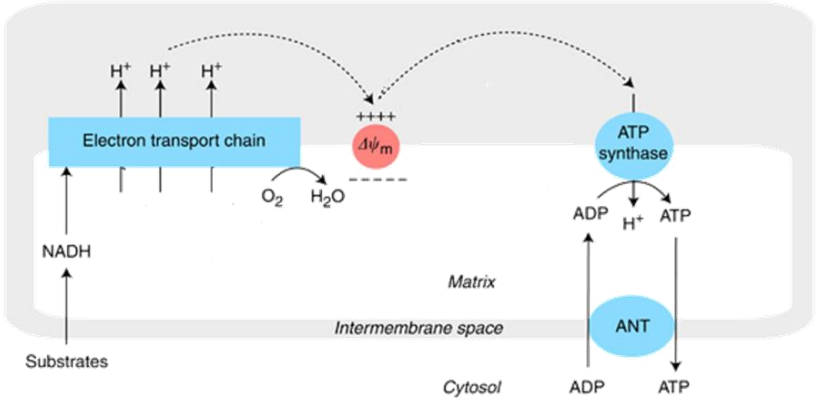
Regulation – Uncoupling
Regulated - Uncoupling proteins (UCPs)

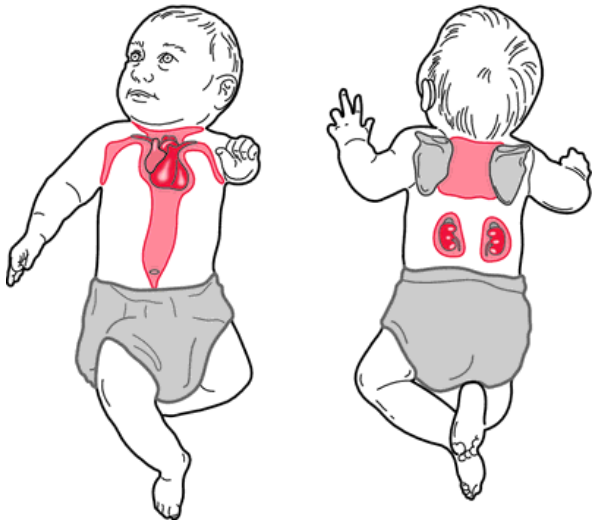
- **Short-circuiting ATP synthase**
- **UCP1 (thermogenin):**
  - ✓ **Brown adipose tissue, non-shivering thermogenesis**
  - ✓ **Infants: neck, breast, around kidneys**
  - ✓ **Fatty acids directly activates UCP1**
  - ✓ **UCP2 (most cells); UCP3 (skeletal muscle); {UCP4, UCP5} (brain)**





Regulation – Uncoupling
Regulated - Uncoupling proteins (UCPs)





- **The complement in this slide:**
  - **You saw the DNP as uncoupler and this DNP is artificial or it is a drug uncoupler, but there is natural uncouplers one of them is called thermogenic UCP1 and it is found in mitochondria in the newly born babies you will see if you notice on their back brown color ;because of the high concentration of mitochondria in that region that contains a lot of thermogenic. So thermogenic as uncoupler, in those newly born babies will produce enough heat to make them warm all the time this is called the natural uncoupler .**

V3: SLIDE 15

ADP instead of ATP