PYRUVATE METABOLISM , TRICARBOXYLIC ACID CYCLE, AND ELECTRON TRANSPORT CHAIN

METABOLISM

1st SEMESTER, 2023

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PYRUVATE METABOLISM

- **Aim: to explain the mechanism and control of pyruvate dehydrogenase, the multienzyme system responsible for the conversion of pyruvate to acetyl-CoA.**
- Content:

1. The reaction **mechanism** involved in the conversion of pyruvate to acetyl-CoA.
2. The organization of the **3 enzymes-E1, E2, E3-** of the multienzyme complex.
3. The **5 coenzymes** involved in the reaction and the 5 B vitamins from which they derived.
4. The **allosteric and covalent modification** of the kinase and phosphatase controlling E1.

OBJECTIVES

1. Write out the reactions involved in the conversion of pyruvate to acetyl-CoA catalyzed by pyruvate dehydrogenase
2. Explain the functions of TTP, lipoate, coenzyme A, FAD, and NAD in the pyruvate dehydrogenase-catalyzed reaction.
3. Demonstrate that you understand how the activity of the enzyme is influenced by insulin and fed state.
4. Demonstrate that you understand how the liver enzyme is controlled in the fasted state when that organ is a glucose producer
5. Explain the **central role of pyruvate** and acetyl-CoA in metabolism.

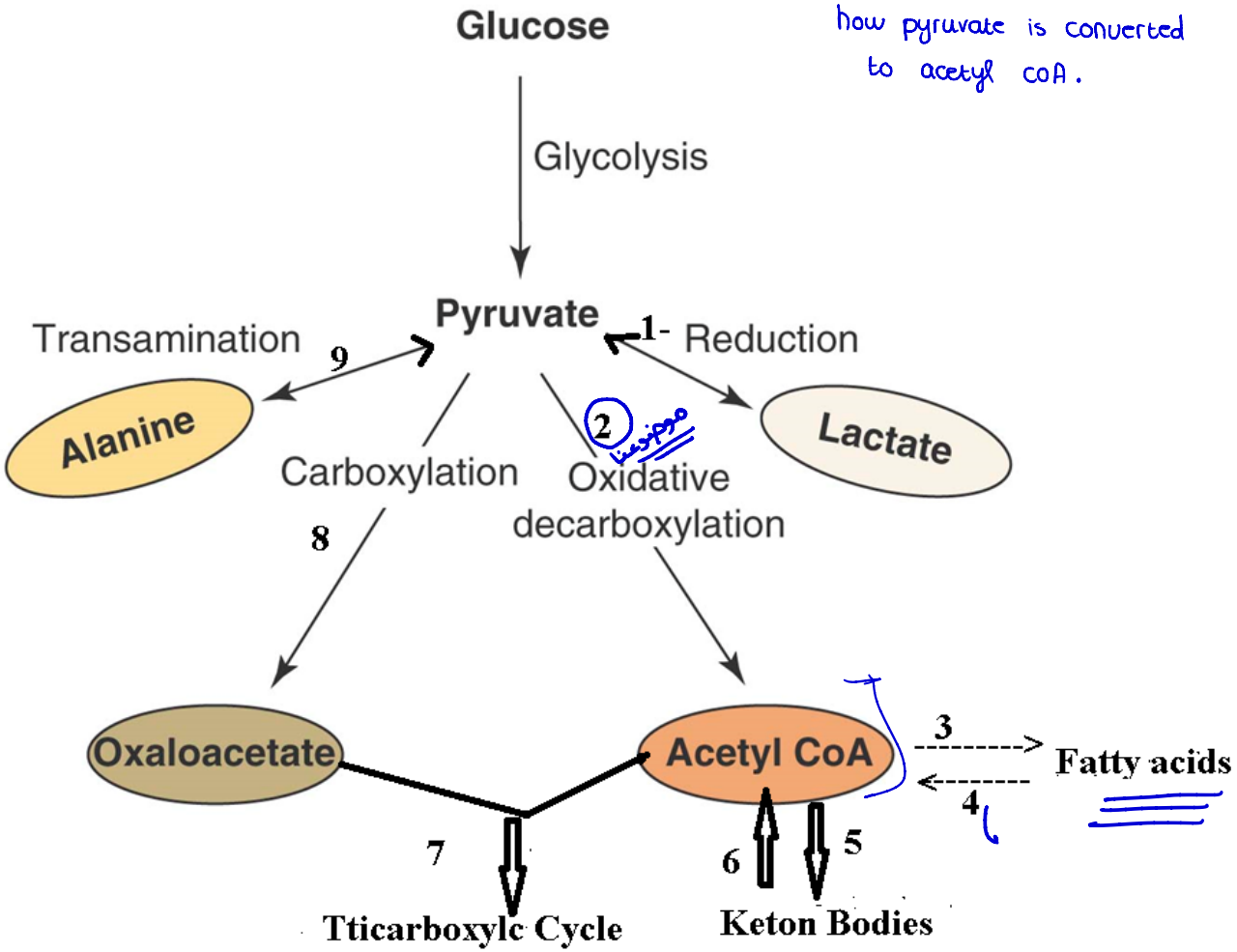
here

What are we going to talk about?

how pyruvate is converted to acetyl coA.

Pyruvate is at important metabolic crossroads

1. Lactate dehydrogenase
2. Pyruvate dehydrogenase
3. Fatty acid synthesis
4. Fatty acid beta- oxidation
5. Ketone body synthesis
6. Ketone body utilization
7. Citrate synthase
8. Pyruvate carboxylase
9. transamination



PYRUVATE DEHYDROGENASE complex

- Oxidative decarboxylation of pyruvate to acetyl CoA.
- The reaction occurs in mitochondrial matrix
- 3 enzymes (or many activities), 5 coenzymes-thiamin pyrophosphate(B1), lipoamide, Flavin adenine dinucleotide (B2), coenzyme A (contain B3), and NAD (niacin)-are required.

if one of them is ↓↓ → The enzyme will **NOT** work.

3 types

E1 : Pyruvate dehydrogenase decarboxylase.

E2 : Dihydrolipoyl transacetylase → transfers an acetyl group to lipoic acid.

E3 : Dihydrolipoyl dehydrogenase → Redox RXNs.

In addition , there are two enzymes, a kinase and a phosphatase, which have key role to play in the control of pyruvate dehydrogenase complex. "By covalent modificarions".

Phosphatase action on E1 **activates** it, **phosphorylation** of E1 by the kinase causes **inactivation**.

- Several key metabolites such as CoASH, acetyl-CoA, NADH affect the activity of the kinase and phosphatase
- **It is important to emphasize the irreversible nature of the reaction catalyzed by the PDH complex. Thus acetyl CoA CANNOT be converted to pyruvate by any known enzyme or pathway:this is the reason that a net conversion of acetyl CoA from fatty acid catabolism to carbohydrate cannot occur in mammals.** NO Glucose from fatty acids.

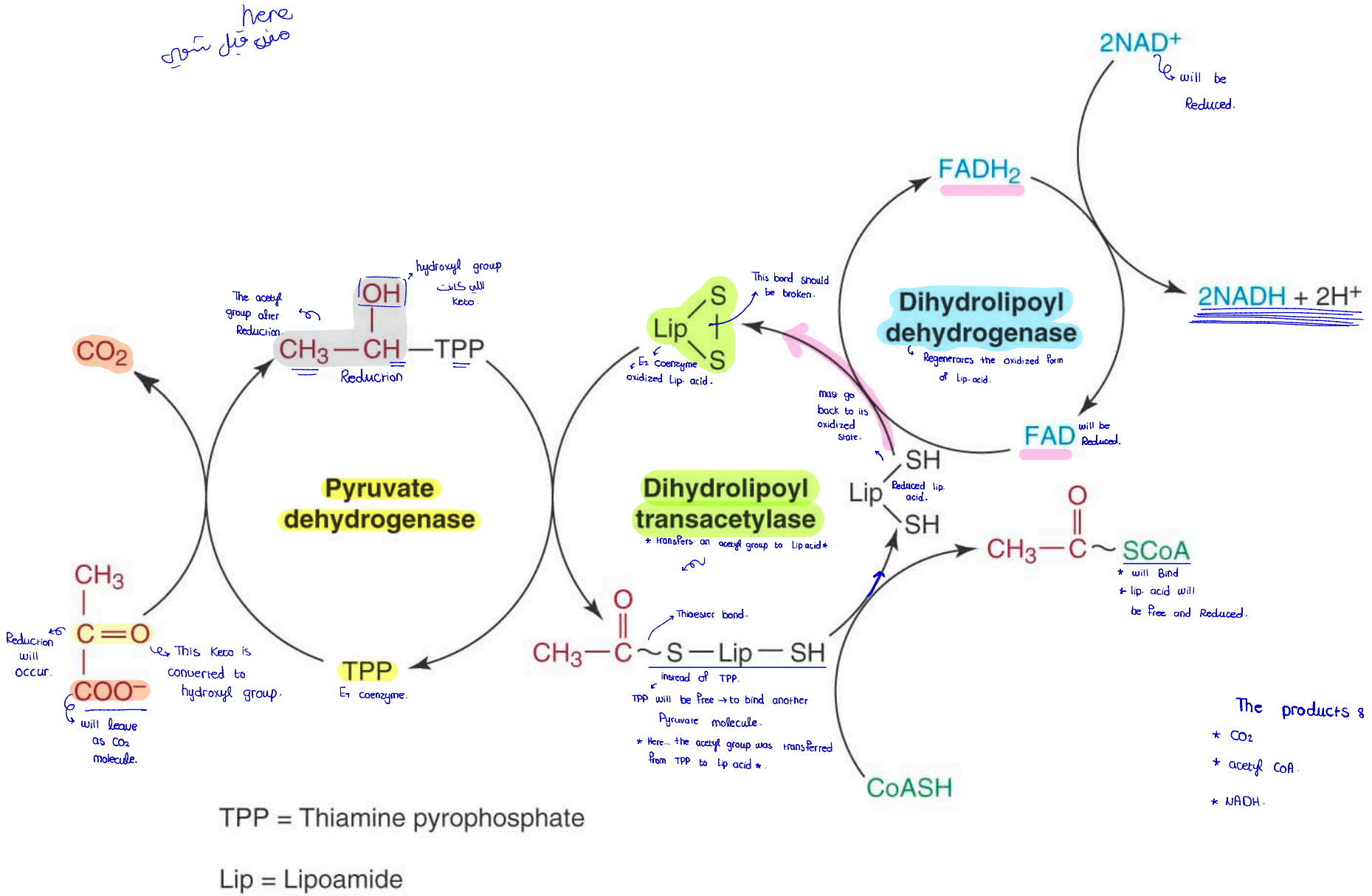


Figure 14.14 Mechanism of the pyruvate dehydrogenase multienzyme complex.

Regulation → Allosteric → Feedback inhibition.
↳ Covalent modification → P, No P. ✓

Are they related ?
* synchronized BUT with different mechanisms.
* NADH → inhibit the complex
↳ activate kinase
with 2 different mechanisms.

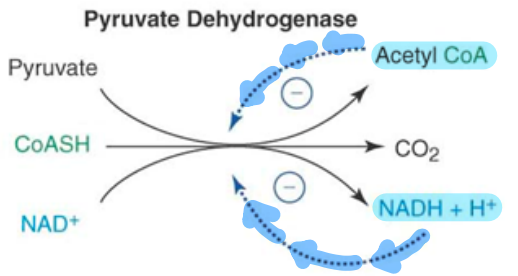
if Pyruvate dehydrogenase is inhibited...
All the mechanism will be reversed → Glu will be synthesized.
like in the fasting state in Pyruvate → Gluconeogen → Glucose.

Acetyl CoA
CAN NOT be converted to Glu.
No enzyme.

Control of Pyruvate Dehydrogenase; E1

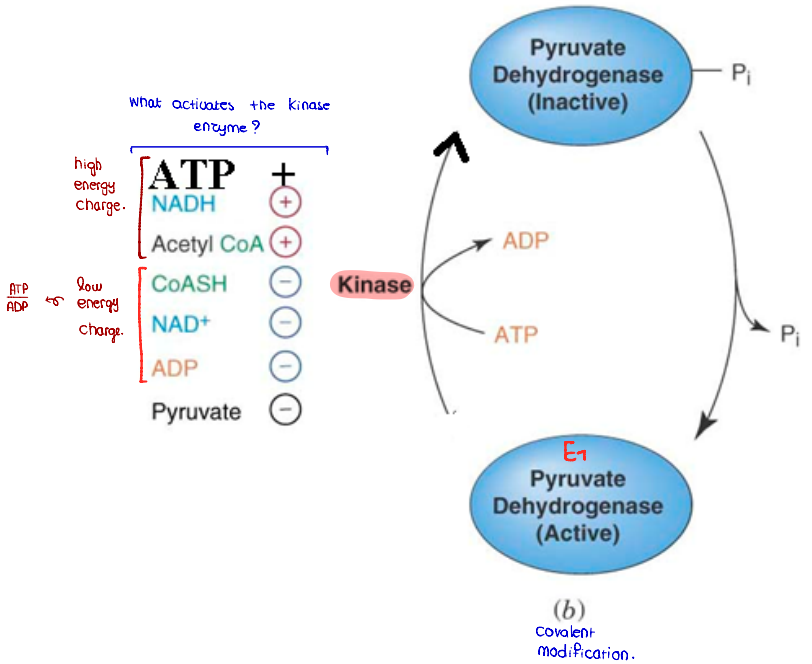
1. In the **fed state** in the liver this rex should be **turned on**" in order that glucose can be efficiently converted to fatty acids.
2. During the **fasted state** this rex should be **turned off** in order that pyruvate will all be driven into gluconeogenic pathway.
3. NADH/NAD, ACETYL COA/COA effects
4. Mg and Ca++
5. Insulin & catecholamines.

Ref: Molecular biology and biochemistry of pyruvate dehydrogenase complexes, Mulchand Patel and Thomas Roche, The FASEB Journal 4: 3224-3233, 1990



The General Reaction.

Feedback inhibition.



What activates the kinase enzyme ?

high energy charge.
ATP +
NADH +
Acetyl CoA +

ATP/ADP ↓ low energy charge.
CoASH −
NAD+ −
ADP −
Pyruvate −

Phosphatase
Mg2+ +
Ca2+ +

Insulin/ in adipocyte ⊕
Catecholamines/ in cardiac ⊕
↳ in cardiac muscles.

After pyruvate complex.

The central metabolic Pathway.
* Stage 3.

TCA(TRICARBOXYLIC ACID) CYCLE, KREB'S CYCLE, CITRIC ACID CYCLE, AND ELECTRON TRANSPORT CHAIN AND OXIDATIVE PHOSPHORYLATION

Aim: To explain the reactions of krebs tricarboxylic acid cycle and the associated electron transport chain and oxidative-phosphorylation.

Contents:

- The reactions of TCA.
- The fate of carbons from OAA and acetyl CoA in the TCA cycle.
- NADH, FADH2 and GTP production.
- Substrate level formation of GTP.
- Succinate dehydrogenase and FAD.
- The control of TCA cycle.
- Shuttles of cytosolic NADH.
- The organization of electron transport chain.
- Iron sulfur proteins, ubiquinone and cytochromes. Cytochrome c oxidase.
- Inhibitors of electron transport chain-action of rotenone, antimycine A, carbon monoxide and cyanide.
- Thiories of oxidative phosphorylation.
- ATP synthase.
- Uncoupling of oxidation and phosphorylation.
- Action of oligomycin. ATP yield from aerobic metabolism of glucose.

Objectives

1. Write the reactions of TCA and follow the fate of the 2-carbon unit in acetyl-CoA.
2. Identify the reactions in which NADH is formed
3. Recognize the reactions of TCA where GTP and FADH2 are generated.
4. Define those reactions of TCA where energy charge and NADH/NAD controls the rate.
5. Demonstrate an understanding of the 5 complexes in the ETC.
6. Identify those reactions in ETC where protons may be generated
7. Demonstrate knowledge of the sites of action of inhibitors of ETC.
8. Explain how proton gradient is generated and its anatomical relationship of ATP synthase.
9. Understand how uncoupler of OXPHOS works and the consequences of its action on respiratory control in mitochondria.
10. Be able to calculate high energy phosphate production associated with aerobic and anaerobic metabolism of carbohydrates and fatty acids

IMPORTANT FEATURES OF TCA CYCLE

- All of the major **nutrients** can be converted to **acetyl CoA** in the first 2 stages of metabolism.
- The complete **oxidation of acetyl group** of acetyl CoA to CO2 and water is accomplished by the enzymes of TCA cycle –stage 3.
- It is a vital pathway for metabolism in all aerobics and occupies of a central position in metabolism because it is the **common pathway** for the oxidation of all major nutrients- carbohydrates, lipids, and proteins.
- It provides **intermediates for the synthesis** of biomolecules- it is **amphibolic**.
- The oxidation of acetyl unit results in the reduction of NAD & FAD to NADH+H and FADH2.
- The hydrogens or electrons of these reduced cofactors, are transferred to oxygen to form water via ETC
- ATP is generated as electrons are transferred to oxygen.
- The reactions of TCA occur in the mitochondrial matrix except succinate dehydrogenase

even lipids.

→ used for: catabolism, oxidation and energy production.
→ also, biosynthesis → its intermediates can be used to synthesize other biomolecules.
eg: excess oxaloacetate can be used to produce glu. via gluconeogenesis.

IMPORTANT FEATURES OF TCA CYCLE

- All the enzymes of TCA are associated with mitochondria (aerobic). Glycolysis is anaerobic and occurs in the cytoplasm.
- OAA acts CATALYTICALLY. There is no net synthesis or degradation of the four carbon intermediates. ↳ very small amounts will be used under normal conditions.
- Each turn of the TCA cycle involves **the uptake of 2 carbon atoms** in the form of acetyl CoA and the release of **2 carbon atoms as CO2** but not the same carbons that were taken upon condensation.
- Each turn of the cycle results in the transfer of 3 pairs of electrons in the form of hydride ions to NAD to form NADH; transfer of 1 pair of electrons in the form of 2 hydrogen atoms to reduce FAD to FADH2.
- There is a substrate level phosphorylation which results in the formation of GTP from GDP and Pi

REACTIONS OF THE TCA CYCLE

tricarboxylic Acid.

1. CITRATE SYNTHASE: Candidate for regulation, Citrate synthesis is necessary for fatty acid synthesis, ΔG0`= -9kcal/mol
   - Favorable RXN.
   - Regulated By → ↑energy compounds ATP, NADH, Acetyl CoA.
   - → Succynyl CoA.

2. ACONITASE: dehydration followed by hydration ΔG0`= +1.5 kcal/mol

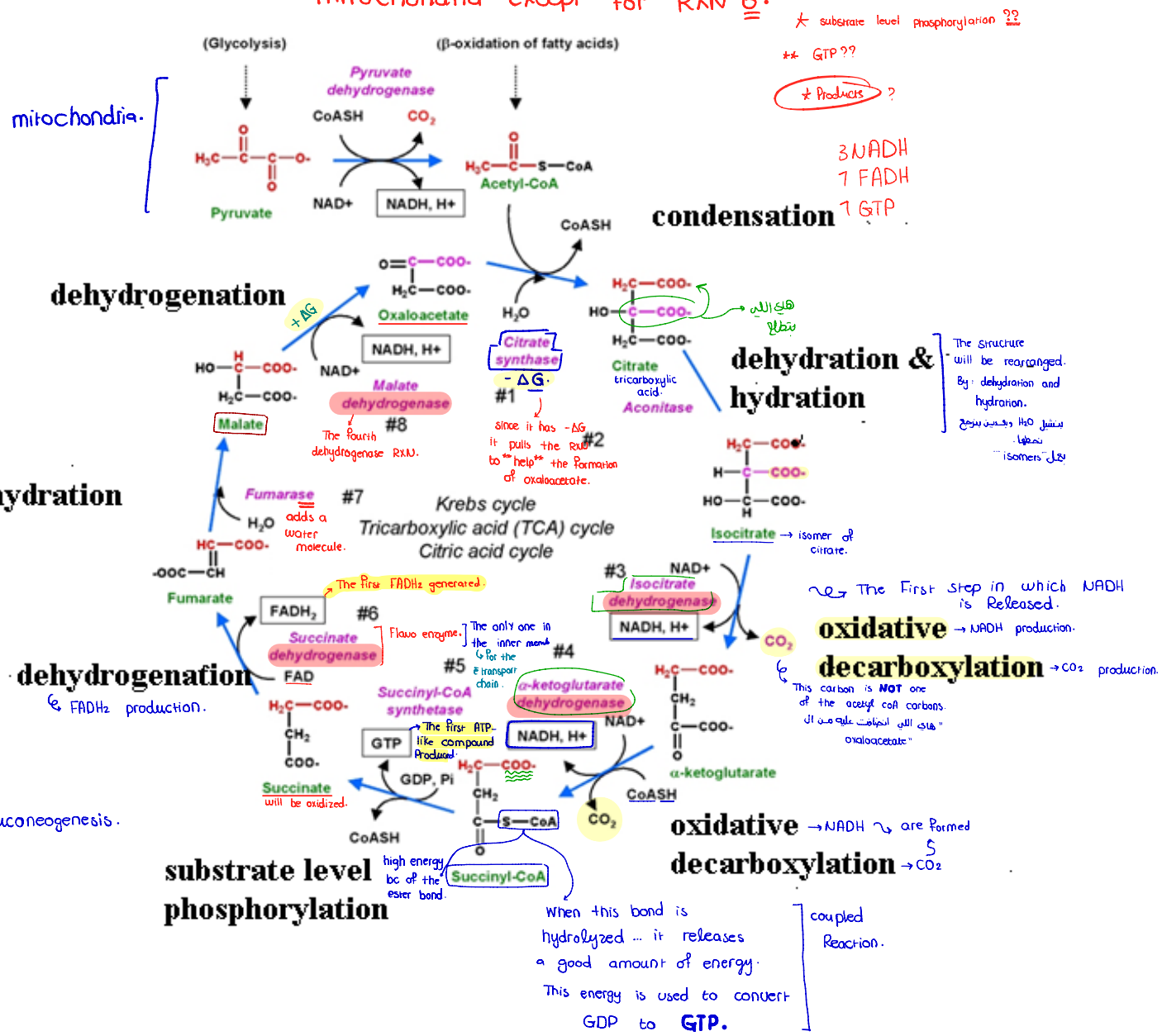
3. ISOCITRATE DEHYDROGENASE: ΔG0`= -5 kcal/mol, oxidative decarboxylation of isocitrate to alpha-ketoglutarate; 1st of four dehydrogenases in the cycle, NADH+H+ formation. AMP& ADP stimulate by lowering km 10 folds. ATP&NADH inhibit the enzyme .inhibition of this enzyme will result in an increase in citrate which can be transported out of mit as substrate for fatty aci synthesis → By gluconeogenesis.

