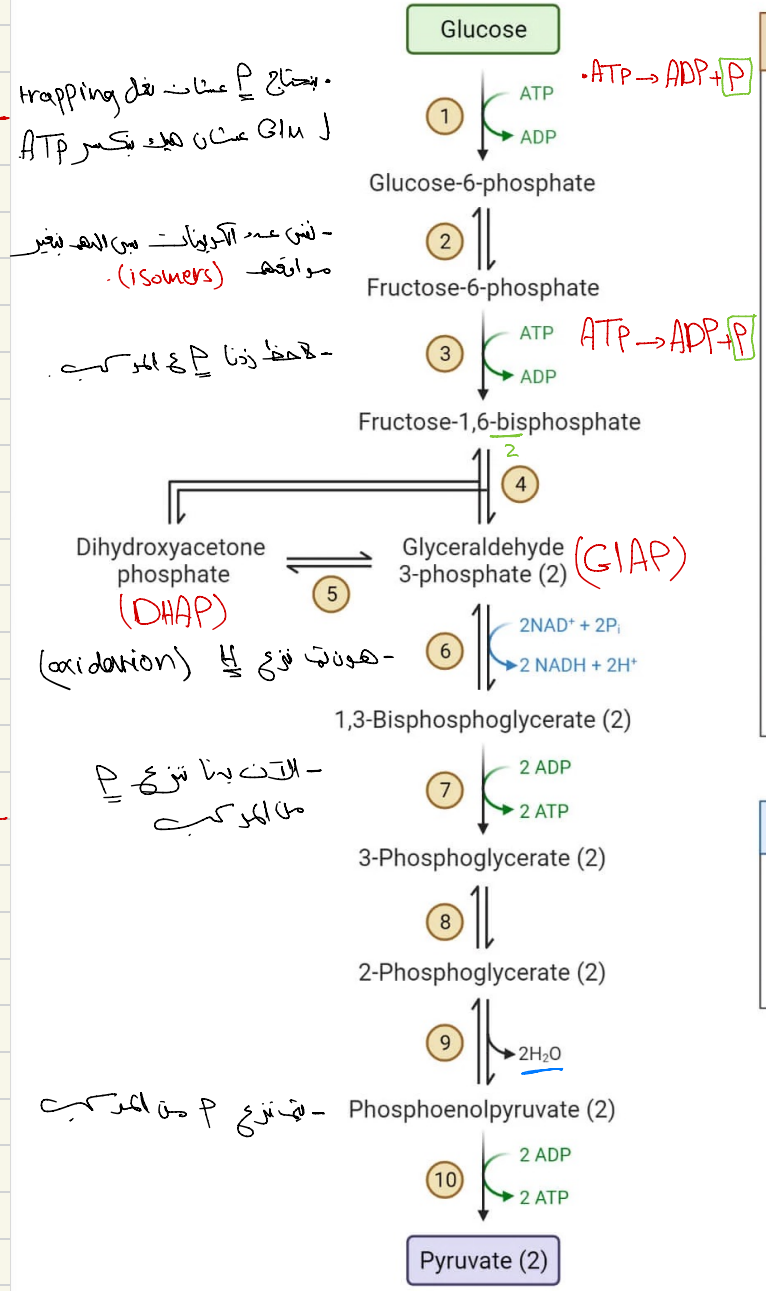
PYRUVATE METABOLISM , TRICARBOXYLIC ACID CYCLE, AND ELECTRON TRANSPORT CHAIN

METABOLISM

1st SEMESTER, 2023

DR. NABIL BASHIR

Glycolysis and Glycolytic Enzymes



ENZYMES	
1	Hexokinase
2	Phosphoglucose isomerase
3	Phosphofructokinase-1
4	Aldolase
5	Triosephosphate isomerase
6	Glyceraldehyde 3-phosphate dehydrogenase
7	Phosphoglycerate kinase
8	Phosphoglyceromutase
9	Enolase
10	Pyruvate kinase