   ليه مش isocitrate ??? Reversible ?

   + high levels of AMP, ADP → means that there is NO ATP Production → We should Stimulate the RXN ⇉ low energy charge. Km is lowered 10 folds →→ The affinity becomes very high.
   Remember : low Km = high affinity
   − high energy charge inhibits the RXN → No need to produce more ATP √√.

4

All these RXNs take place within the matrix of the mitochondria except for RXN 6.



4. α-KETOGLUTARATE DEHYDROGENASE COMPLEX : → oxidation and decarboxylation.

- ΔG0`= -8 kcal/mol .
- 2nd molecule of CO2, and the 2nd NADH+H formation.
- TPP, lipoic acid, CoAsh, FAD, and NAD are involved.
- ATP, GTP, NADH, and succinyl CoA inhibit the complex, AMP is a positive effector, calcium is positive effector.
  - inhibit ← energy charge.
  - AMP ← low energy charge.
  - calcium ← Ca^{++} needs ATP for → muscles contraction.
- The complex consists of α-ketoglutarate dehydrogenase, dihydrolipoyl transsuccinylase and dihydrolipoyldehydrogenase. → it looks like the pyruvate dehydrogenase complex . in structure + mechanism.
- α-ketoglutarate represents a significant point of convergence in metabolism. Several aa are converted to glutamate which if transaminated or oxidatively deaminated yields alpha ketoglutarate.

* NOTE → this enzyme can't undergo covalent modifications.
  ↳ no phosphorylation or dephosphorylation.

REACTIONS OF THE TCA CYCLE

5. **SUCCINYL THIOKINASE ,ΔG0`**= -8 kcal/mol: cleavage of thioester bond is coupled to phosphorylation of GDP to GTP- **substrate level phosphorylation .**

↳ The first ATP like molecule.

Nucleoside diphosphate kinase:

GTP + ADP ⇄ GDP + ATP (1)

Adenylate kinase: AMP + ATP ⇄ 2ADP…(2)

SUM: GTP + AMP ⇄ GDP + ADP

to regenerate GDP.

AMP promotes the formation of GDP for the continuation of the cycle.

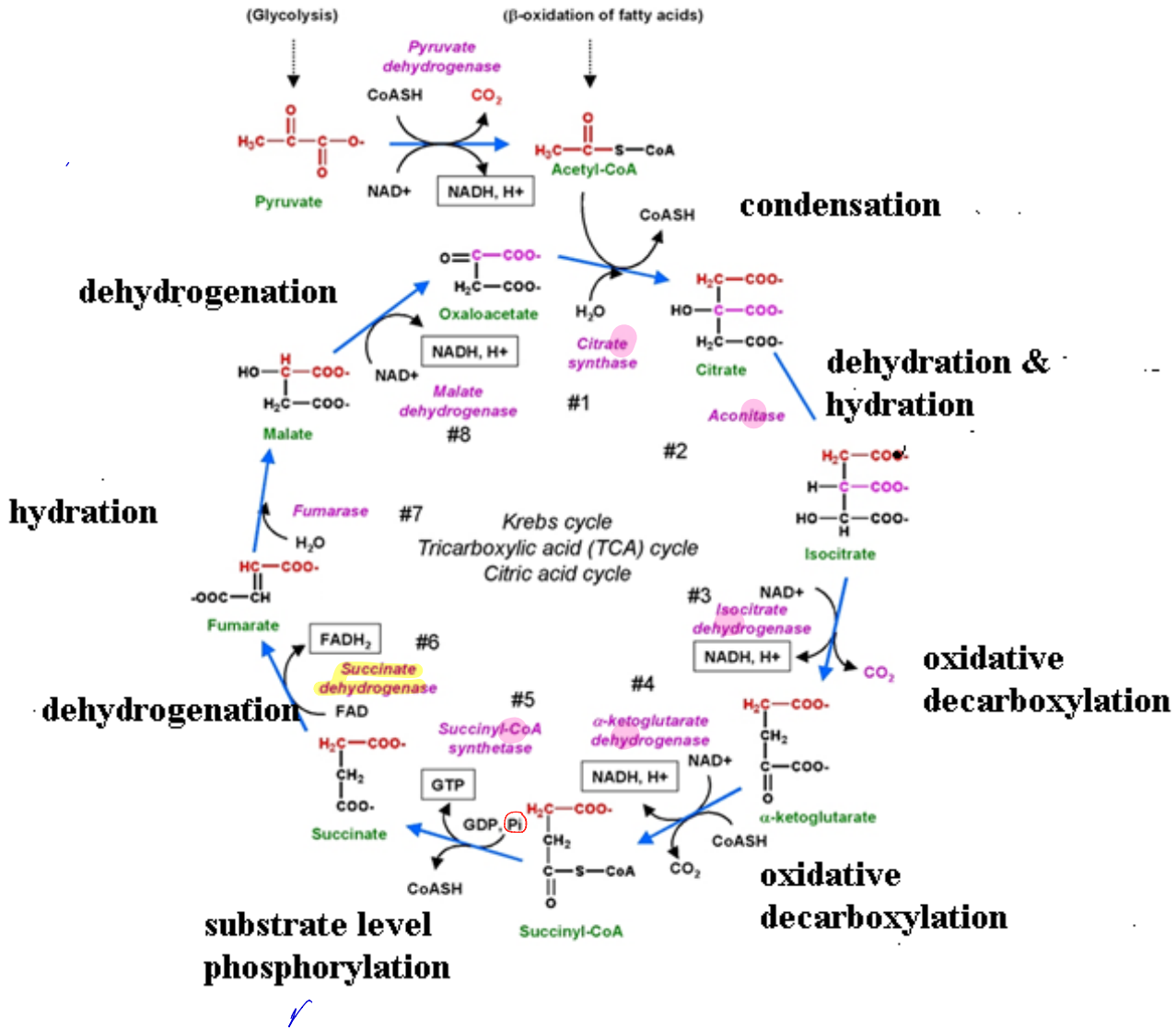
6. **SUCCINATE DEHYDROGENASE ΔG0`**=0 : the only dehydrogenation in TCA cycle that is not NAD-linked, but FAD to form FADH2.malonate is a competitive inhibitor

* Flavo enzyme.
* The ONLY step that happens in the inner mitochodrial membrane.

7. **FUMARASE ΔG0`**=0.9 : reversible hydration of fumarate to L-malate, this enzyme is specific for the trans and L-isomers of the unsaturated and hydroxy acids, respectively.

↳ Does NOT produce D- isomers.

If L-isomers (↓↓) the TCA will stop.



* The energy... ATP → will be formed in the ē- transport chain and oxidative phosph → except Glycolysis + in Krebs cycle in the form of GTP.

REACTIONS OF THE TCA CYCLE

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8. MALATE DEHYDROGENASE:

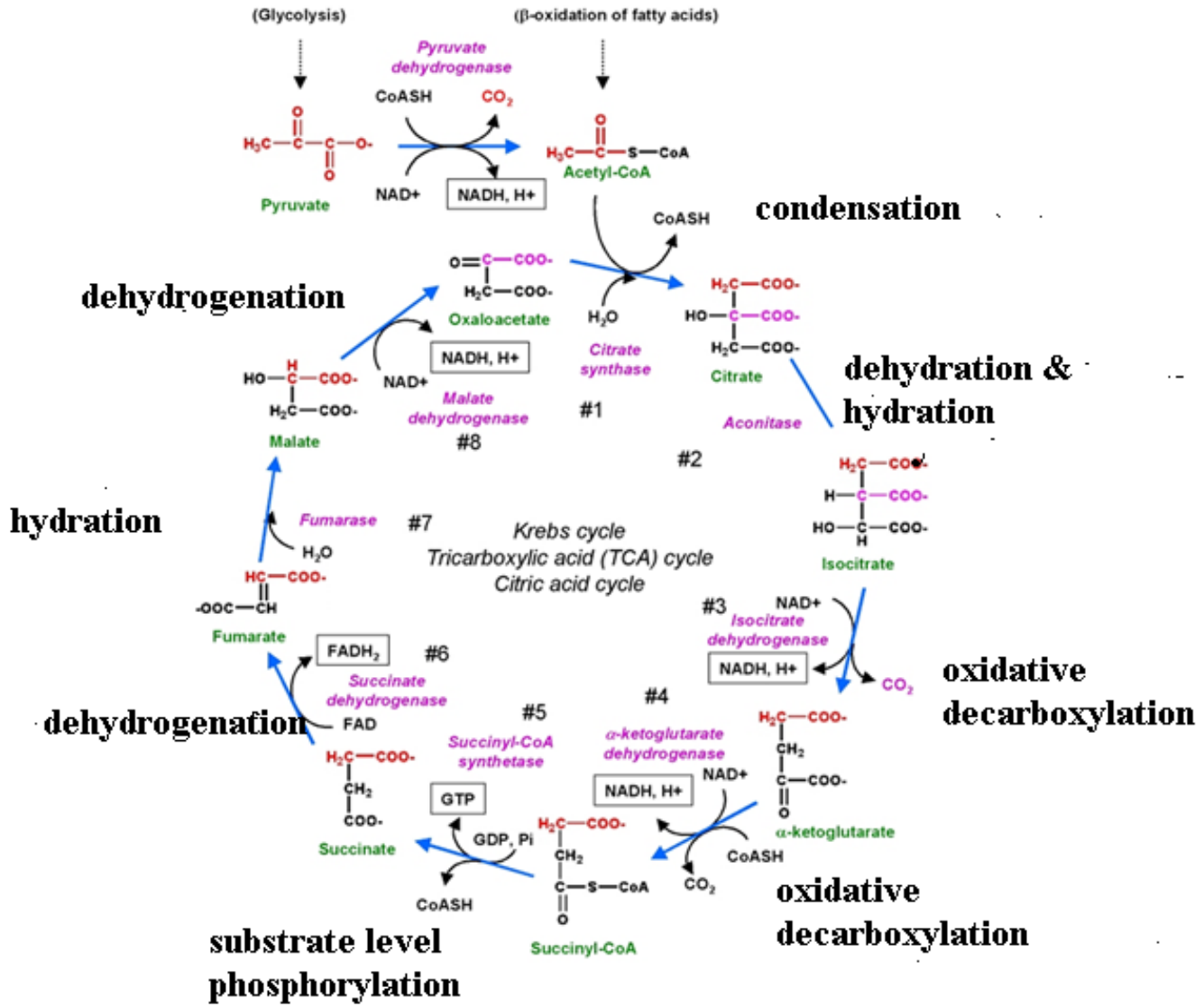
ΔG0`=+7.1. completes the cycle by regenerating OAA-A REGENERATING SUBSTRATE. It is the final of three reactions in which NADH+H is produced.

The equilibrium greatly favors the reverse reaction , the reduction of OAA. However, citrate synthesis is closely associated with the dehydrogenase and removal of OAA assists in pulling the malate dehydrogenase reaction towards the formation of OAA. OAA can be reversibly transaminated to aspartate

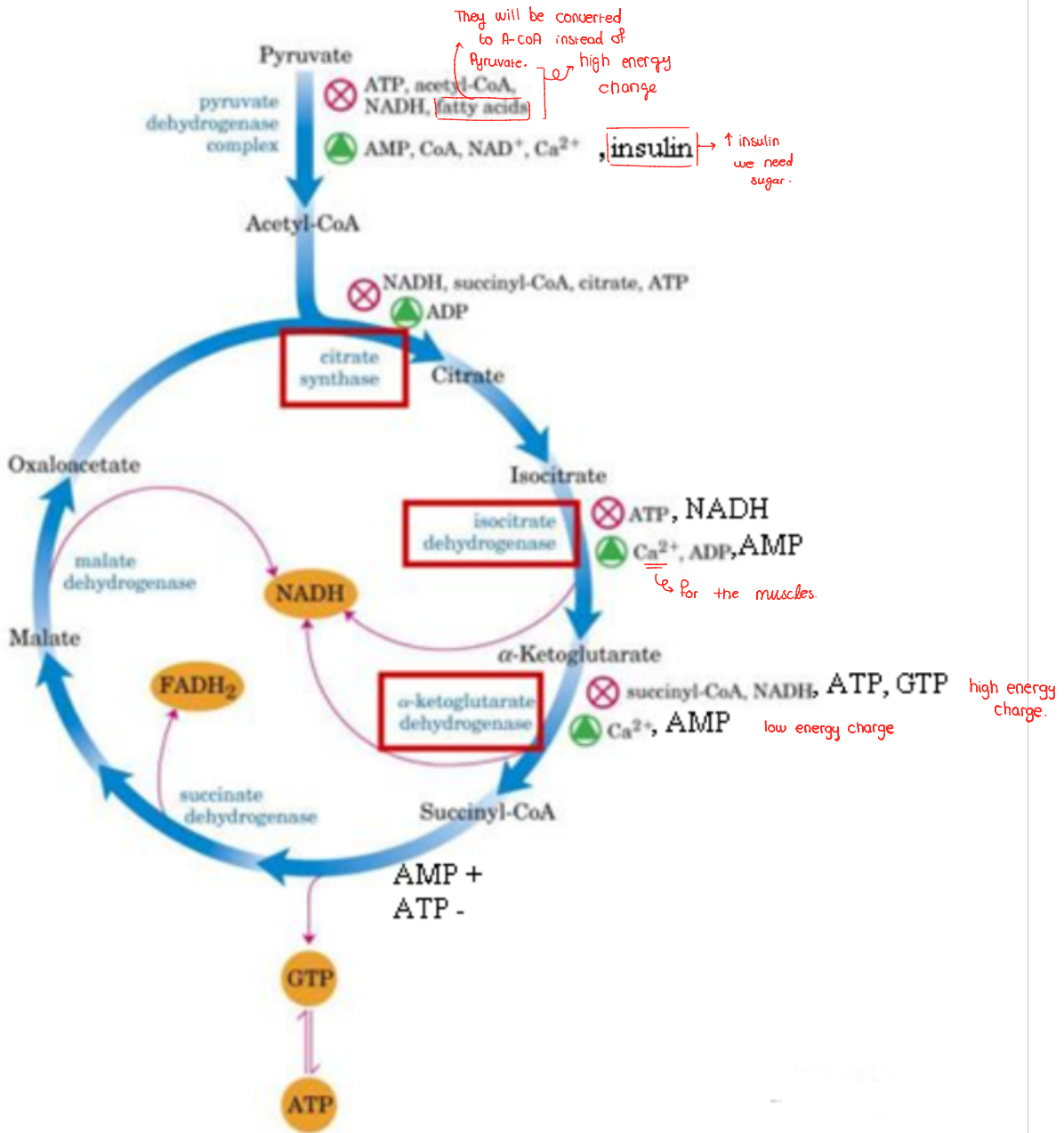
SUM: The -ΔG RXN pulls the +ΔG RXN forward.

AcetylCoA+3NAD+FAD+GDP+Pi+2H2O ⟹

2CO2+3NADH+2H+FADH2+GTP+CoASH



Regulation of Citric Acid Cycle



The citric acid cycle is regulated at its three exergonic steps

uses all high energy molecules from all sources.

اللي بيحصلوا في ال cytosol لازم يتنقلوا لل mitochondria.

What are the sources of Reducing powers ?
1- Glycolysis → 2 NADH + H+.
2- The T.S → 2 NADH + H+
3- TCA → 6 NADH + H+ + 2 $FADH_2$.

all should be transferred to the inner mitochondrial membrane.

ELECTRON TRANSPORT, SHUTTLES, AND OXIDATIVE PHOSPHORYLATION

- Products of TCA cycle include NADH+H+ and FADH2 which are energy rich molecules because they contain a pair of electrons of high transfer potential.

NOT $NADH_2$. Reducing powers Because they are able to Reduce other substances.

They should reach oxygen and Reduce it.

- Transfer of these electrons to oxygen thru a series of carriers results in the release of a large amount of energy which can be used to generate ATP.

أكبر كمية طاقة هون بتتعل. مش من عريس.

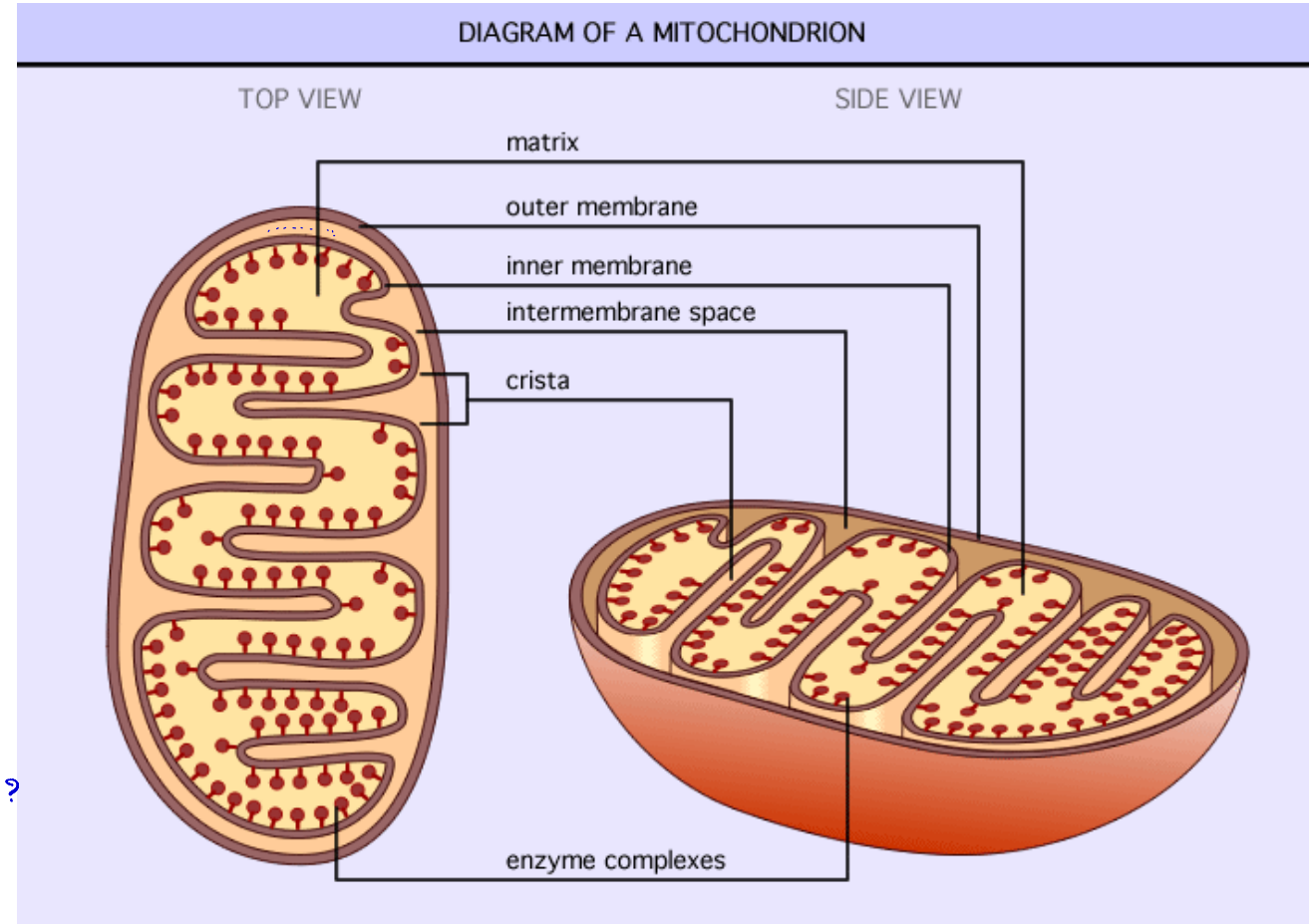
- oxidative phosphorylation is the process in which ATP is formed as electrons are transferred by this series of carriers from NADH+H+ and FADH2 to O2.