PRODUCTS	
2 ATP	2 Pyruvate
2 NADH	

Microbe Notes

شرح مبسط لعملية ال glycolysis

STAND With GAZA

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 phosphorase

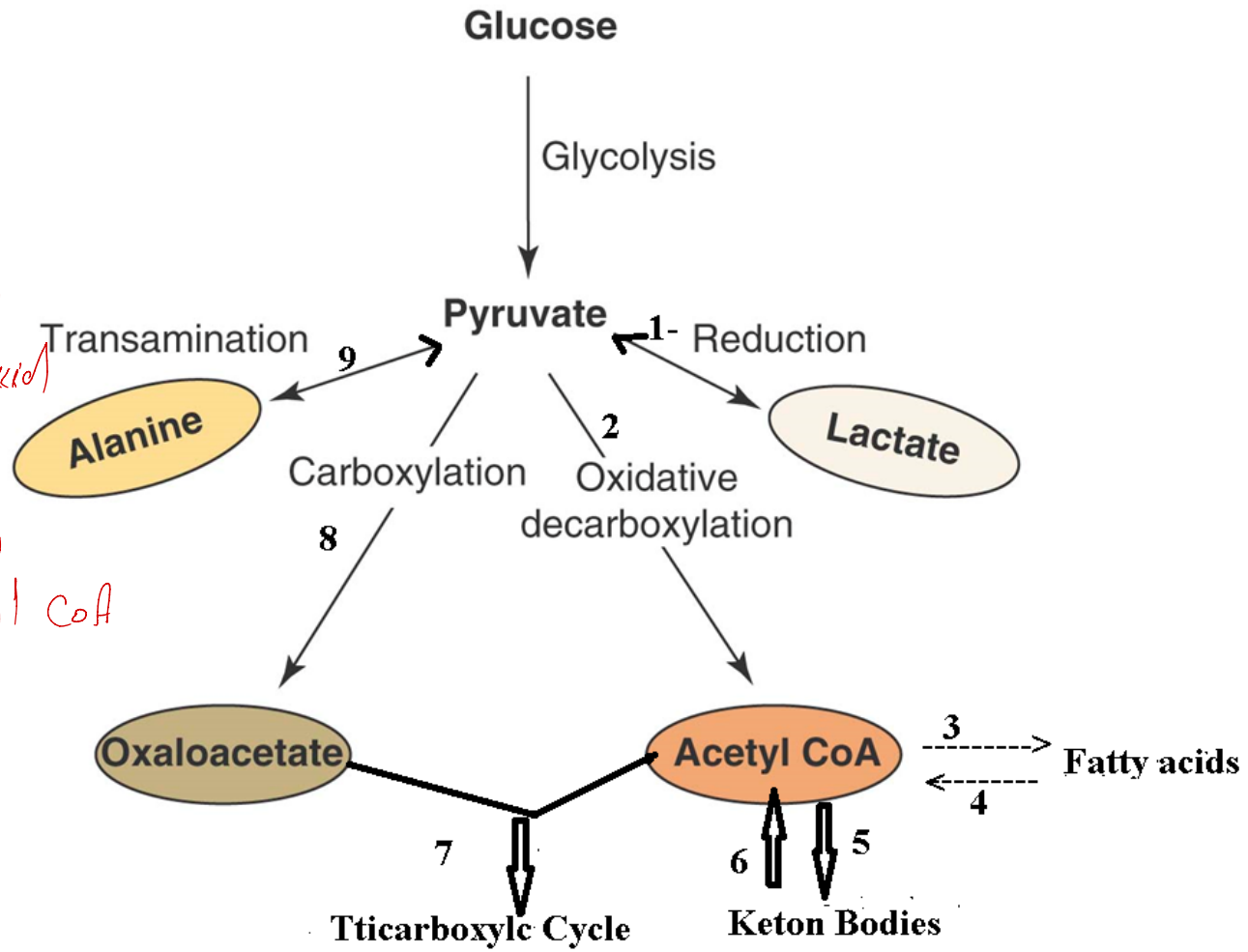
OBJECTIVES

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.

*pyruvate could come from glycolysis (oxidation of glucose), from transamination of some amino acid.

Pyruvate is at important metabolic crossroads

1. Lactate dehydrogenase pyruvate ⟷ lactate.
2. Pyruvate dehydrogenase pyruvate → acetyl CoA
3. Fatty acid synthesis acetyl CoA → fatty acid
4. Fatty acid beta- oxidation fatty acid → Acetyl CoA
5. Ketone body synthesis Acetyl CoA → Keton body
6. Ketone body utilization Keton body → acetyl CoA
7. Citrate synthase
8. Pyruvate carboxylase pyruvate → OOT
9. transamination pyruvate ⟷ alanine



How pyruvate convert to acetyl CoA?