By ATP synthase.

→ After the e transport chain.

here.

- OXPHOS takes place in the mitochondria of the cell
- Mitochondria consist of 2 membranes-the outer and the inner membranes.
- The outer is freely permeable to molecules MW<10K
- The intermembrane space contains the enzymes that catalyze the interconversions of adenine nucleotides ?? Glu to Asp ?
- The inner membrane space has many folds directed towards the mitochondrial matrix. → to increase the surface area → More ATP.



here.

* enzymes with the same functions are located together.
↪ Markers of each site.

Location of the various mitochondrial enzymes in mitochondrial compartments.

Outer membrane	Intermembrane space	Inner membrane (e trans. proteins.)	Matrix (TCA)
NADH cytochrome b5 reductase	Adenylate kinase	NADH-Coenzyme Q reductase	PDH
Cytochrome b5	Nucleoside diphosphokinase	Succinate-Coenzyme Q	ALPHA-KG DH
Monamine oxidase	nucleosidemonophosphokinase	Coenzyme QH2-cytochrome c reductase	CITRATE SYNTHASE
Glycerophosphate acyltransferase	Sulfite oxidase	Cytochrome oxidase	ACONITASE
Fatty acid elongation system		Oligomycine-sensitive ATPase	MALATE DH
		Beta-hydroxyl butyrate DH	ISOCITRATE DH
		Carnitine palmitoyl transferase	FUMARASE
			GLUTAMATE DH
		Carbamoylphosphate synthetase I	PYRUVATE CARBOXYLASE
			FATTY ACYL-COQ DH
			ENOYL HYDRASE
			BETA-HYDROXYACYL-

→ All TCA enzymes are located in the Matrix except for : Succinate D.H.

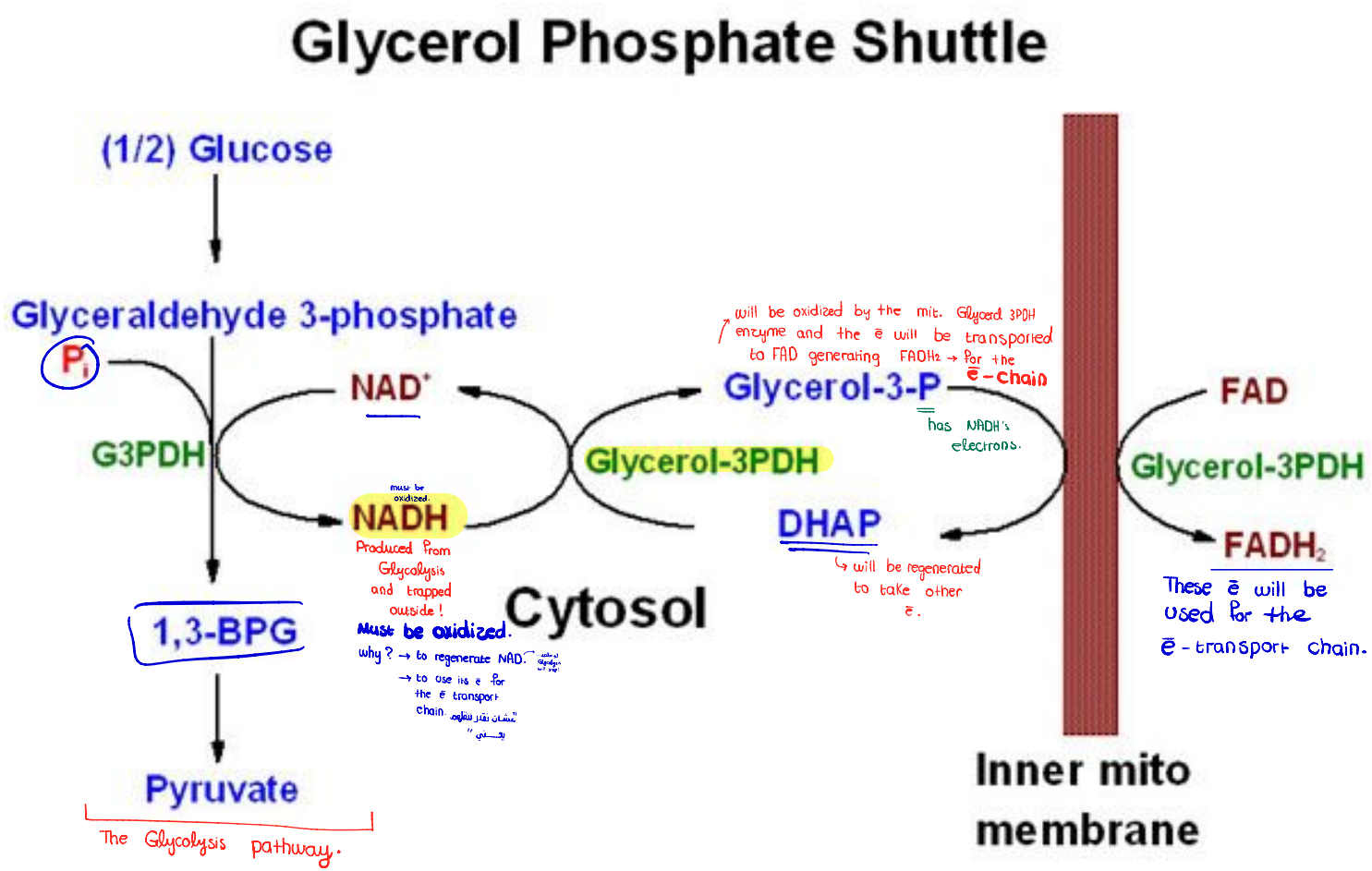
كون لازم نوقف انت :
enzymes with the same function are located in the same place ✓

Why do we need shuttles ? to transfer the ē of NADH from the cytosol to the mitochondrial matrix.

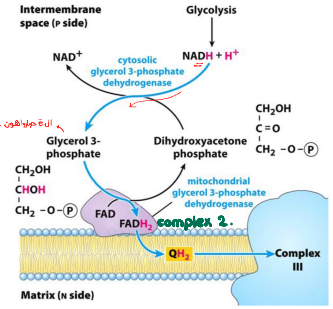
These NADH molecules were produced from glycolysis... But NADH can't pass the mitochondrial membrane to reach the ē transport chain, and be used in oxidative phosphorylation.

① α-Glycerol Phosphate-Dihydroxyacetone Phosphate shuttle

- DHAP is reduced to glycerol-3-phosphate
- Glycerol-3-P is oxidized to DHAP by FAD-dependent glycerol-P-dehydrogenase(mit) ✓
- NADH(cyt)+FAD(mit) → NAD(cyt)+FADH2(mit) ✓
- Operation in muscle



usePul NOTES :



(1) Cytoplasmic Reactions (Figure 2)

- **Dihydroxyacetone phosphate (DHAP)** gains hydride ions from NADH forming **glyceral-3-phsophate** and NAD^+
- Glyceral-3-phosphate has a specific channel into the mitochondrial matrix.

(2) Mitochondrial Matrix Reactions (Figure 2)

- Glcyceral-3-phosphate reduced **FAD to $FADH_2$**.
- Meanwhile FAD oxidizes glyceral-3-phsophate back to DHAP
- Catalyzed by **glyceral-3-phosphate dehydrogenase**
- Generated $FADH_2$ can react with Complex II
- $FADH_2$ transport electrons via Complex II to the next component coenzyme Q until final acceptors are reached.

* Remember that: energy produced from $FADH_2$ (2 ATP) is less than $NADH+H^+$ (3ATP). So, cells that use this shuttle will lose some energy while transporting the electrons.

② Malate-Aspartate Shuttle

Why?

OAA(cyt) is reduced to malate by NADH-dependent malate dehydrogenase.

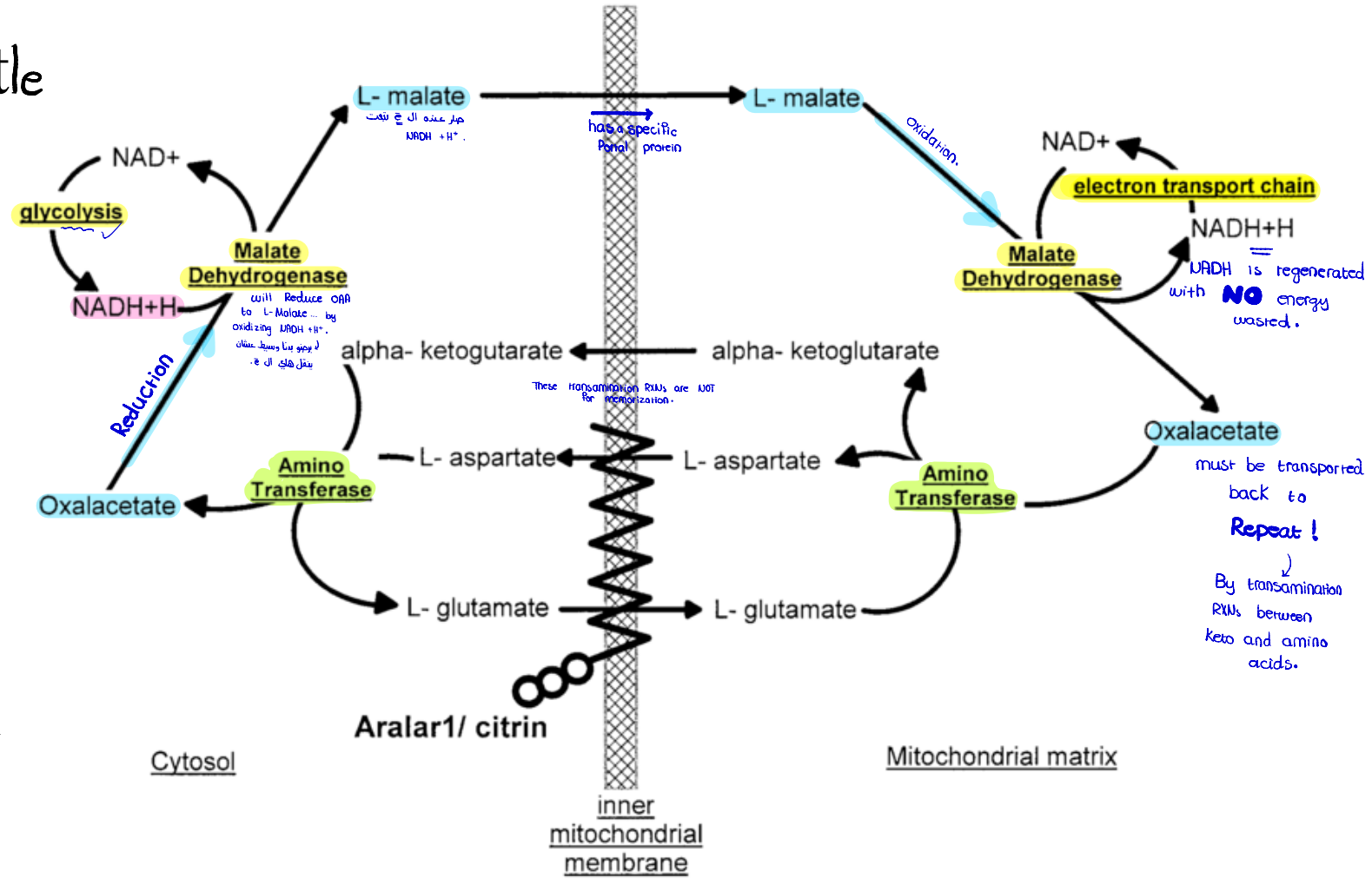
Malate is transported to mitochondria where NAD is reduced to NADH+H+ and OAA is regenerated.

A NADH+H (cyt) has been changed to NADH+H+(mit)

OAA cannot transverse the mit, however, transaminases and antiporters result in return of OAA to cytoplasm.

NADH(cyt)+NAD(mit)□NAD(cyt)+NADH(mit).

Operational in liver and heart



* The NADH of the cytoplasm will be transferred as NADH → NO energy loss.

Shuttle System	Malate-Aspartate	Glycerophosphate
Two electrons transferred	NADH to OAA	NADH to DHAP
Through	Cytosolic malate dehydrogenase	Cytosolic glyceral-3-phosphate dehydrogenase
Product	Malate	Glyceral-3-phosphate
Oxidized by	Mitochondrial malate dehydrogenase	Glyceral-3-phosphate dehydrogenase
Oxidized as	NAD^+	FAD *energy loss*

here

Carriers of Electron Transport Chain

The chain of carriers is called : Electron Transport Chain Or Respiratory Chain.

carrier.

soluble "mobile" that is **NOT** Protein in nature.

① Coenzyme Q: it has long isoprenoid tail which enables the molecule to diffuse rapidly in the hydrocarbon phase of the inner mitochondrial membrane.

mobile.

* carries 2 ē , 1 ē at a time.
* can donate these ē to other protein complexes
* more Q → more ē (more energy more ATP.

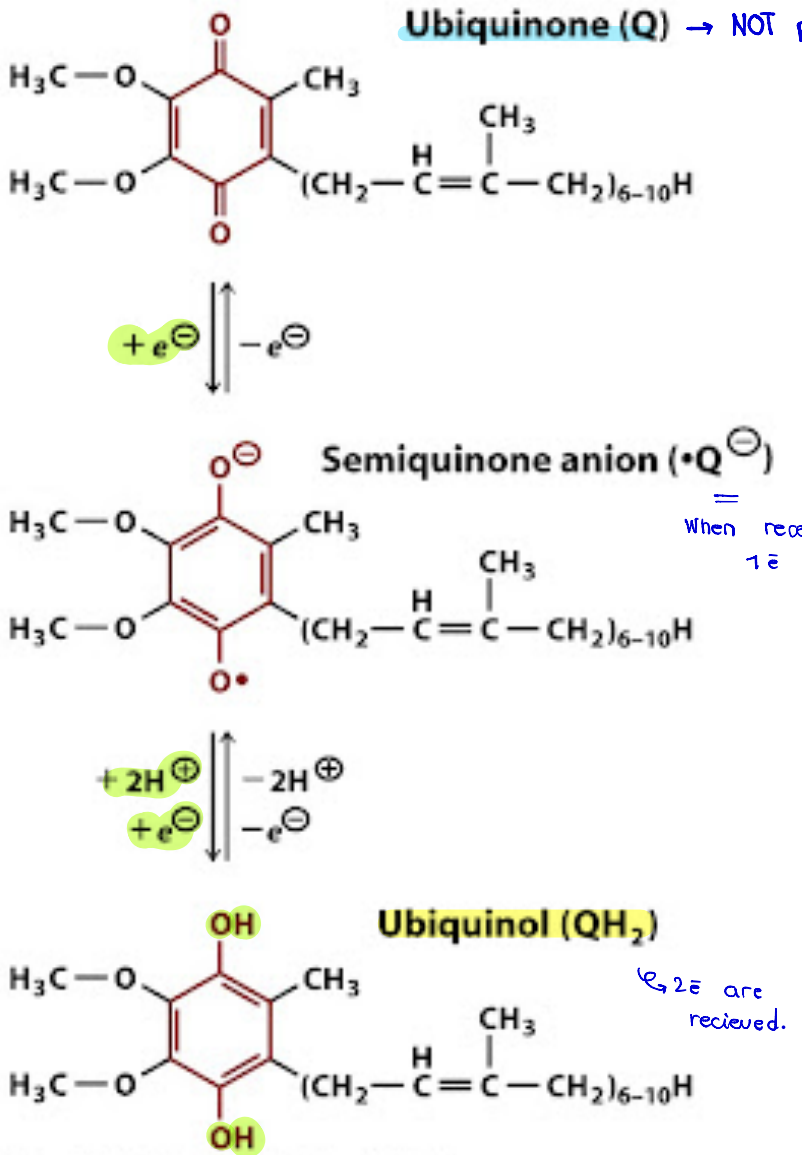


Figure 7-31 Principles of Biochemistry, 4/e
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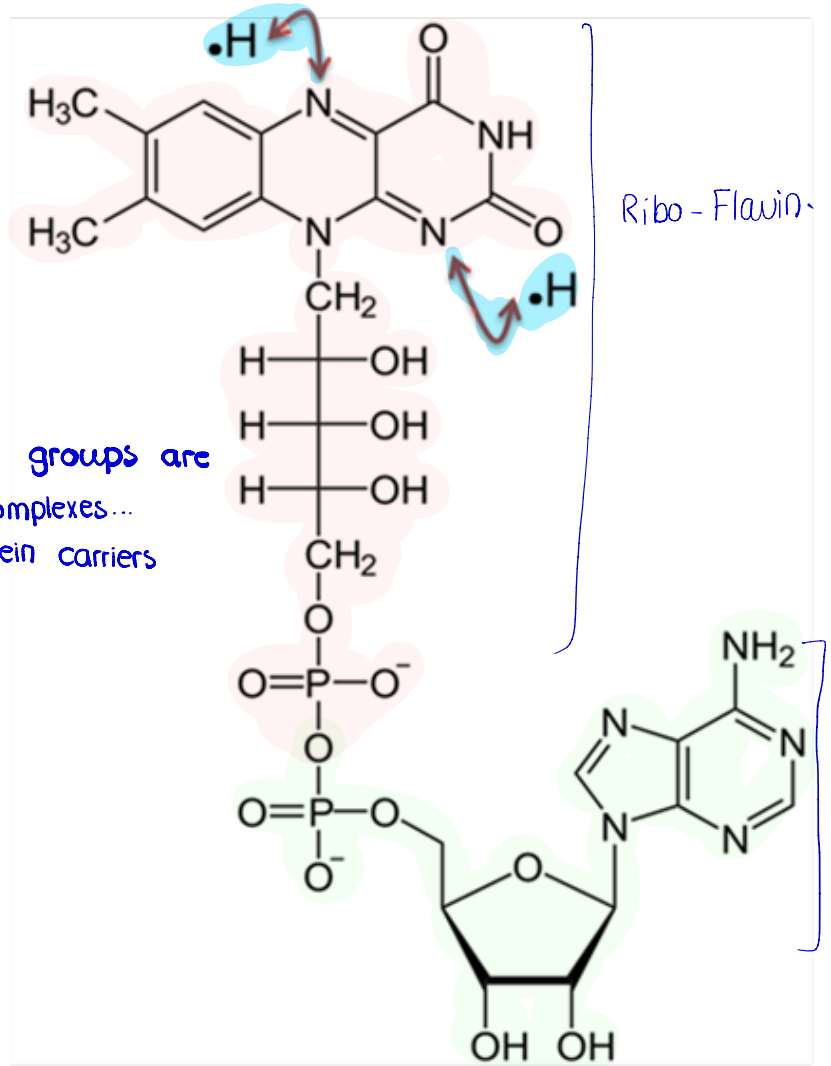
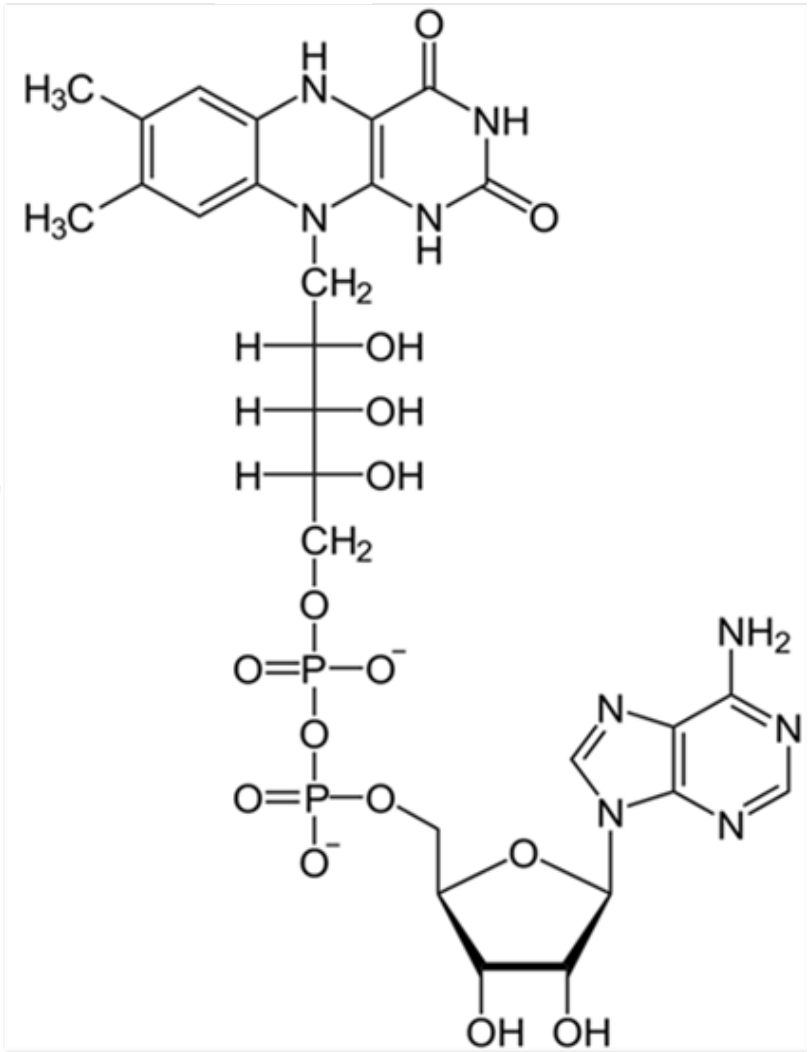
Prosthetic groups.

in General:

These prosthetic groups are found within the complexes... They help the protein carriers to accept and donate $\bar{e}$ between them.