Then we will see what will happen to Acetyl CoA in Krebs cycle.

PYRUVATE DEHYDROGENASE

- Oxidative decarboxylation of pyruvate to acetyl CoA.
- The reaction occurs in mitochondrial matrix
- 3 enzymes, 5 coenzymes-thiamin pyrophosphate(B1), lipoamide, Flavin adenine dinucleotide (B2), coenzyme A (contain ~~B3~~ B5), and NAD (niacin)-are required.

E1 : Pyruvate dehydrogenase → to decarboxylate pyruvate (remove the C atom in the form of CO_2)

E2 : Dihydrolipoyl transacetylase → to transfer the acetyl group to a lipoeic acid which coenzym

E3 : Dihydrolipoyl dehydrogenase → to do some oxidation, reduction reactions.

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

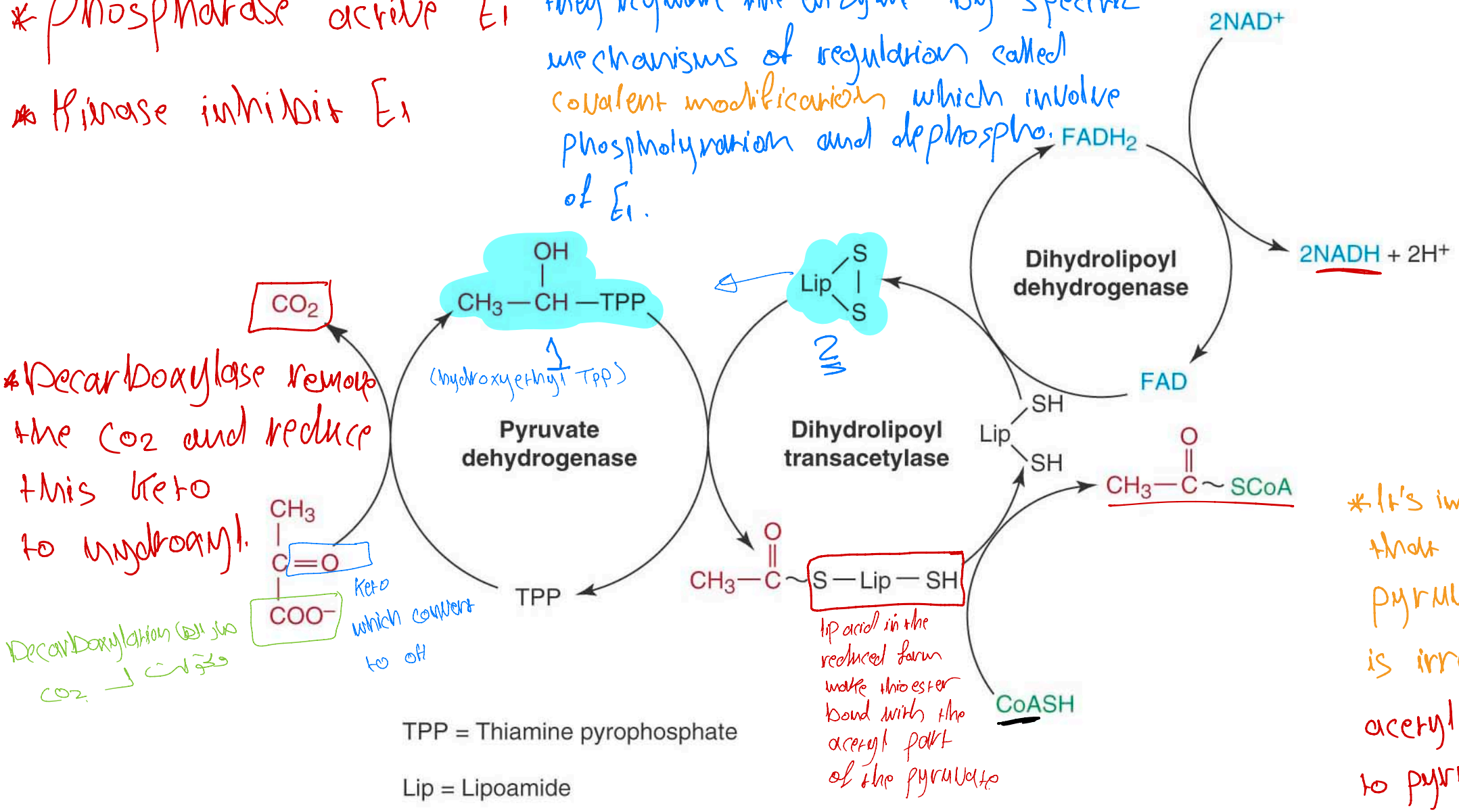
Phosphatase action on E1 **activates** it, **phosphorylation** of E1 by the