- ↳ Fe-S centers
- ↳ FAD, NAD
- ↳ heme
- ↳ and many others





① **FAD**

- ↳ dinucleotide.
- ↳ oxidized form.
- ↳ Can bind 2 H^+. → $FADH_2$.
- ↳ can covalently bind to some $\bar{e}$-chain complexes.

$FADH_2$

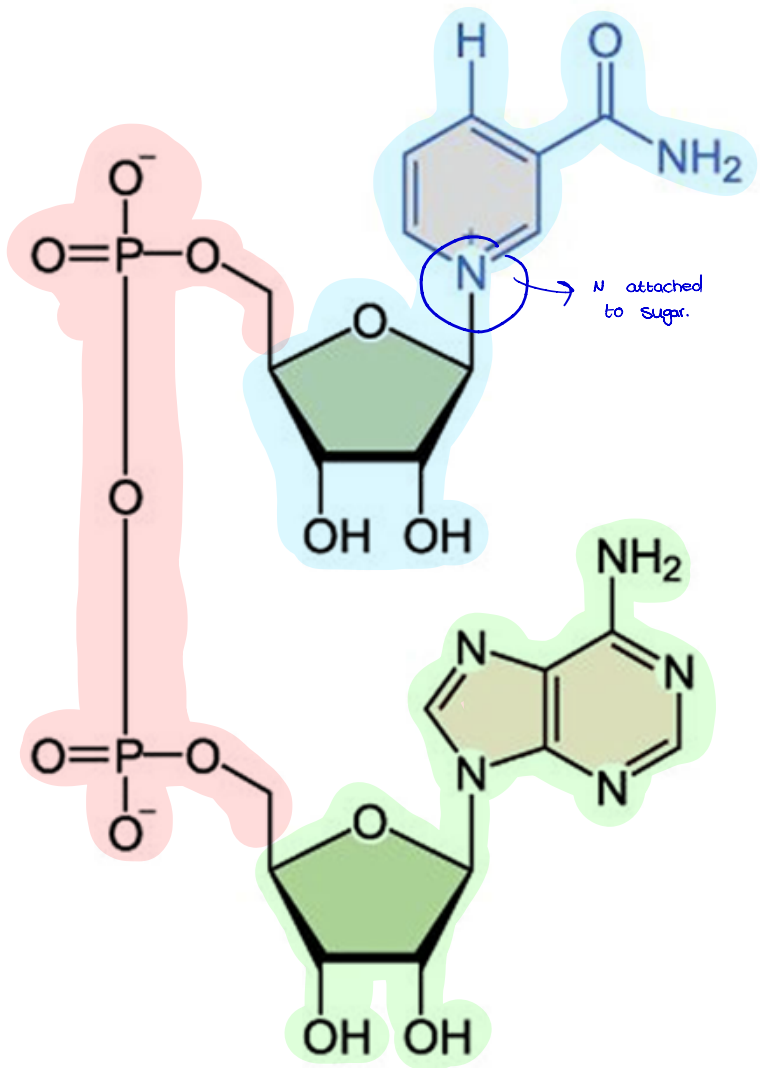
- ↳ Donates its $\bar{e}$ after being reduced to FAD.

2

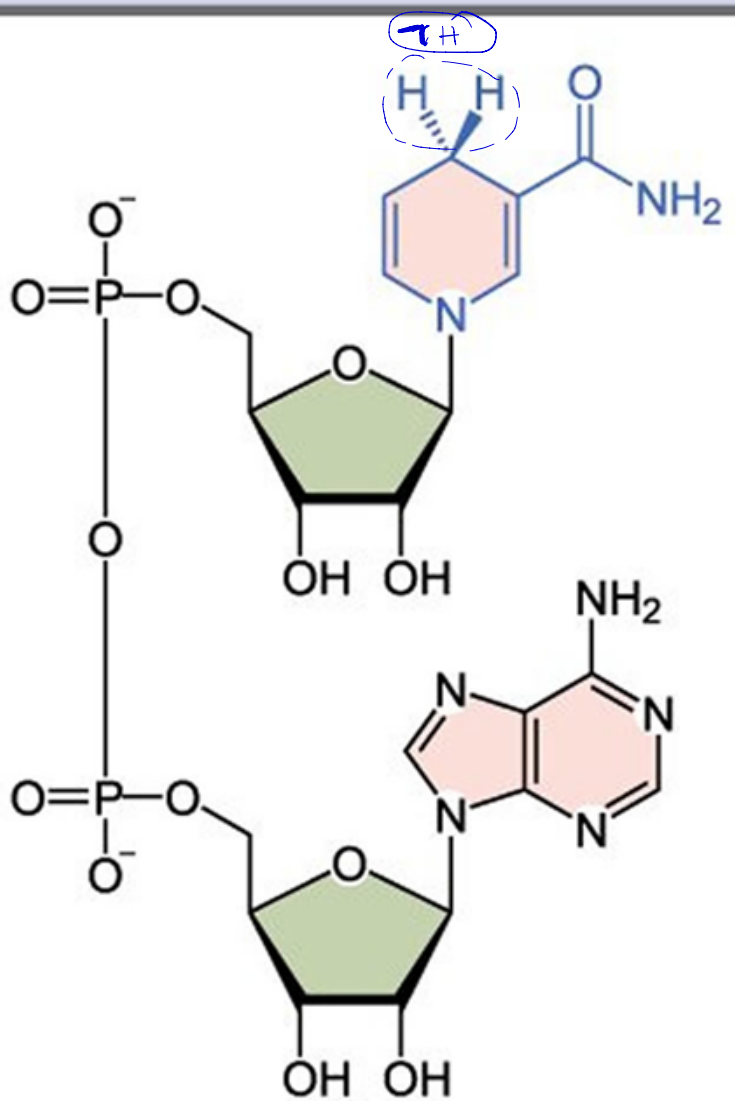
Nicotinamide ٦ Adenine 2 dinucleotide 3

NAD^+

→ the oxidized form
→ can bind ١ proton, $2\bar{e}$ → NADH + H^+



NADH



Protein carrier.

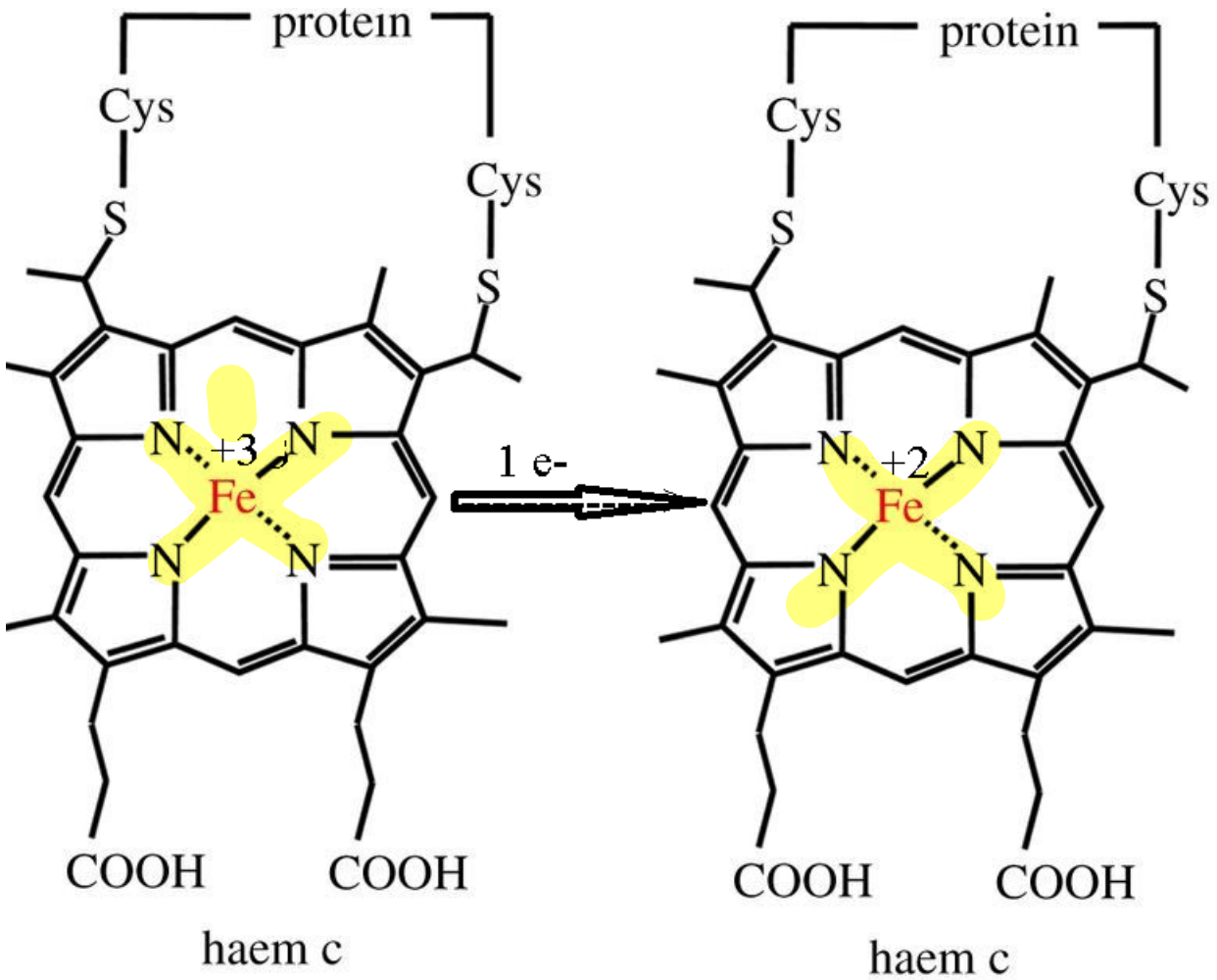
Cytochromes (heme proteins) → Prosthetic group.

③

different types.

Cytochromes (heme proteins): electron transfer proteins which contain heme group and accept a **single electron** in contrast to NAD, FAD, and coenzyme Q which are 2 electron carriers.

* heme ? → 4 pyrrole rings
  → iron atom "Ferric state" +3
  Ferrous ← لما يكتسب e
* how do cytochromes differ ?
  their heme groups have different compositions of the double bonds conjugation + side chains → attached to the pyrrole rings
  Producing different hemes, different cytochromes



haem c

haem c

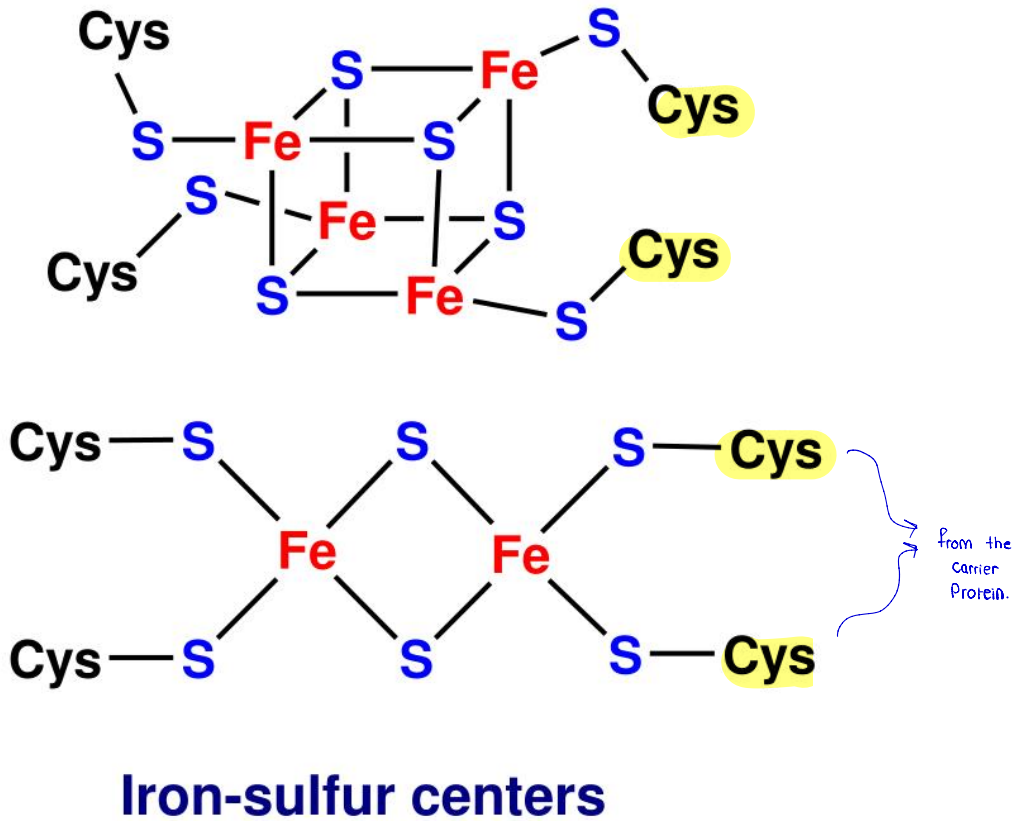
4

Iron Sulfur Centers

a prosthetic group → complexes 1 and 2.

Iron sulfur proteins contain two or four iron atoms bound to an equal number of sulfur atoms and to cysteine side chains.

One electron carriers.



Iron-sulfur centers

5

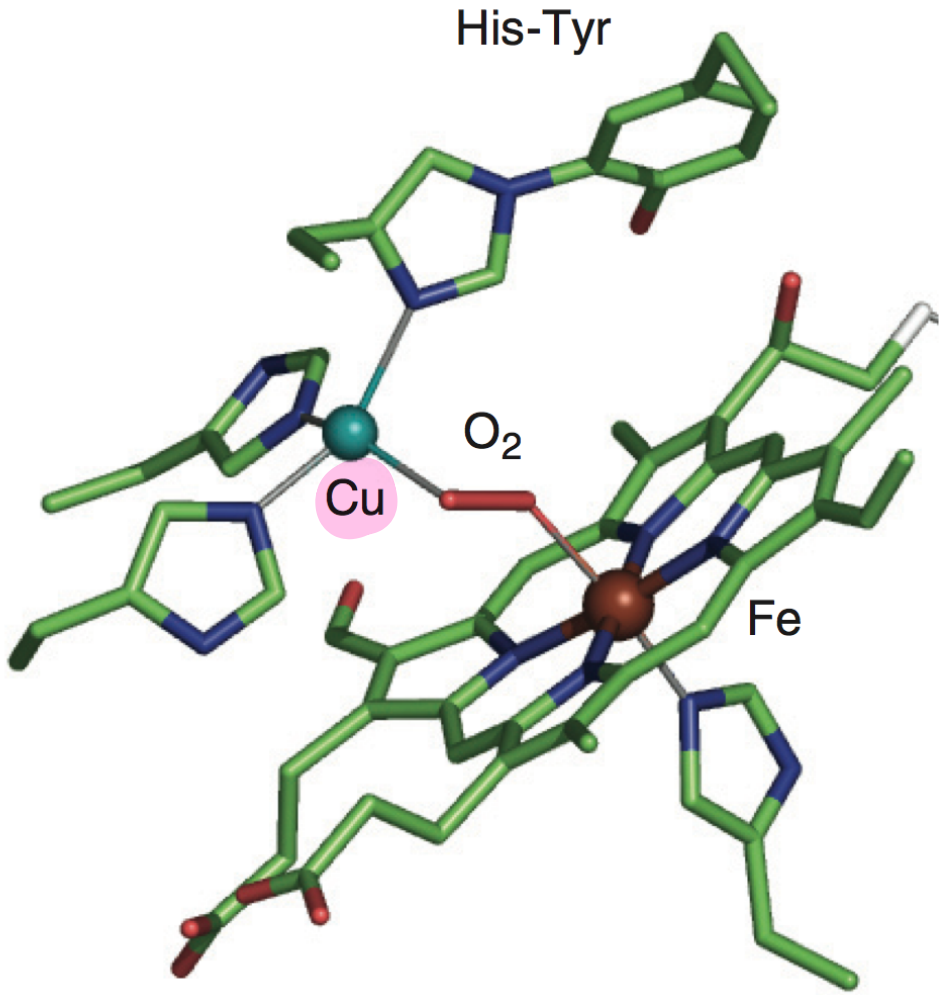
↗ Prosthetic group.

Copper Containing Proteins

In addition to the heme, they contain copper which participate in electron transfers.

1 e-

Cu2+----------□Cu1+



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ANATOMY OF THE RESPIRATORY CHAIN → contains 4 complex proteins. "integral protein"

1. Complex I: NADH Reductase.

NADH + e-> CoQ

- binds NADH and oxidize it
- it has Fe-S cener. No heme.
- FMN will be Reduced.
- Gives its ē to coenzyme Q.

2. Complex II: Succinate-CoQ Reductase,

* takes ē from $FADH_2$ ↳ taken by Fe-S centers → to FMN → to CoQ.
* contains prosthetic groups + succinate dehydrogenase.

Succinate + ē → CoQ.

3. Complex III, Cytochrome C Reductase, → Reduces Cyt c.

CoQ + ē → Cyt c → binds CoQ.

4. Complex IV, Cyt Oxidase,

Cyt c + ē → Oxygen → ē then to complex 4

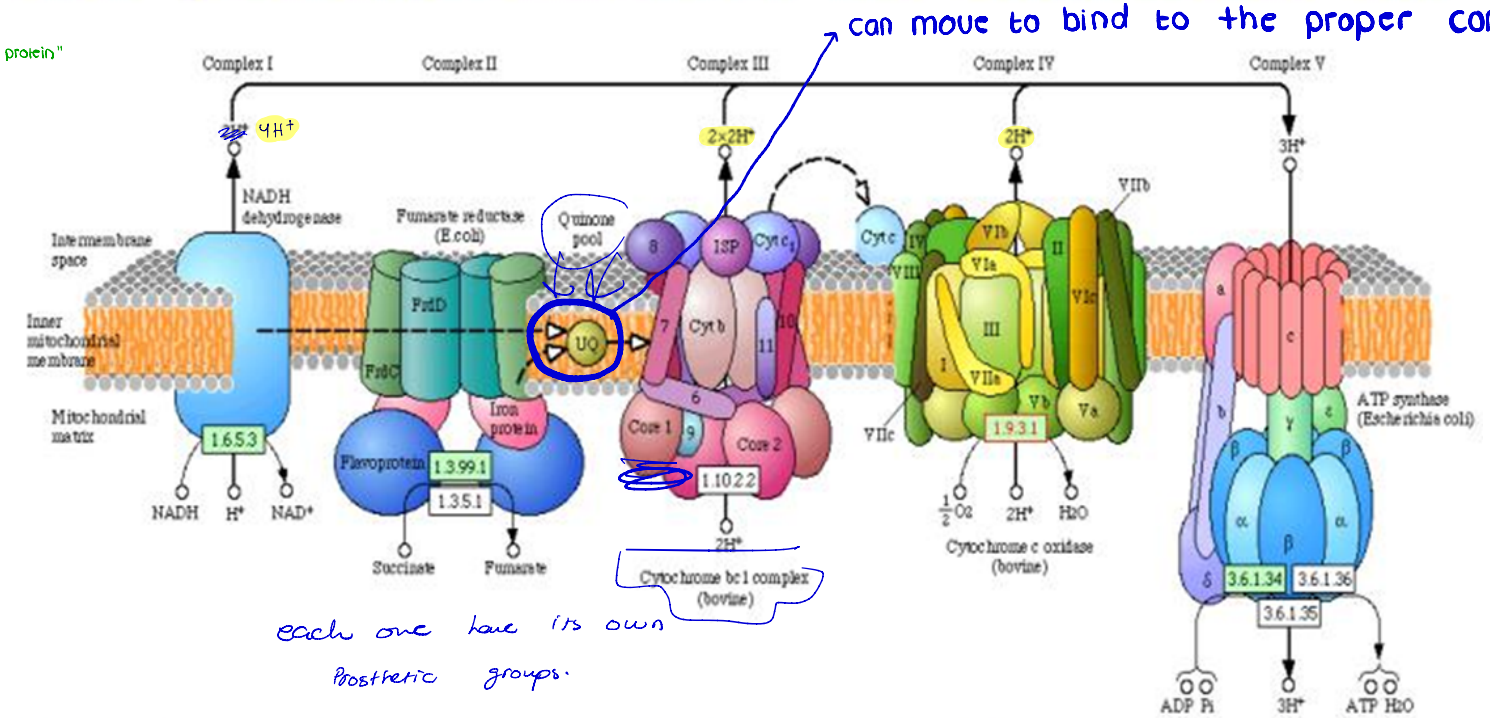
*copper
* cyt a
* cyt a_3

↳ Then to $O_2 \rightarrow H_2O$

5. Complex V: ATPase

* complexes 1, 3, 4 → have pumping activity. (① ③ ④)
* Both complexes (1+2) give their ē to CoQ.
  CoQ → 3 → cyt C → 4

Respiratory chain subunits encoded by two genomes: Nuclear and Mitochondria



each one have its own prosthetic groups.

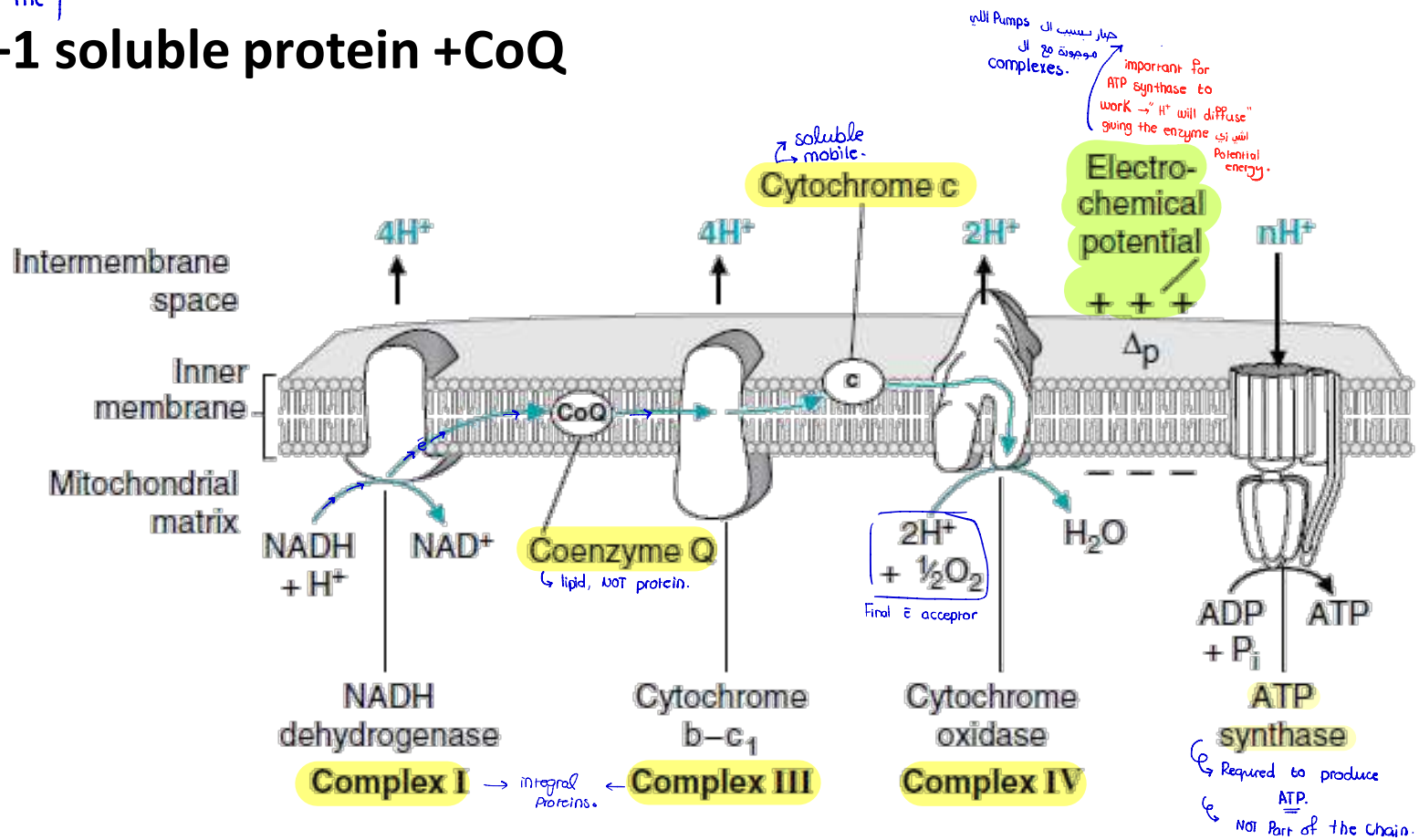
complex	I: NADH DH	II: SDH	III: cyt c red	IV: COX	V: ATPase
Mito/ nuclear	7/43	0/4	1/11	3/13	2/13

↳ NOT a part of the ETC.

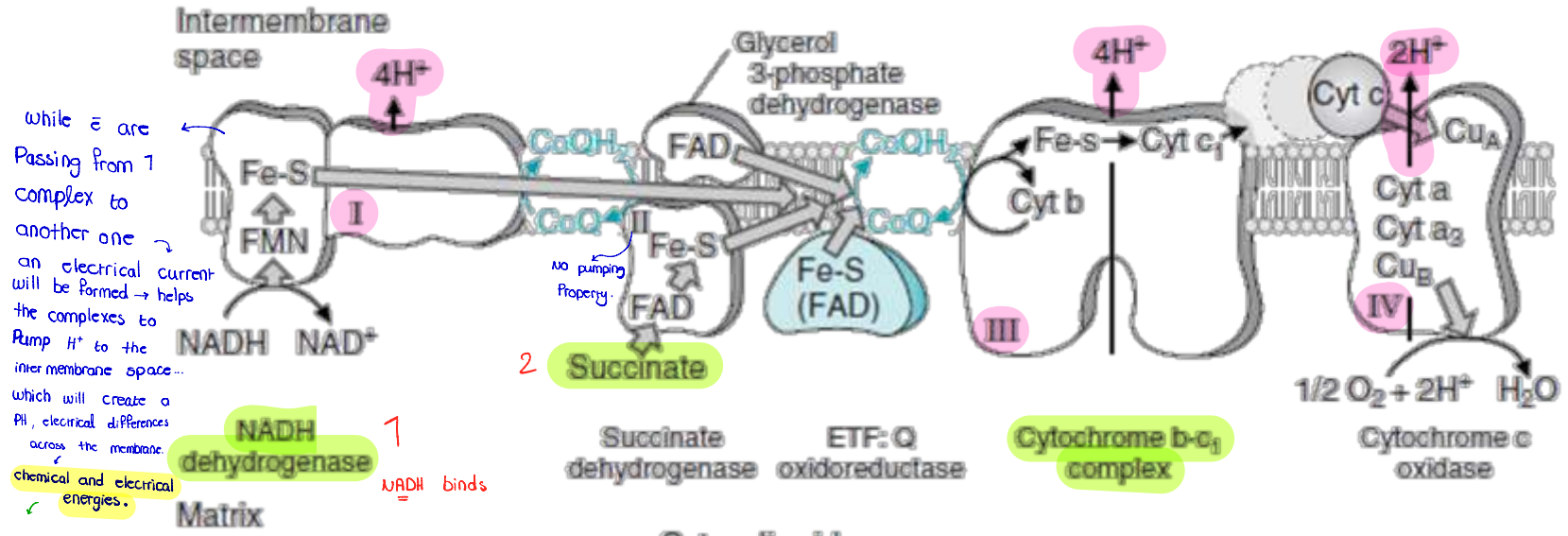
Requirements of OxPhos

- **Redox reaction: electron donor (NADH or FADH2) & electron acceptor (O2)**
- **An intact IMM** → so there will be some insulation between the inner mitochondrial membrane and the matrix. (helps ATP-synthase.)
- **ETC of proteins: 4 complexes+1 soluble protein +CoQ**
- **ATP synthase**

* The best ē acceptor → oxygen.
* Why is the process step-wised ?
  in each step there might be an energy release that the cell will use to do some work. → More beneficial than →1 step



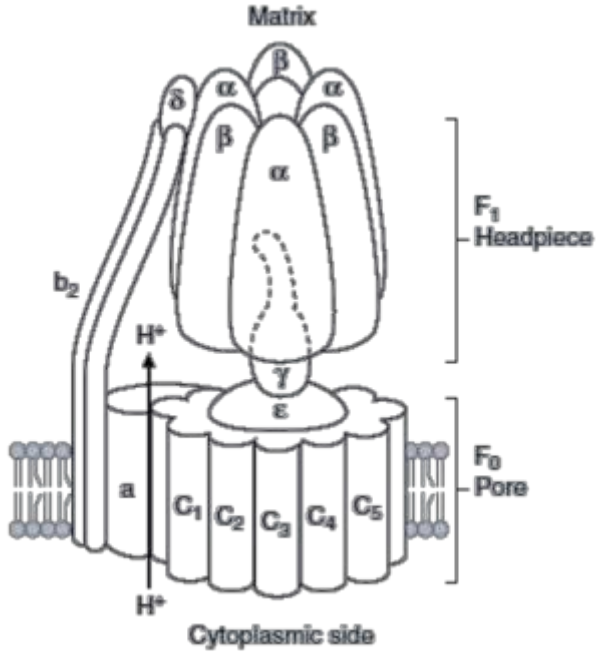
ET to O2, how does the process occurs? "The chemi-osmotic theory"

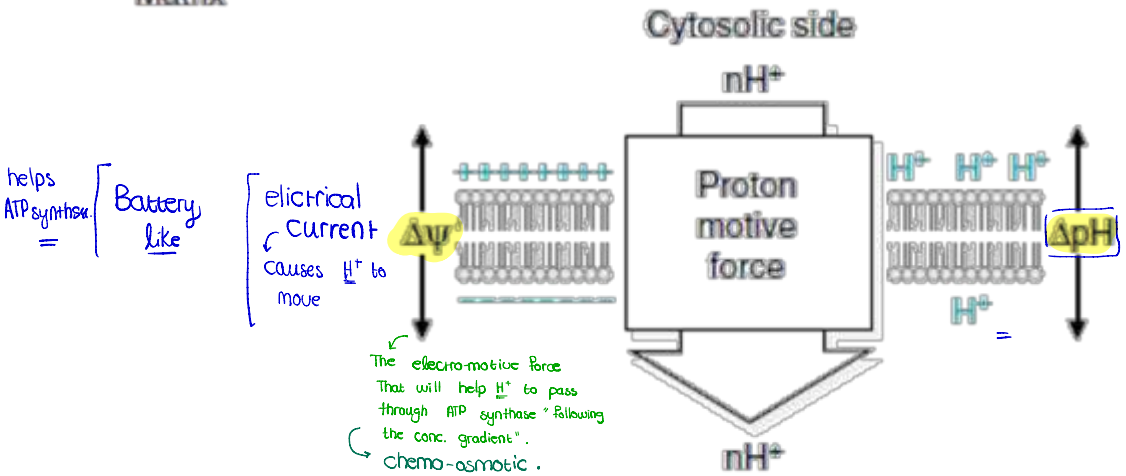


while ē are Passing from 1 complex to another one ⇝ an electrical current will be formed → helps the complexes to Pump H^+ to the inter membrane space... which will create a PH, electrical differences across the membrane. ↙ chemical and electrical energies.

no pumping Property.

1 NADH binds





helps ATP synthase = Battery like

electrical current causes H^+ to move

The electro-motive force That will help H^+ to pass through ATP synthase "following the conc. gradient". ↙ chemo-osmotic.

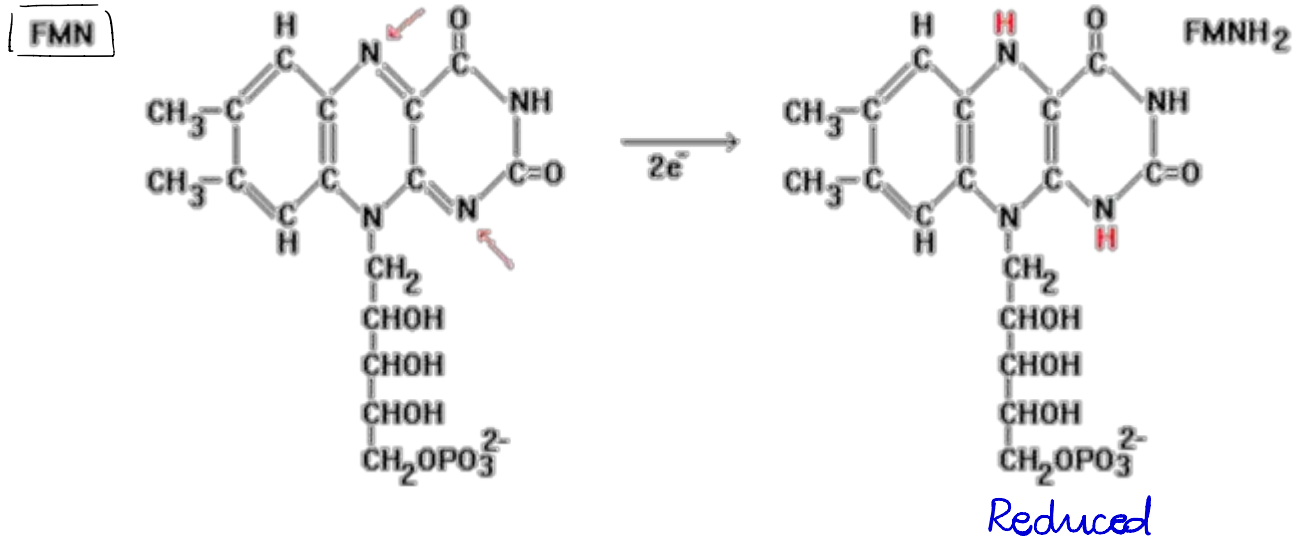
* What determines the direction of the ē flow?

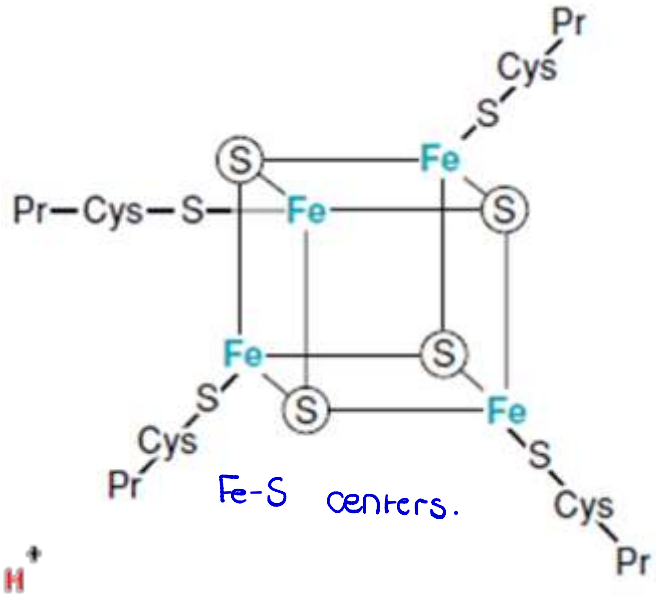
The Redox potential. → from smaller to larger ↙ from the most negative to the most positive. The Redox potential of NADH is much smaller than that of O_2. So ē flow From NADH to O_2.

Oxi–Red Components of the ETC
"NADH Dehydrogenase" OR oxidase – Complex I

NADH-Q oxidoreductase → Reduces complex Q.

* More than 25 polypeptide chain "very complex"
* A huge flavoprotein membrane-spanning complex → There is FMN or FAD tightly bound to the complex.] No FAD in complex 1.
* The FMN is tightly bound
* Seven Fe-S centers of at least two different types
* Binds NADH & CoQ (oxidized / Reduced)
* 4 H+ → will be pumped from the matrix to the inter-membrane space.
* This complex spans the membrane and has a domain that extends to the matrix of the mitochondria. "where NADH binds"



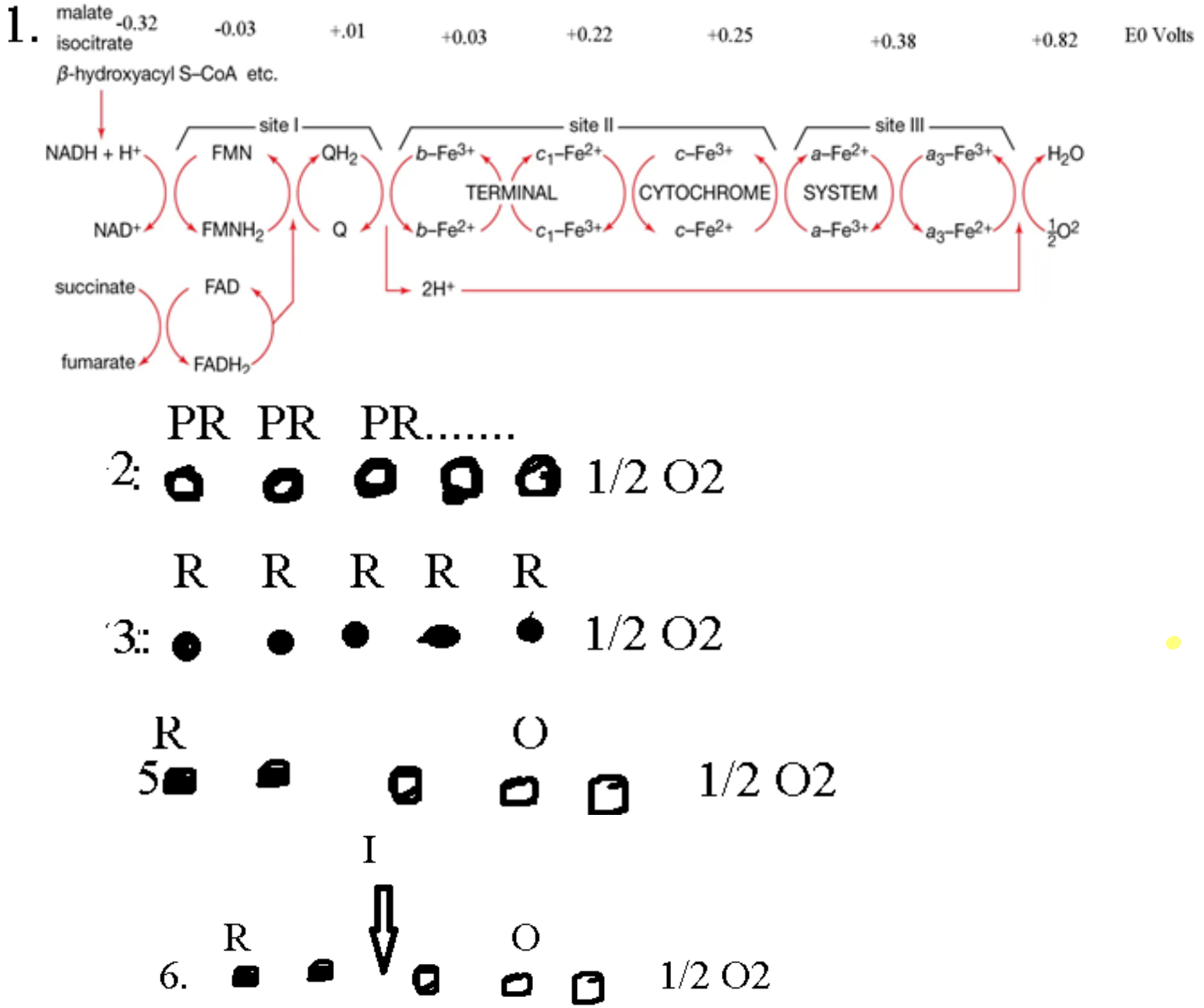


ELECTRON TRANSPORT

- Reactions that involve transfer of electrons are called oxidation-reduction reactions or REDOX reactions.
- A molecule that gains electrons is reduced, and a molecule that loses electrons is oxidized.
- The tendency of redox reaction to proceed depends upon the difference in energy of transferable electrons of the two molecules,
- Consider a pair of electrons that is transferred to NAD to produce NADH+H+
- By convention the reduced form is written to the right: $NAD^+ + 2e + 2H^+ \rightarrow NADH + H^+$
  $E0' = -0.32$ volts.
- The energy of the transferred electrons under standard conditions is expressed as $\Delta E0'$
- A **strong reducing** agent has a **negative redox** potential, whereas a strong **oxidizing agent** has a **positive** redox potential. A positive redox potential means that a substance has a higher affinity for electrons than does a substance with less positive redox potential.