*Phosphatase active E_1

*Kinase inhibit E_1

they regulate the enzyme by specific mechanisms of regulation called covalent modification which involve phosphorylation and dephospho. of E_1.

نواتج الحلقة
2NADH
CO_2
acetyl CoA



*Decarboxylase remove the CO_2 and reduce this keto to hydroxyl.

Decarboxylation ازالة ثاني اكسيد الكربون CO_2

Keto which convert to OH

lip acid in the reduced form make thioester bond with the acetyl part of the pyruvate

*It's important to know that conversion of pyruvate to acetyl CoA is irreversible.

acetyl CoA can't convert to pyruvate in mammalian system and this is why we can't synthesis Glucose from lipids.

Figure 14.14 Mechanism of the pyruvate dehydrogenase multienzyme complex.

How to bit these 3 enzymetic activities with 5 co-enzyme ?

- first TPP which is the first co enzyme will bind with pyruvat and form complex number 1, TPP with some reduction (because of hydrogenase)

convert this kero to hydroxyl group and CH_3 will remain the same

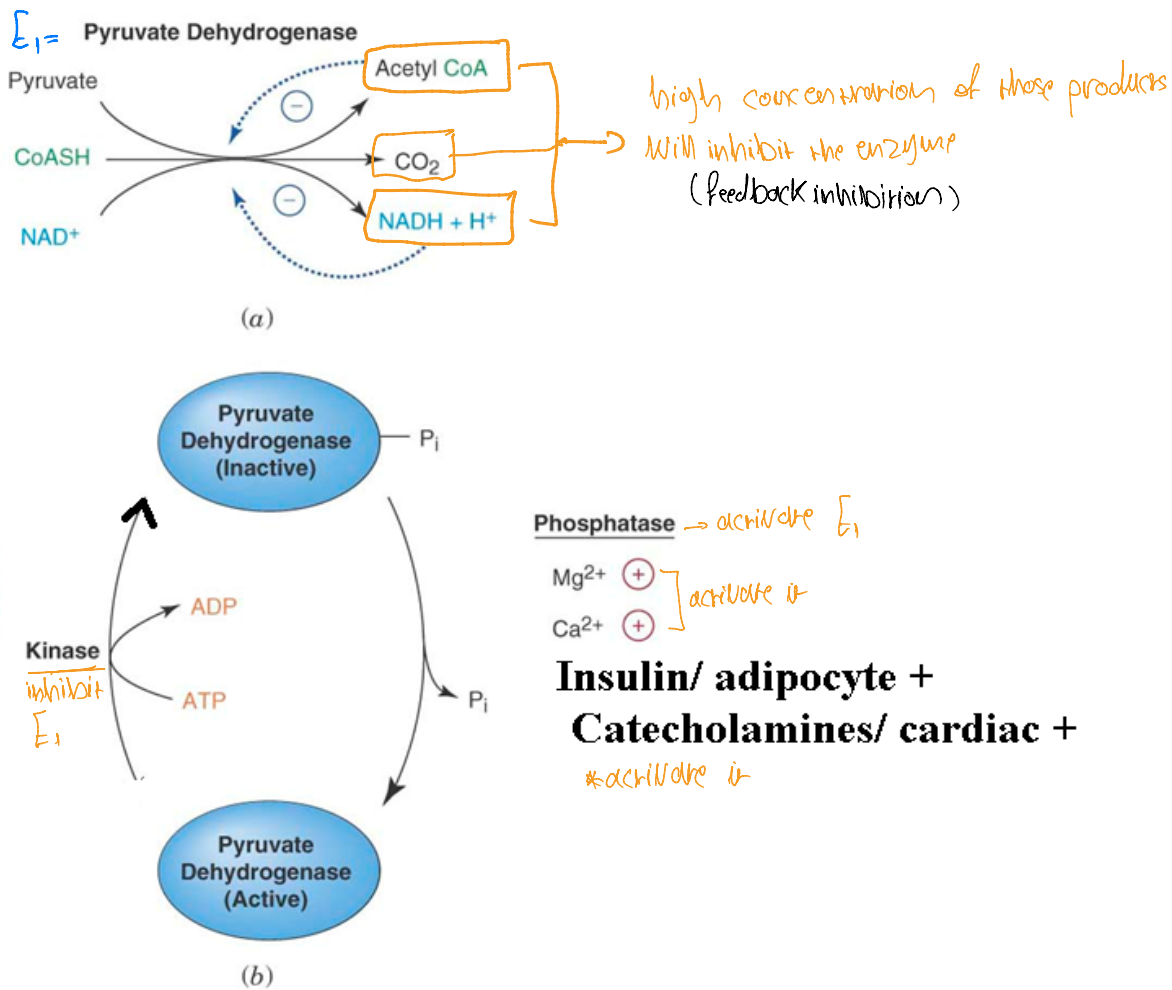
- Second the second Co-enzyme lipoeic acid will react with the TPP complex catalyzed by Dihydrolipoyl transacetylase transferring the acetyl group from TPP complex to lipoeic acid, regenarating the TPP to take another molecule of acetyl CoA.
- In the precense of the coA there will be a release of thioester bond and CoA will bind to acetyl producing the acetyl Co-A
- The reduced lip acid must be generation in the oxidized form to produce molecule like number 2 and this is done by Dihydrolipoil Dehydrogenase that requires FAD. FAD will be reduced and lip acid will be oxidized and as a result of reduce FAD $\longrightarrow$ $FADH_2$ will generated but this FAD must be generated as FAD to do this reaction So in the presence of NAD^+ and this enzyme, this $FADH_2$ is going to oxidized as FAD and the NAD will reduced as $NADH^+$.