Sequence of carriers in ETC

1. The order is consistent with E0, carriers with more positive E0 as electrons pass from substrate to oxygen.
2. Under normal conditions, all carriers are in their partially oxidized state
3. Under anaerobic conditions, and in the presence of substrate, all carriers are in their fully reduced state:
4. The extent of oxidation of the carriers can be monitored as they exhibit a distinct spectra which differ in their oxidized and reduced state.
5. Upon sudden addition of oxygen, carriers become oxidized .the carrier nearest oxygen becoming oxidized first
6. Addition of specific inhibitor causes the carriers between the block and oxygen to become more oxidized. The upstream carriers become more reduced.

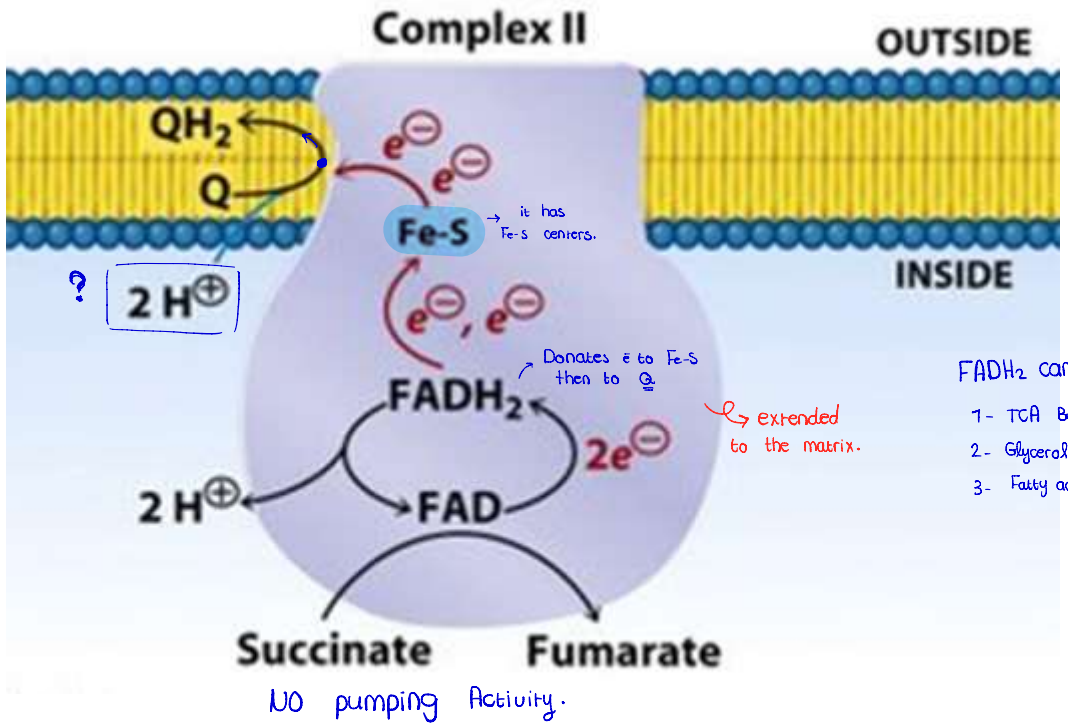


Oxi–Red Components of the ETC "Succinate Dehydrogenase" – Complex II

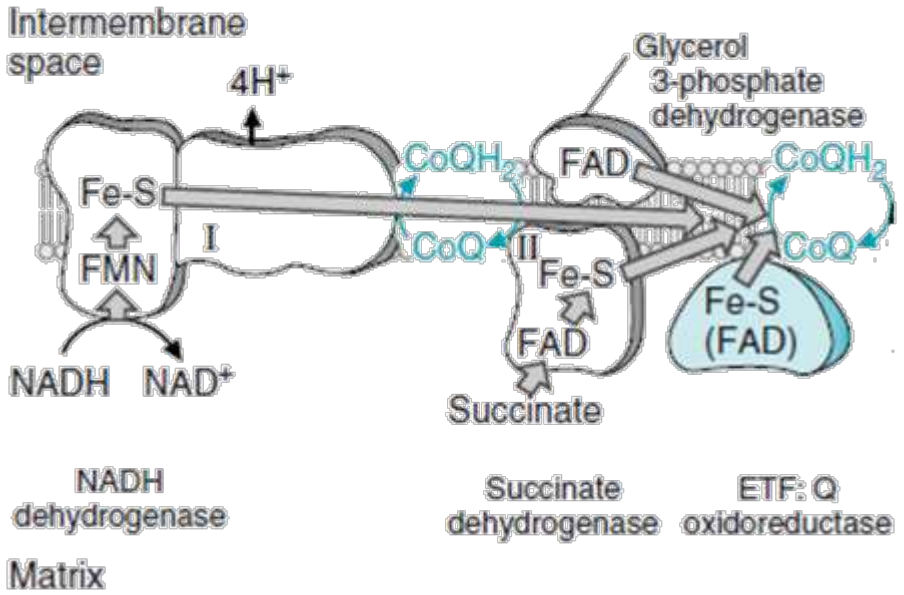
- **Succinate Dehydrogenase & other flavoproteins** "FMN"
- **TCA cycle**
  - ✓ **ETF-CoQ oxidoreductase (ex. fatty acid oxidation)**
  - ✓ **≈Substrates oxidized by FAD-linked enzymes bypass complex-I**
  - ✓ **Three major enzyme systems:** All produce NADH
  - ✓ **Succinate dehydrogenase**
  - ✓ **Fatty acyl CoA dehydrogenase**
  - ✓ **Mitochondrial glycerol phosphate dehydrogenase**
  - ✓ **0 kcal, H+?** → NO H+ Pumping activity.

* has Fe-S centers.

★ Since complex 2,1 accept 2 e they should have 2 Fe-S centers



$FADH_2$ can be formed from:
1- TCA By succinate dehydrogenase.
2- Glycerol 3-P dehydrogenase.
3- Fatty acids metabolism.

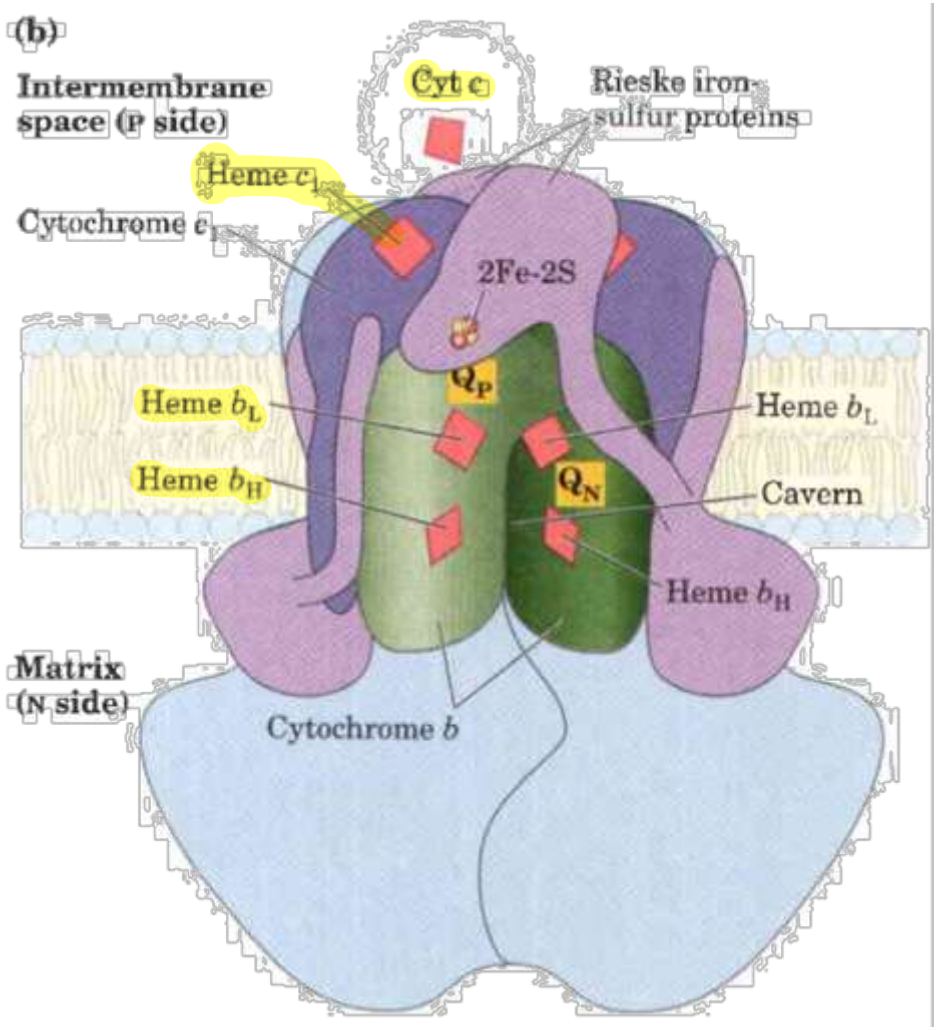


Oxi–Red Components of the ETC

"Cytochrome bc1" – Complex III

has cyt c1 and cyt b Prosthetic groups.

- **Also called: Q-cytochrome c Oxidoreductase**
- **Catalyzes the transfer of electrons from QH2 to cytochrome c**
  - ↳ The fully reduced form.
- **11 subunits including two cytochrome subunits**
- **Contains iron sulfur center**
- **Contain three heme groups in two cytochrome subunits**
- **Contain two CoQ binding sites**
- **4H+**



* at the beginning CoQ will give 1 ē for a specific prosthetic group, the other ē will be given in a different pathway → will parricipate in the Q cycle. In order to complete the Q cycle ... we need another coQ.
  ↳ until the 4 H^+ are pumped
  مرتين راح يعطي في كل مرة 2 H^+ ... ويروح Q.
*

? Fe-S oxidase

Oxi–Red Components of the ETC

"Cytochrome c oxidase" – Complex IV

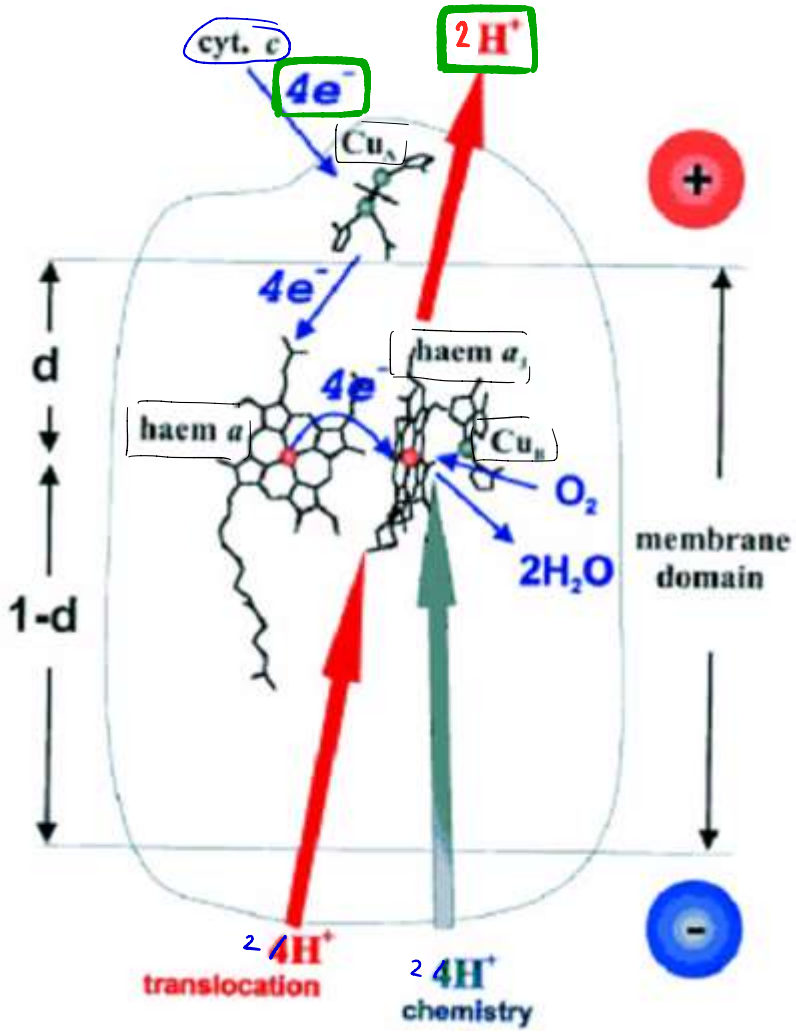
- **Passes electrons from Cytocrome c to O2**
- **Contains cytochrome a and a3**
- **Contains two copper sites**
- **Contains oxygen binding sites** "The final acceptor"
- **O2 must accept 4 electrons to be**

  reduced to 2 H2O (2H+/2e-)
- **Cytochrome c is one electron carrier** معناها هاد كله يصير ٤ مرات كل مرة ٦٤ .

Cyt c_{red} + $4H^+$ + O2 → Cyt c_{ox} + $2H_2O$

1e

Multi-step



2 H^+ at a time are taken from the matrix.

↳ 2 H^+ will be pumped out.

* What inhibits the ē flow ?

Both inhibit complex 1.

1. **Amytal.**(sedative)-inhibits NADH-Q Oxireductase
2. **Rotenone.**(insecticide)-inhibits NADH-Q Oxireductase
3. **Antimycin A**: inhibits electron flow between cyt b and c1, which prevents continued ATP synthesis at sites I and II as the carriers. InhibitsQ-cyttochromr c oxireductase,once reduced can not be oxidized.

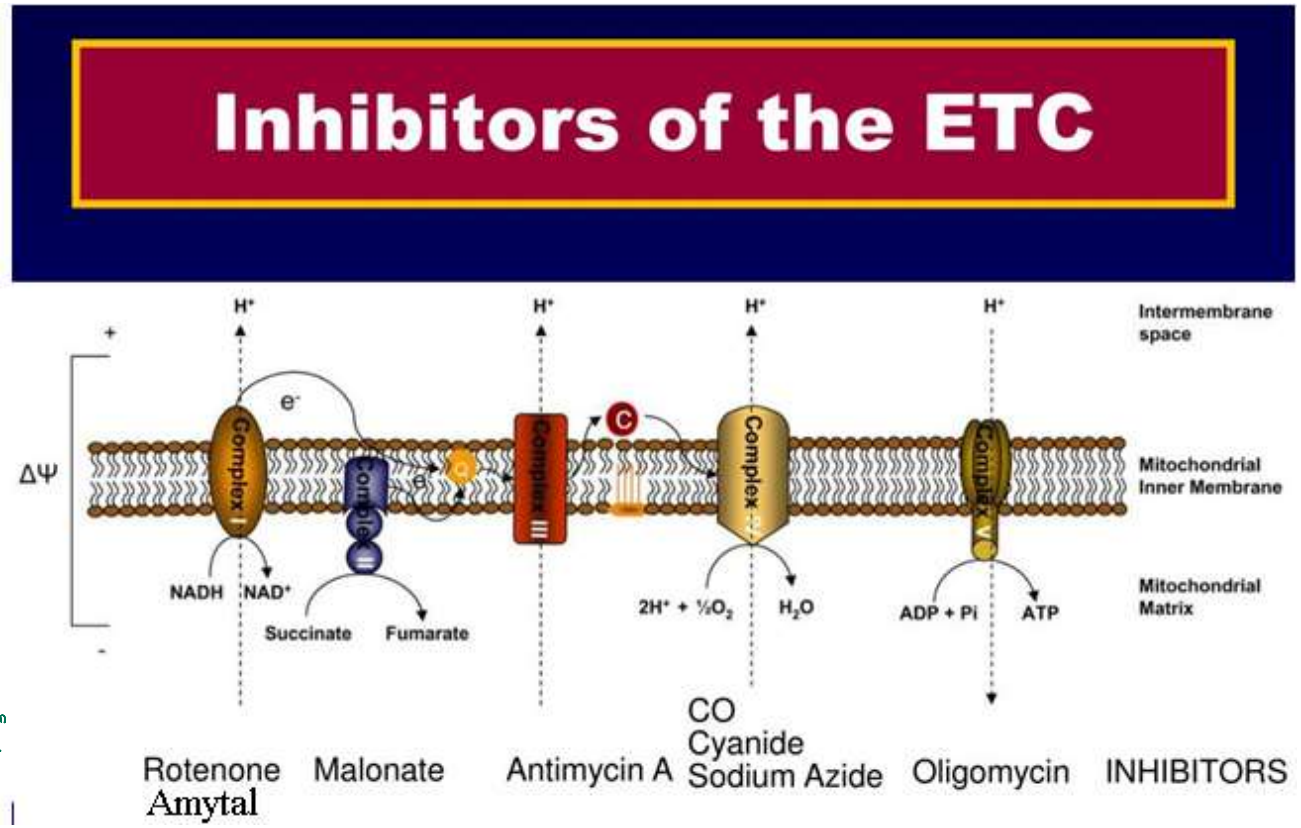
complex 3 inhibitor — اللي هم within complex 3.

complex 3.

* NO accepting ē from coQ
* NO oxidation of cyt C.

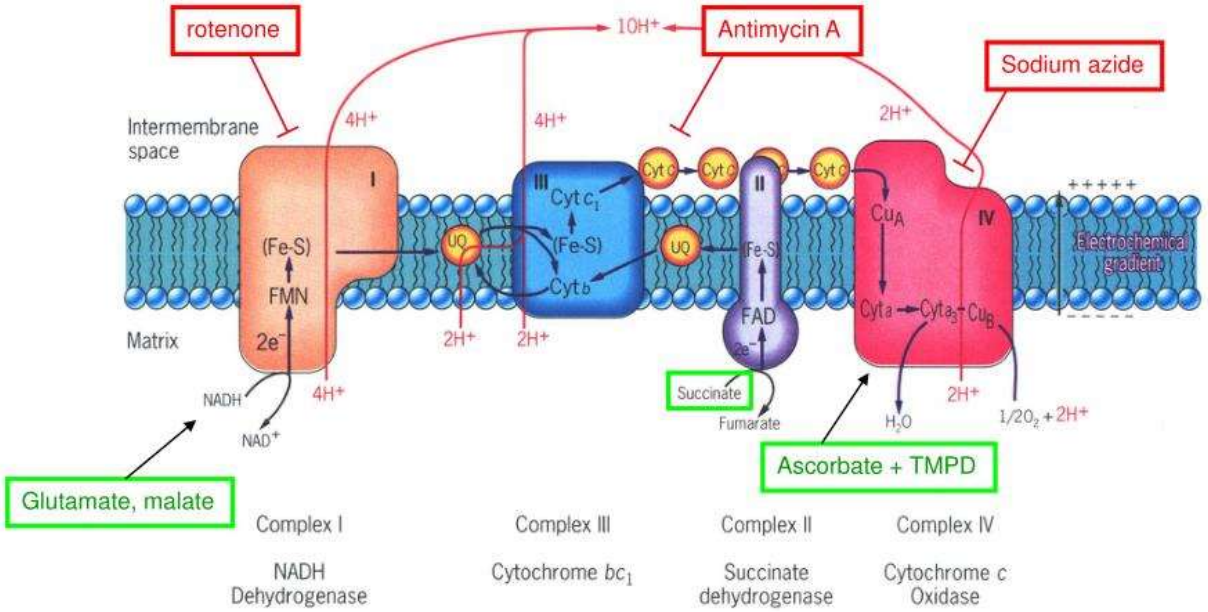
4. CO. –inhibit cytochrome c oxidase
5. Sodium Azide . –inhibit cytochrome c oxidase
6. Cyanides. –inhibit cytochrome c oxidase
7. Oligomycin—inhibits ATP synthase

complex 4 inhibitors.
→ NO oxygen Reduction.