pyruvate is the substrate, the products :- Acetyl CoA, NADH and CO_2

Control of Pyruvate Dehydrogenase;

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 mechanism how NADH inhibit the complex in feedback inhibition is differ than how NADH activate kinase in allosteric regulation.

- when you are fasting and you finish your storage glycogen, pyruvate dehydrogenase will inhibited, the pyruvate insted of converted to acetyl CoA, it will be used in gluconeogenisis.

** ال Acetyl CoA لا يمكن تتحول لغلوكوز ، لأنه ما لنا enzyme يحول acetyl CoA لـ Pyruvate

extra info:-

kinase will add phosphate to enzyme from the reaction of ATP hydrolysis ATP → ADP + P

phosphatase will remove phosphate in order to use it in ATP synthesis ADP + P → ATP

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.

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

TCA cycle is very important because it's a central pathway, it's oxidizing acetyl CoA that come from any where to produce energy for survival

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**. → It could be used for catabolism and oxidation and energy production and it could be used in biosynthesis
- The oxidation of acetyl unit results in the reduction of NAD & FAD to NADH+H and FADH2.

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.
- 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

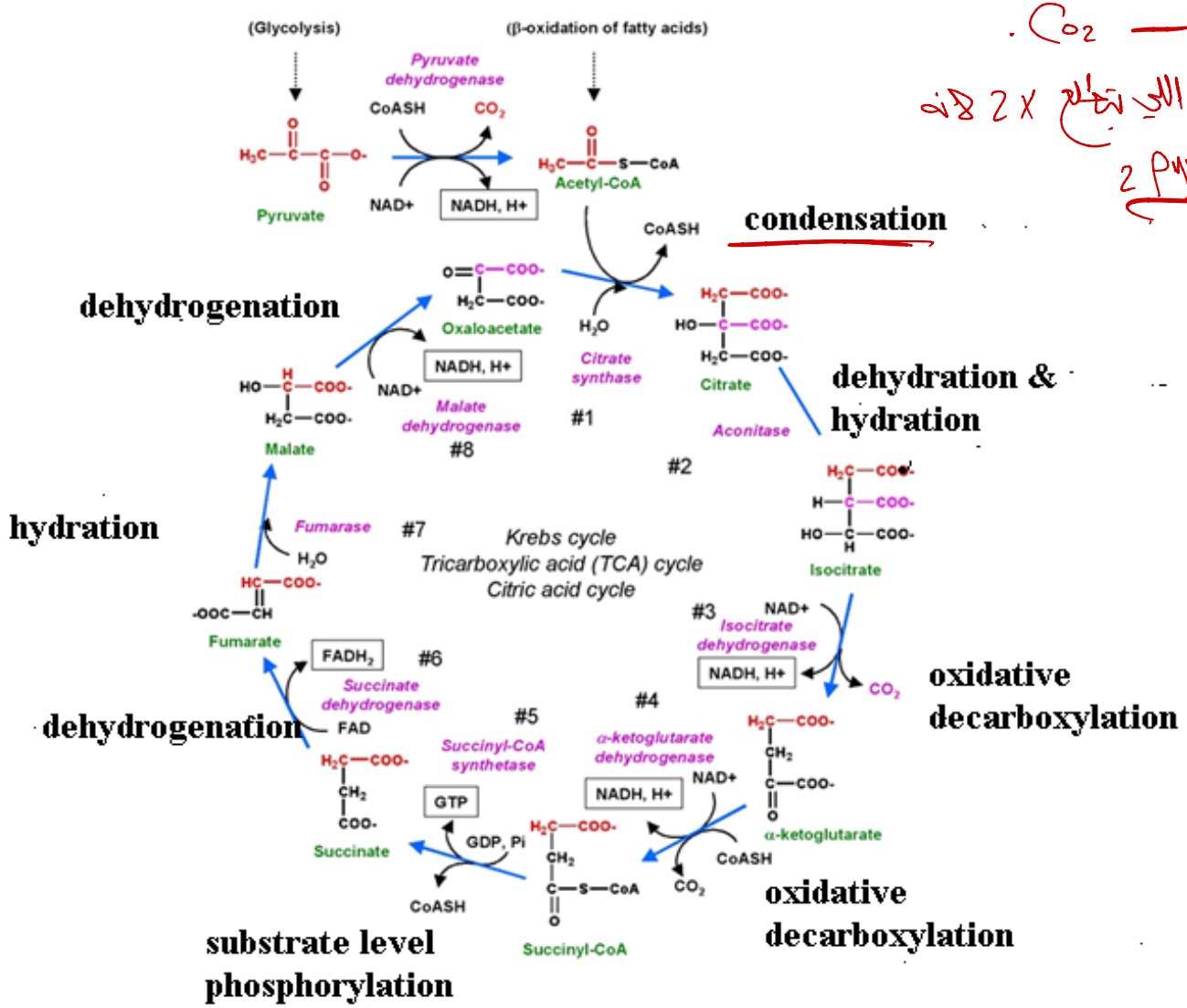
REACTIONS OF THE TCA CYCLE

1. CITRATE SYNTHASE: Candidate for regulation, Citrate synthesis is necessary for fatty acid synthesis, ΔG0`= -9kcal/mol

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

4. α-KETOGLUTARATE DEHYDROGENASE COMPLEX : ΔG0`= -8 kcal/mol 2nd molecule of CO2, and the 2nd NADH+H formation; TPP, lipoic acid, CoAsh, FAD, and NAD are involved. ATP,



شرح مبسط و مرتب ل TCA

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 .**

Nucleoside diphosphate kinase:

GTP + ADP□GDP+ ATP (1)