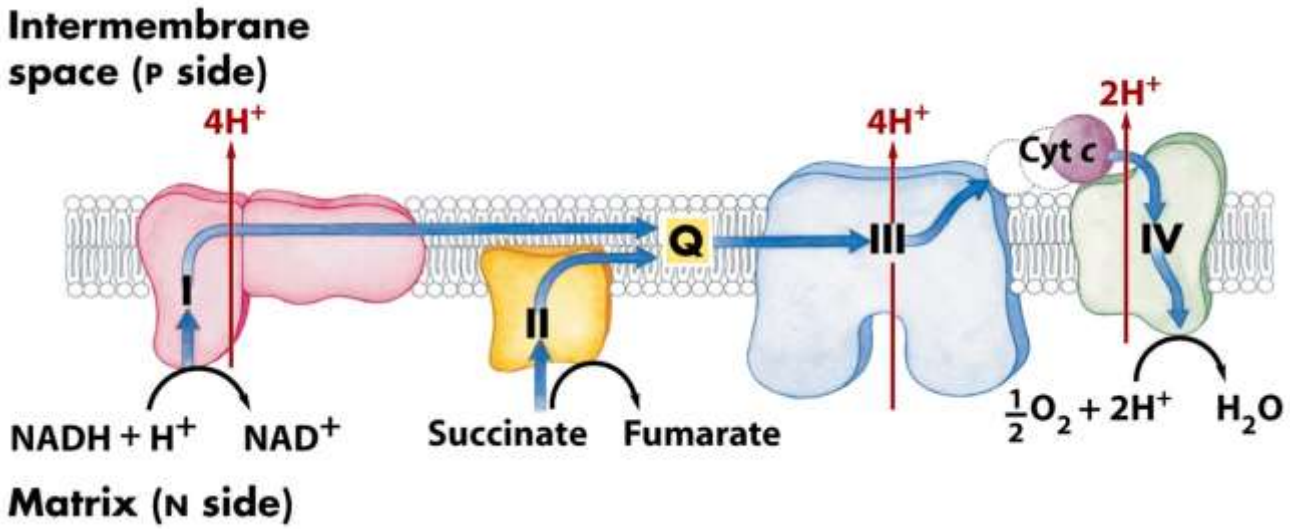
1. Complexex I, III, IV all have large enough E0 for the transfer of 2 electrons to support the synthesis of one ATP.
2. Complex I, III, IV are recognized as phosphorylation sites I, II, and III.
3. Oxidation of 1 molecule NADH+H+ or FADH2 corresponds to the synthesis of 3 or 2 molecules of ATP, respectively, and the reduction of one atom of oxygen.
4. Oxidation of NADH + H+ and FADH2 occurs with P/O ratio of 3 and 2, respectively.
5. Using ascorbate as substrate and TMPD as artificial electron carrier, a P/O ratio =1.
6. P/O ratio is the number of moles of Pi incorporated into ATP per atom of oxygen utilized.
7. P/O for malate=3, succinate=2, ascorbate=1

Electron transport chain inhibitors and substrates



Pumping of Protons

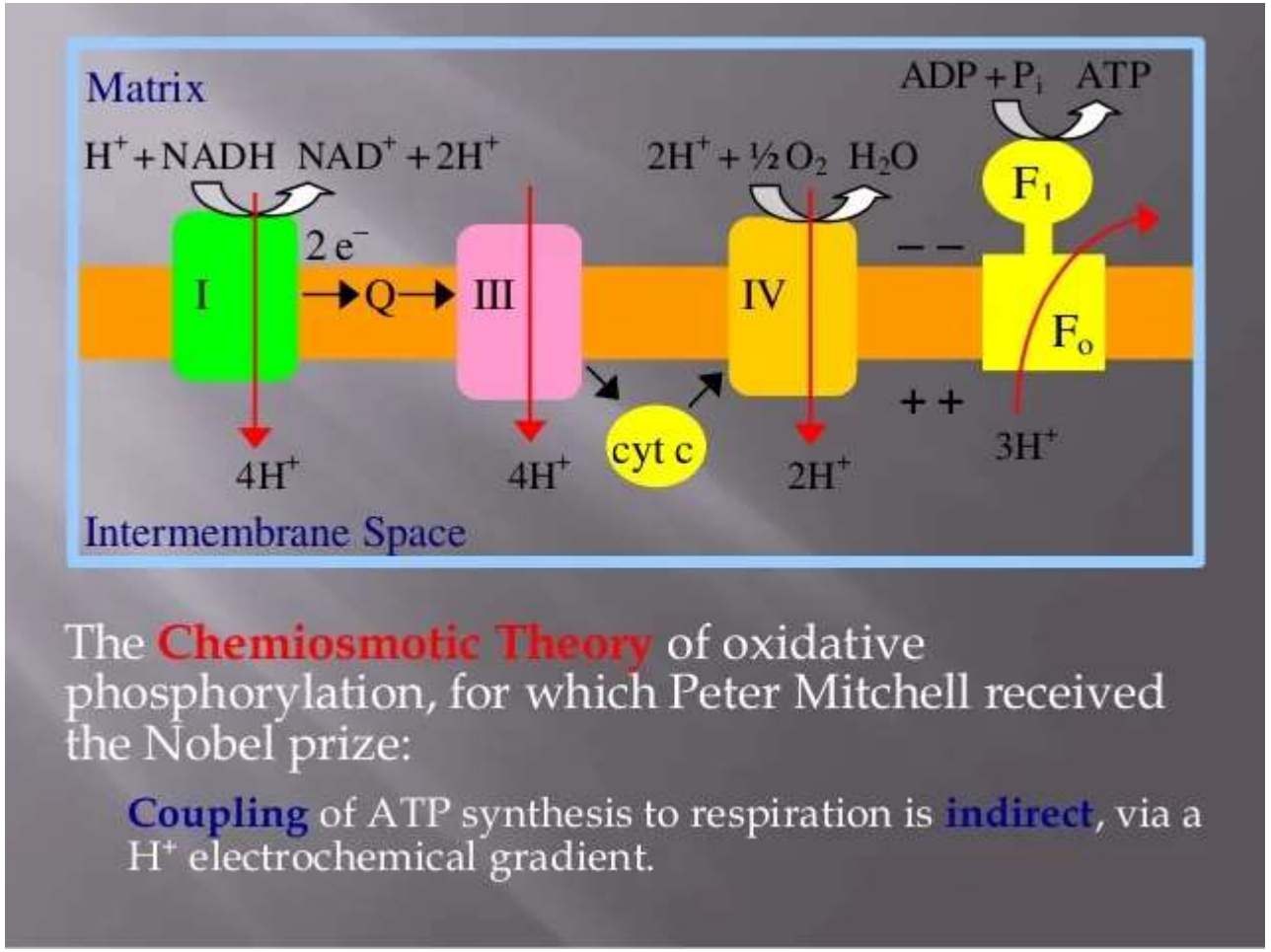
- **For every 2 electrons passing:**
- **$4H^+$ (complex I); oH^+ (complex II); $4H^+$ (complex III), $2H^+$ (complex IV**



ē path.

Chemiosmotic hypothesis:

- a proton gradient is generated by a proton pump in the inner membrane of the mitochondria.
- The proton pump is operated by electron flow and causes protons to be expelled through the membrane from the matrix space.
- Protons flow back into the matrix down their electrochemical gradient and the energy released is used to drive the synthesis of ATP.



Chemiosmotic hypothesis:

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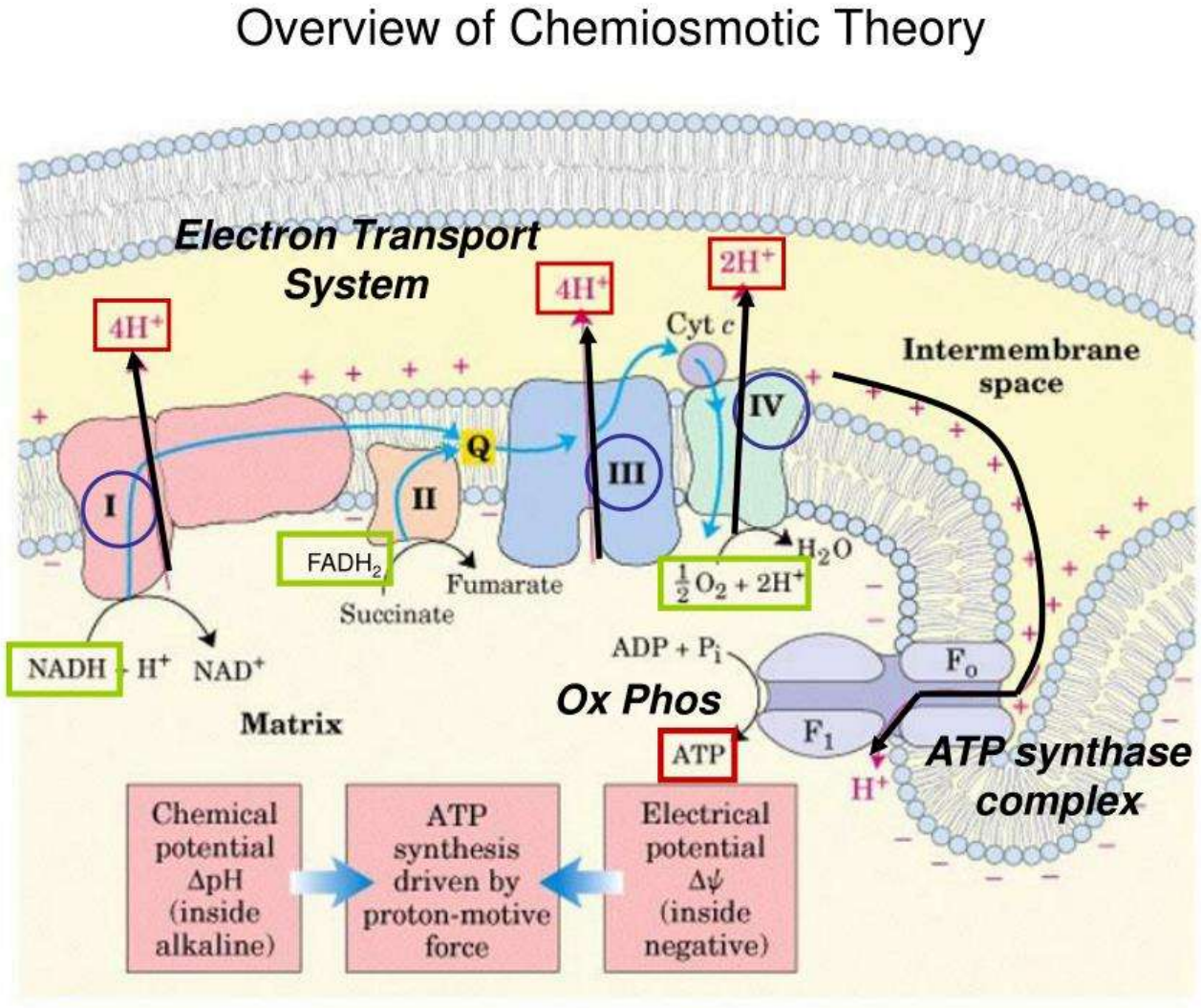
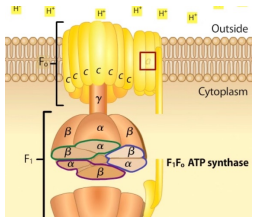




STRUCTURE AND MECHANISM OF ATP SYNTHASE-COMPLEX V

1. F0 is the proton channel of the complex → inhibited by oligomycin.
2. F1 hydrolyzes ATP in the absence of proton gradient "multi-subunits"

The catalytic subunit.
↳ has 3 catalytic sites within β subunits.
α → NO catalysis "structural".

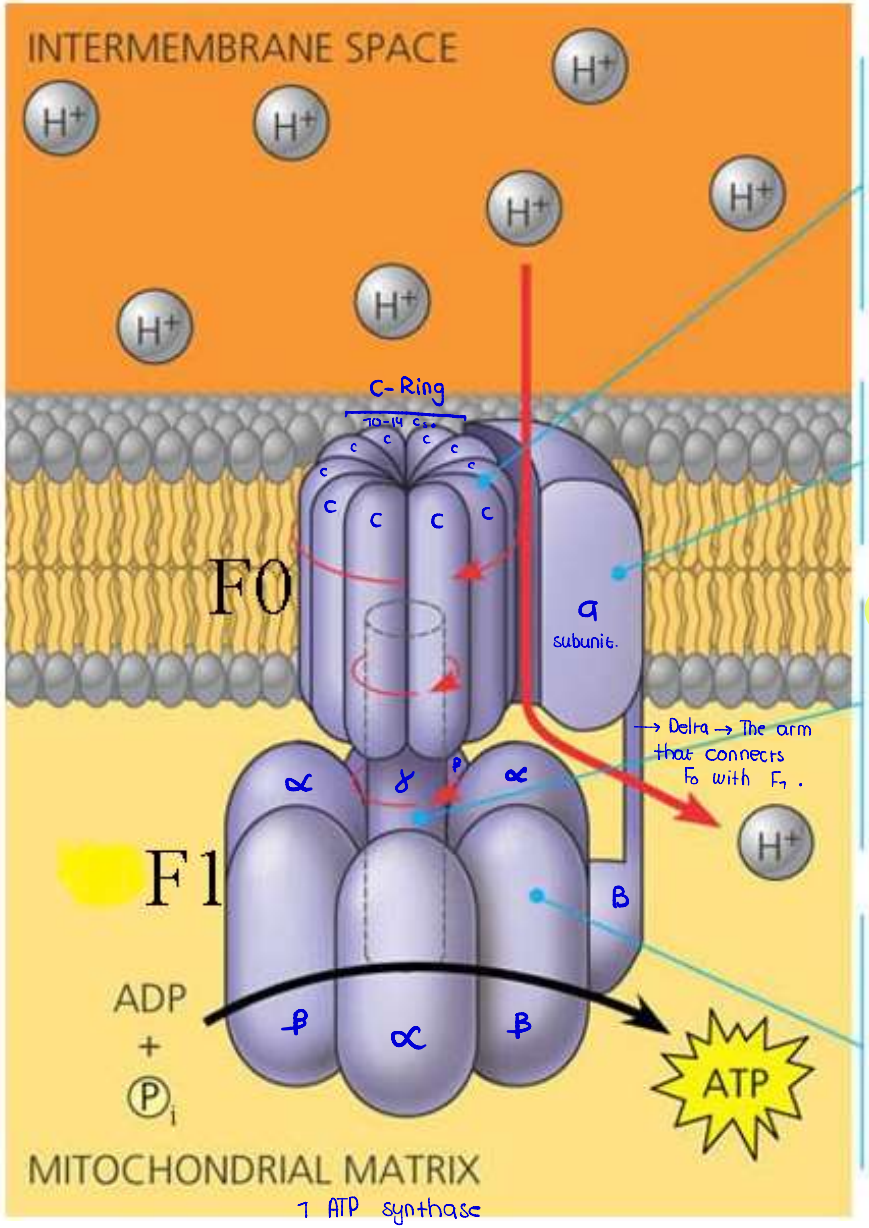
3. The stalk between F1 and F0 contains several proteins, one of which is sensitive to oligomycin. This antibiotic inhibits ATP synthesis by interfering with the utilization of the proton gradient.
4. ATP SYNTHASE catalyzes the reaction:

ADP +Pi---→ ATP +H2O

https://youtu.be/U26Jz3K1w2k

? Rotation مين الجزء اللي يدل
The C-ring of Fo with the gamma-epsilon stalk of F1.

here



A rotor within the membrane spins clockwise when H+ flows past it down the H+ gradient.

A stator anchored in the membrane holds the knob stationary.

Fo subunit is composed of:
① The C-Ring
↳ The proton Passage from inter-membrane space to the matrix.
② The a subunit

A rod (or "stalk") extending into the knob also spins, activating catalytic sites in the knob. → The gamma-epsilon subunit.
↳ Rotates with the C-Ring of Fo

→ Delta → The arm that connects Fo with F1.

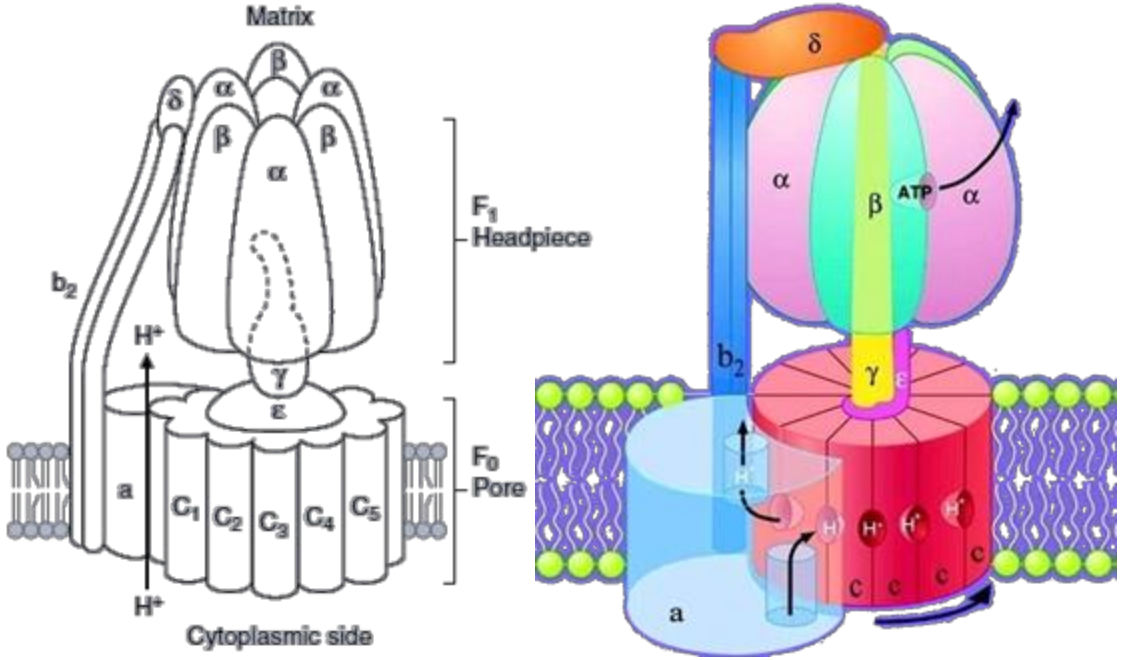
Three catalytic sites in the stationary knob join inorganic phosphate to ADP to make ATP.

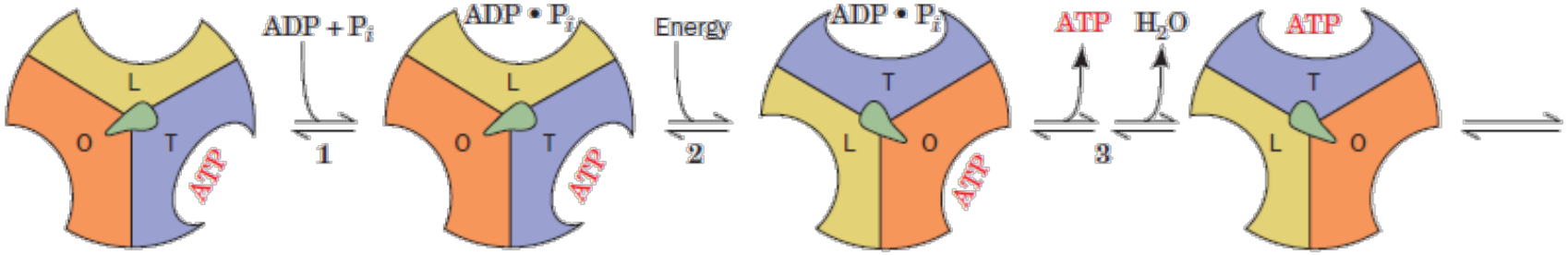
F1:
This part is composed of α3 β3 hexamer. or 3 pairs of α-β.
Both α and β DO NOT Rotate.
α → Structural
β → catalytic.

1 ATP synthase
↳ 3 α
↳ 3 β
↳ 1 γ + 1 delta.
↳ 1 ep

ATP Synthase

- F1:
  - "γ" subunit: rotates
  - "β" subunit: binds "catalytic".
  - "α" subunit: structural
  - 3 conformations: tight (T), loose (L), open (O)
- Fo:
  - "a" subunit: point of entry & exit
  - "c" subunit rotates
  - 4H+/ATP
- Can run backwards





هاد هو شغلها.