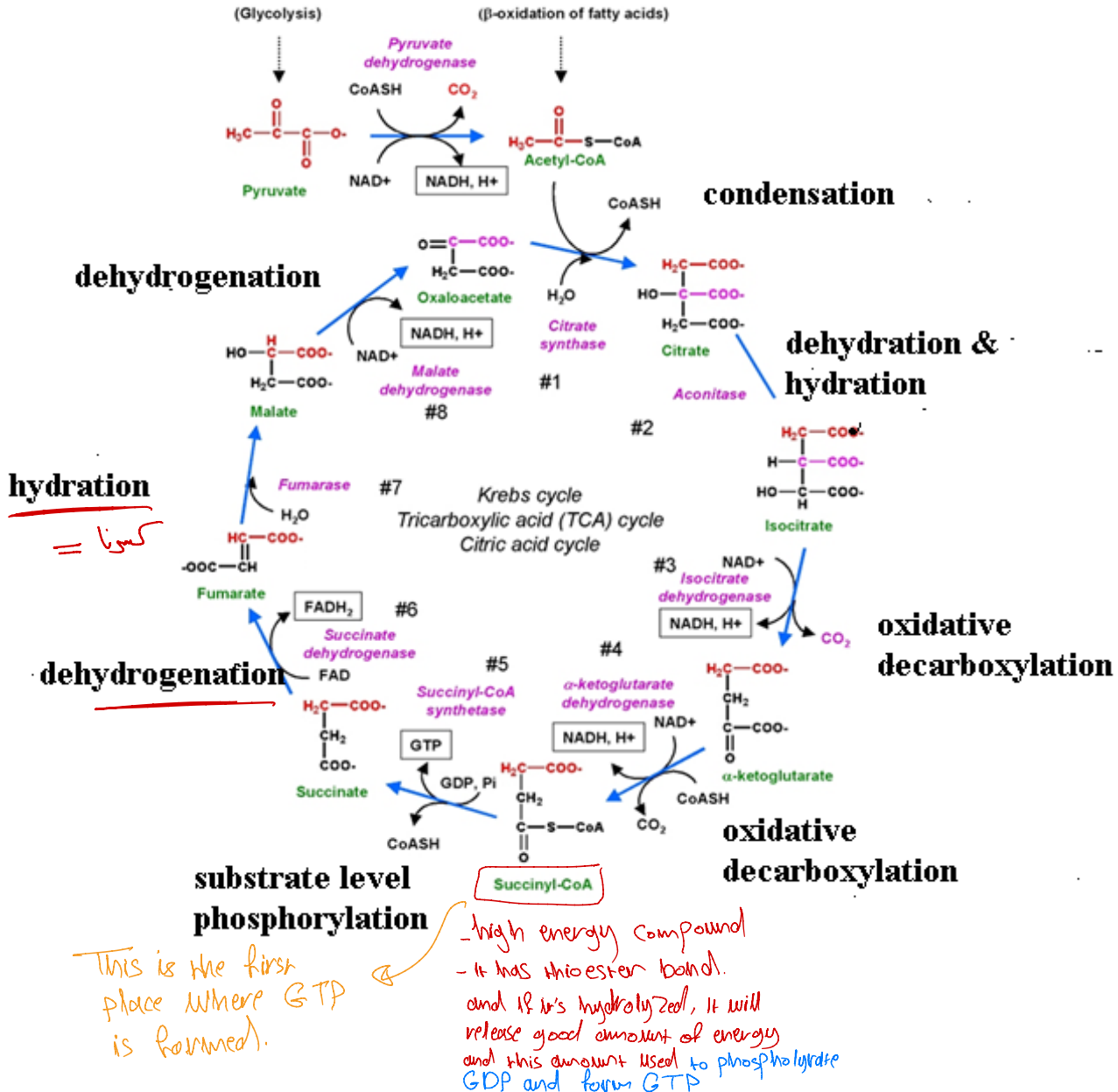
Adenylate kinase: AMP+ATP□2ADP…(2)

SUM: GTP+AMP□GDP+ADP

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

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.



REACTIONS OF THE TCA CYCLE

.

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.