From F_o to F_1

H^+ path through membrane

c subunit | subunit a

c ring & a subunit structure

- each c subunit has 2 membrane-spanning a helices
  - midway along 1 helix: *asp* "aspartate"
  - COOH↔COO^-
    aspartic acid — aspartate.
- a subunit has 2 half-channels

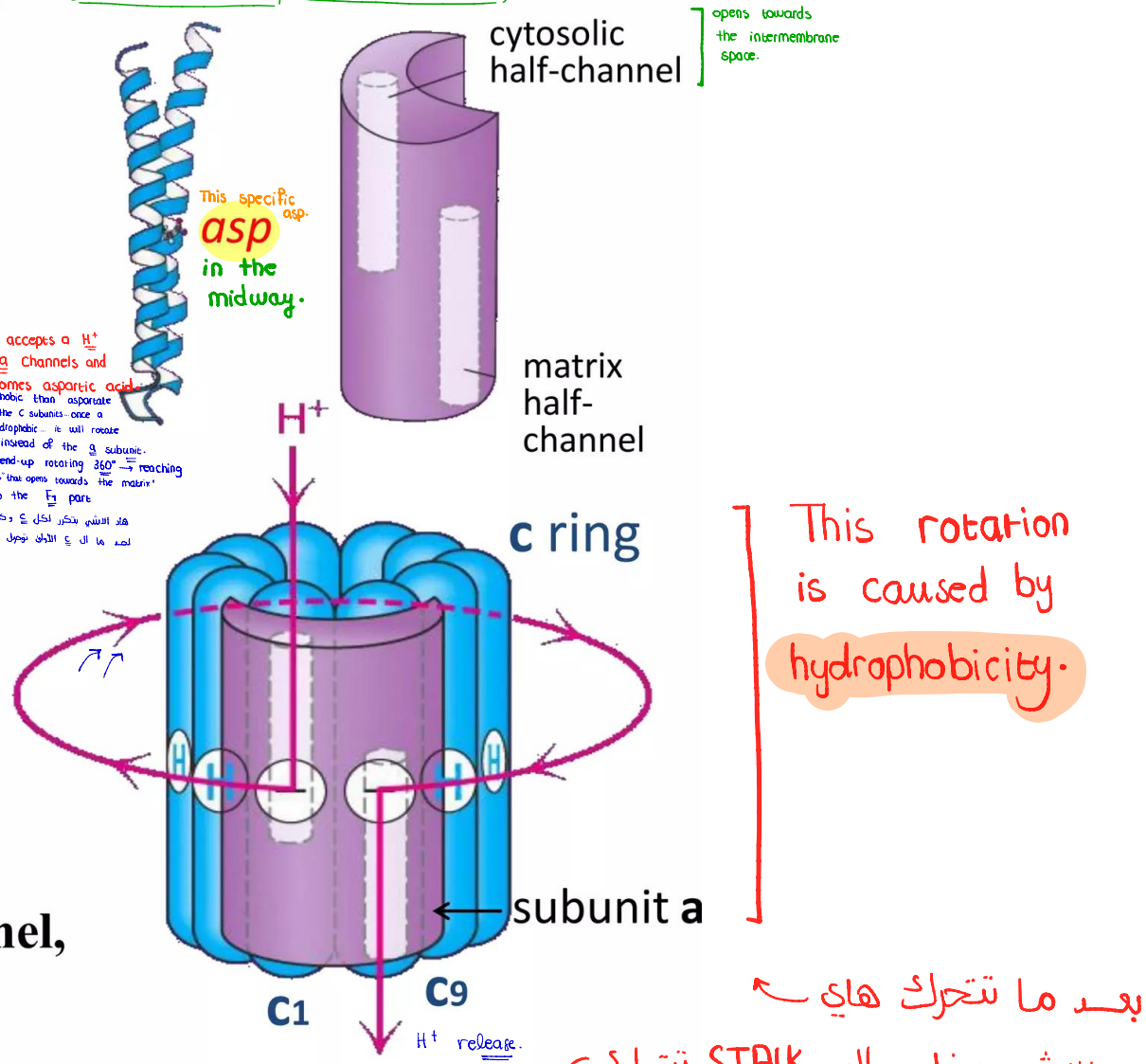
Point of entry and exit.

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 ∨∨ ← Because it became more hydrophobic.
- c9 now interfaces matrix half-channel, allowing H^+ to diffuse into matrix ∨∨

و بترجع

→ Negatively charge. → accepts a H^+ that is coming from a Channels and binds to it, and becomes aspartic acid.
• aspartic acid is more hydrophobic than aspartate
this causes the ROTATION of the c subunits. once a c-subunit becomes more hydrophobic... it will rotate to face the membrane instead of the a subunit.
after some time it will end-up rotating 360° → reaching the other half Chanel ~ that opens towards the matrix
releasing the H^+ to the F_1 part
هاد الاشي بتكرر لكل c وكل c بتجيب H^+ بتلف شوي
2nd chanel لحتى ما ال c الاولاني توصل ال



This rotation is caused by hydrophobicity.

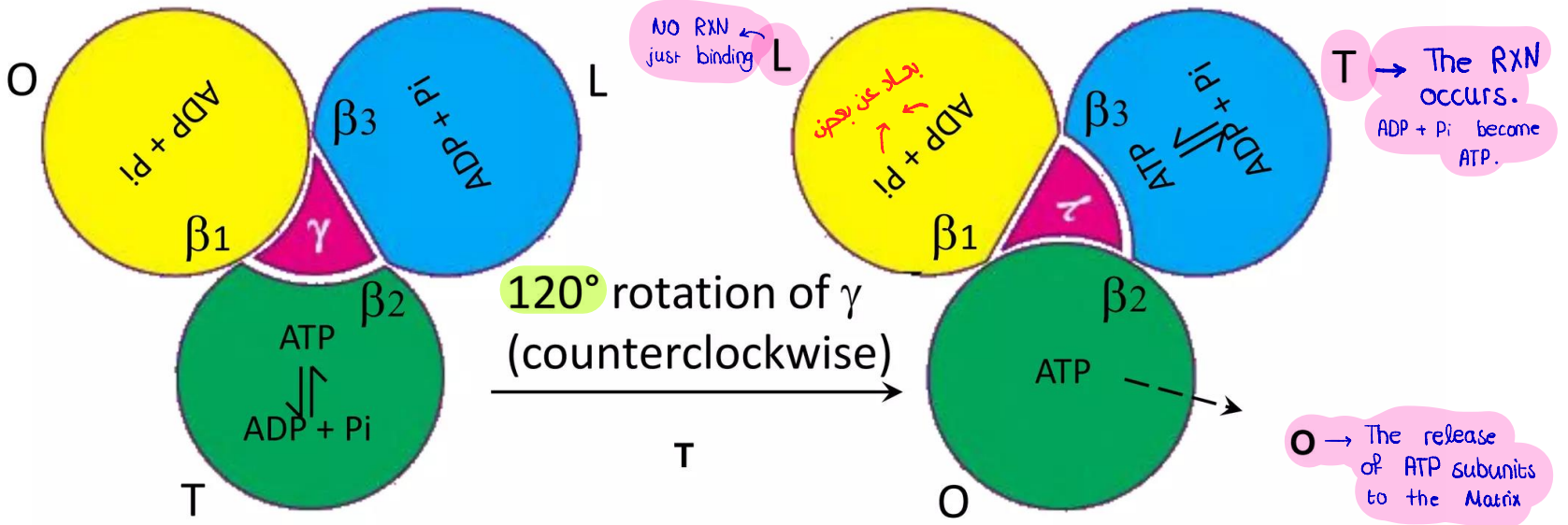
بعد ما تتحرك هاي ← هاد الاشي بخلي ال STAIK تتحرك

causing The conformational interconversion of β subunits Releasing ATP.

Binding-change mechanism of ATP synthesis

* ∝ + β Do NOT rotate.

- 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.



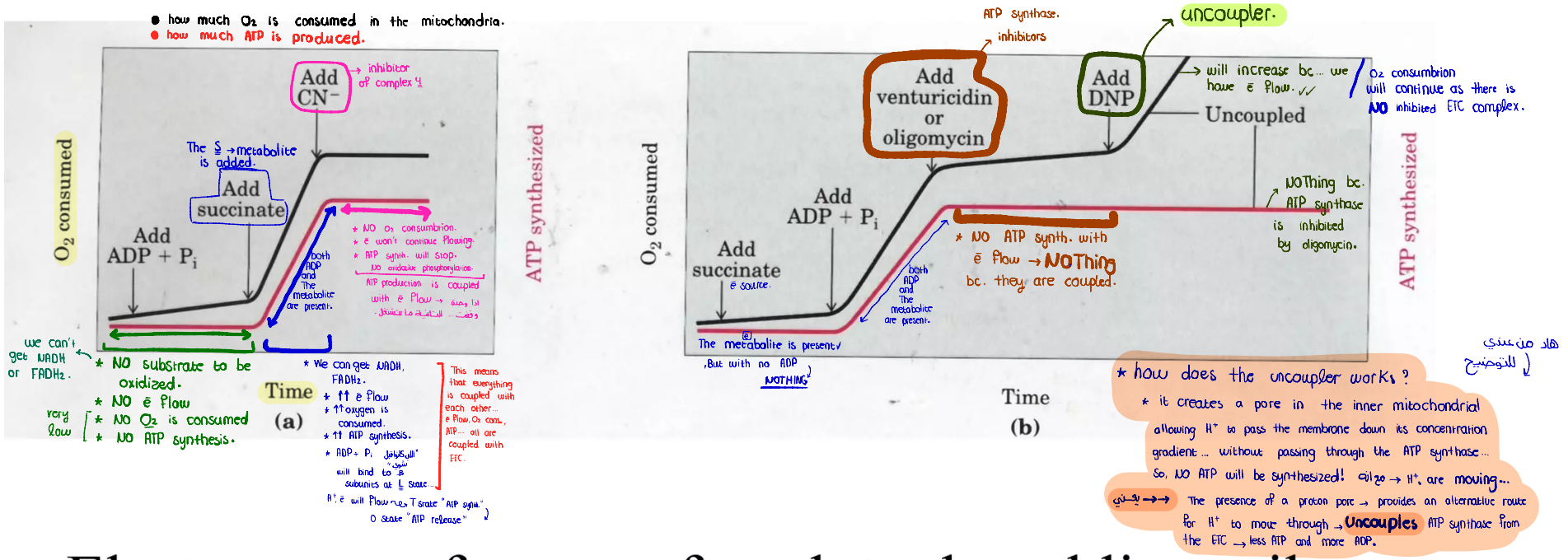
NO RXN just binding

T → The RXN occurs. ADP + Pi become ATP.

O → The release of ATP subunits to the Matrix

T+L
No Release

Respiratory control. → The effect of ADP on ETC and oxidative phosphorylation.



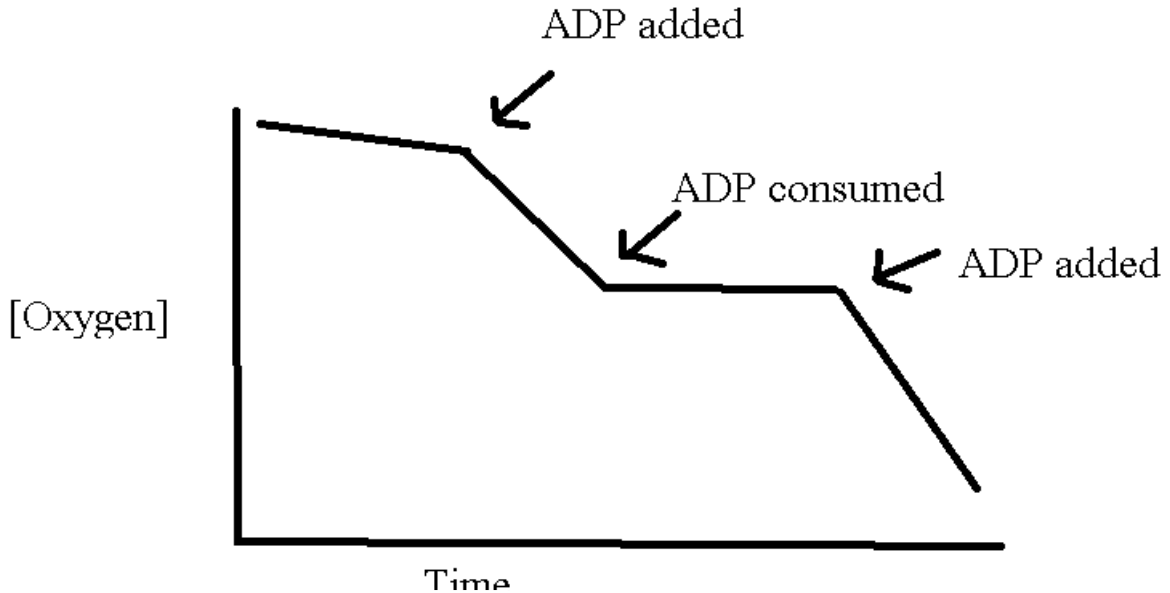
Electron transfer was found to be obligatorily coupled to ATP Synthesis in isolated mitochondria suspensions:
neither occurs without the other.

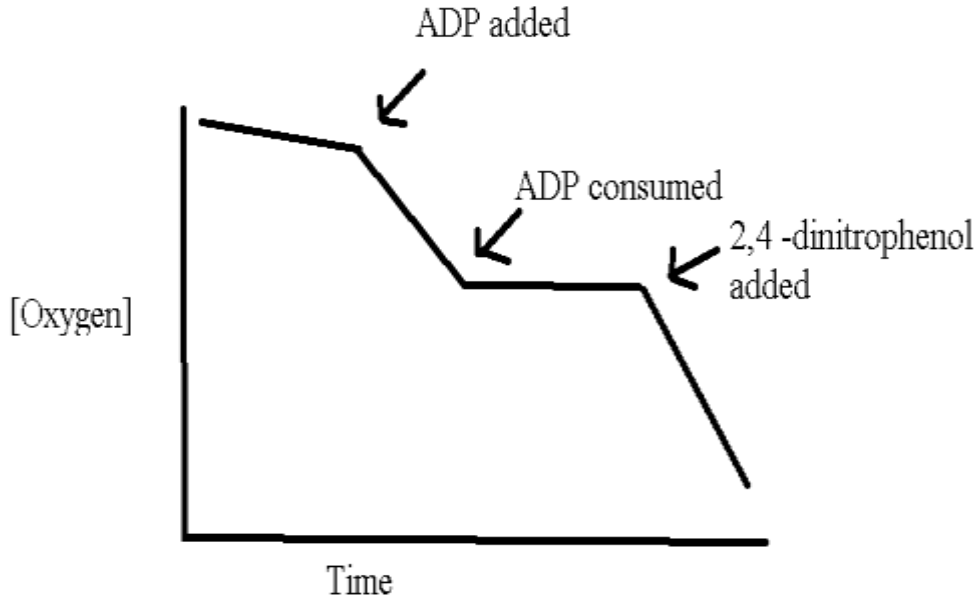
قبل فترة من الزمن... كانوا الناس يستعملوا هاي الـuncouplers to lose weight... → Since they have ↑ ADP, they will have ↑ TCA activity → ↑ metabolic rate but with NO ATP → ↑ heat..
وأنوع جديدين ابن الجسم بحيس يستخدم الـ Fats اللي عنده عشان يولد طاقة.

Natural uncouplers
↳ to produce heat
في السلاليات اللي بجد.

RECEPTOR OR ACCEPTOR CONTROL

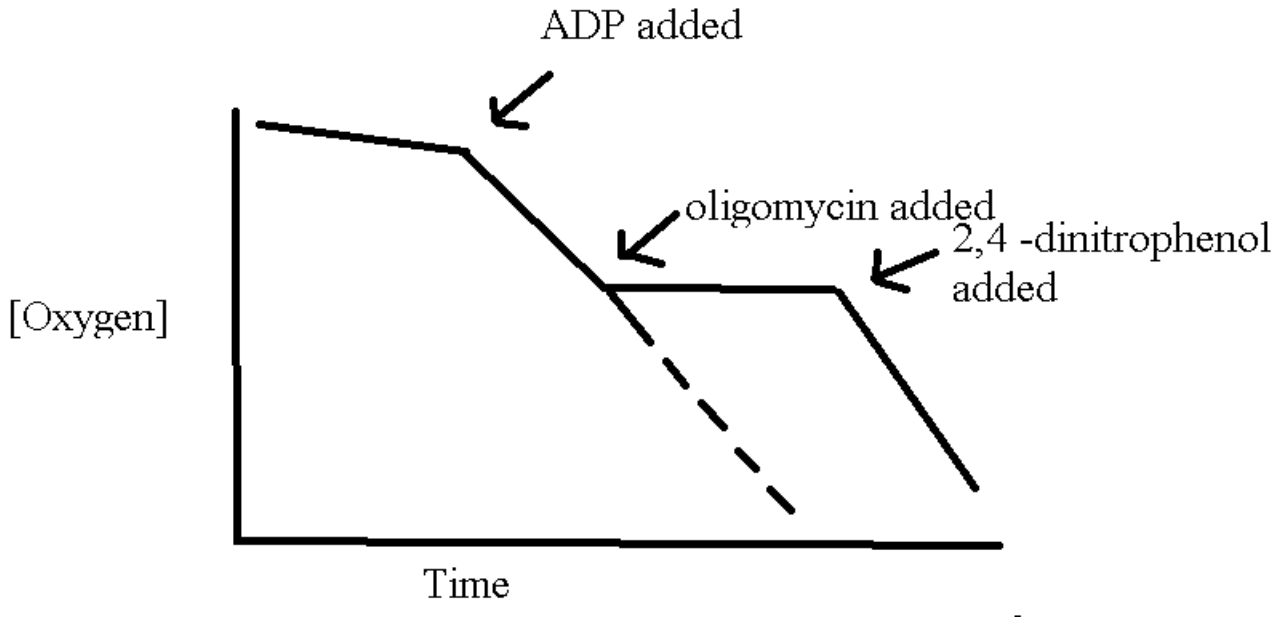
1. Electron transport is normally tightly coupled to oxidative phosphorylation so that electrons do not flow through the respiratory chain unless ADP is simultaneously phosphorylated to ATP.
2. Uncoupling agents, such as 2,4-dinitrophenol, collapse the proton gradient as they are able to channel protons across the membrane. Under this condition, electrons transport runs unchecked at its maximal rate in the absence of the acceptor ADP.





RECEPTOR OR ACCEPTOR CONTROL….

1. Oligomycin inhibits the increased oxygen consumption stimulated by the addition of ADP: phosphorylation of ADP to ATP is also inhibited under these conditions.
2. Oligomycin prevents the utilization of the proton gradient.
3. Uncouplers relieve the inhibition of oxygen consumption.
4. 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.



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!**

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:
- malate → With NADH it is 3 → more than $FADH_2$ → 2 ATP
- With succinate (→ 1 $FADH_2$) it is 2
- With ascorbate it is 1
- The overall equation for respiratory chain phosphorylation:

NADH+ H+ +3ADP+3Pi--→ NAD + H2O + 3ATP

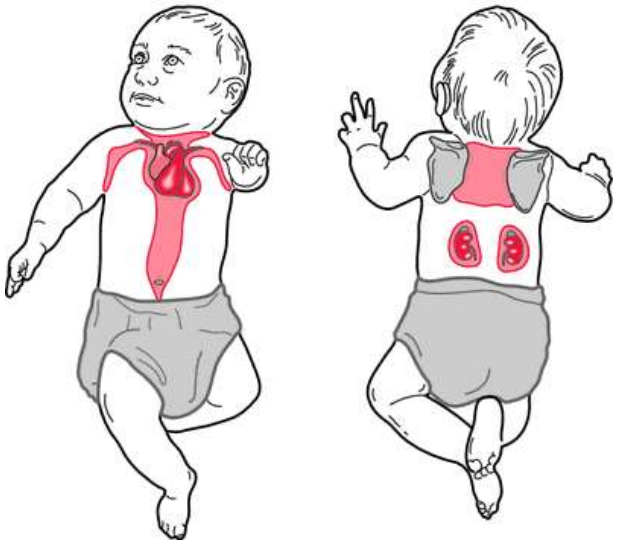
What is the P:O ratio of 1 pyruvate?

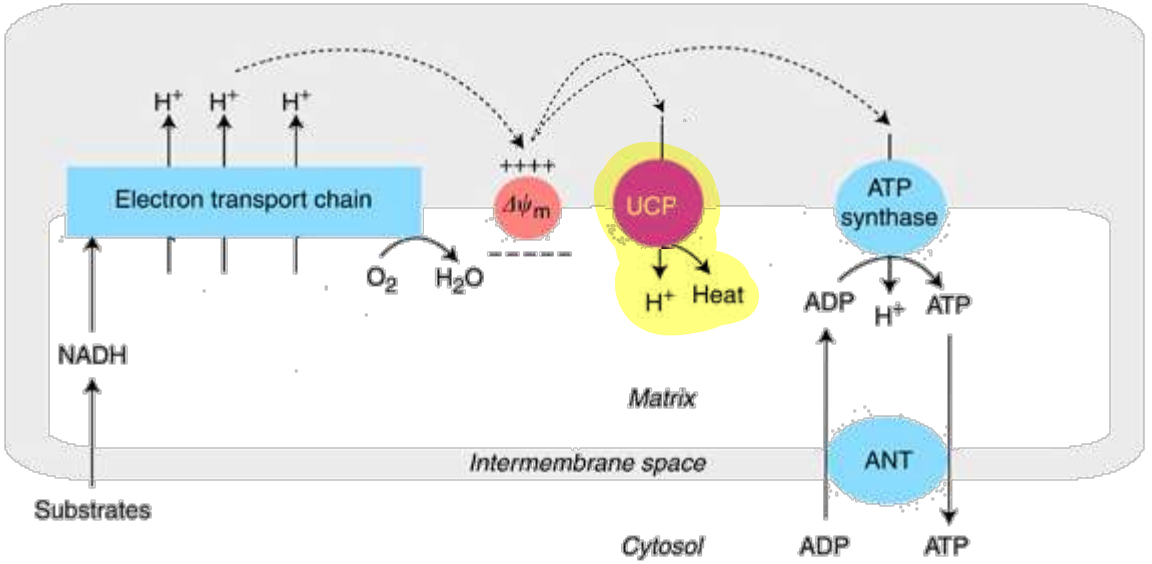
Regulation – Uncoupling
Regulated - Uncoupling proteins (UCPs)

- **Short-circuiting ATP synthase**
- **UCP1 (thermogenin):** ↳ found in babies.
  - ✓ **Brown adipose tissue, non-shivering thermogenesis**
  - ✓ **Infants: neck, breast, around kidneys**
  - ✓ **Fatty acids directly activates UCP1**

= ↳ creates a pore inside the membrane → uncoupled H^+ movement → ↑↑ heat → instead of ATP.

- **UCP2 (most cells); UCP3 (skeletal muscle); {UCP4, UCP5} (brain)**





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