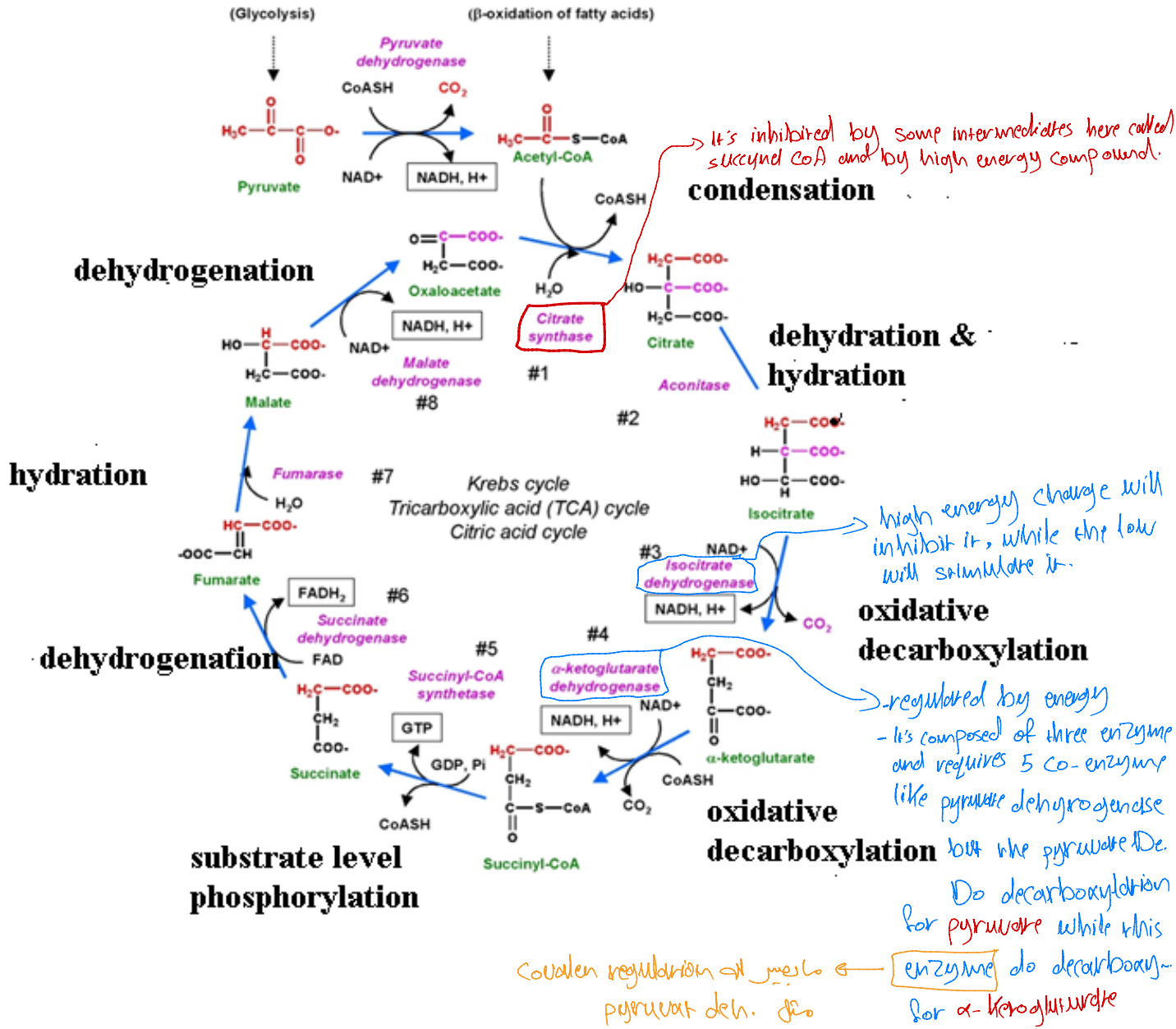
The equilibrium greatly favors the reverse reaction , the reduction of OAA. However, citrate synthesis is closely associated with the dehydrogenase and removal of OAA assists in pulling the malate dehydrogenase reaction towards the formation of OAA. OAA can be reversibly transaminated to aspartate

SUM:

AcetylCoA+3NAD+FAD+GDP+Pi+2H2O□

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

*citric acid is a tricarboxilic acid, and because of this the cycle called tricarboxilic acid cycle (TCA)



It's inhibited by some intermediates here called succynel CoA and by high energy compound.

high energy charge will inhibit it, while the low will stimulate it.

- regulated by energy
- It's composed of three enzyme and requires 5 co-enzyme like pyruvate dehydrogenase but the pyruvate De. Do decarboxylation for pyruvate while this enzyme do decarboxy- for α-ketoglutarate

covalen regulation of pyruvat deh. etc

What happen in TCA ?

- Acetyl CoA will be condensed with oxaloacetic acid which generated in TCA as catalytic form "very small amount will be generated" through a series of enzymatic reaction, such as (citrate synthase) in TCA. This condensation between Acetyl CoA and the few amount of oxaloacetate "as a catalytic" convert to citrate (the first compound in TCA)
  which have $-\Delta G$

- After that the structure will be rearrange by Dehydration and hydration removal water molecule then adding water molecule in order to change the structure (when you are dehydrating you are making double bond, and when you hydrating you are adding double bond off and H) the cell will change the place of hydroxyl group from C_2 to C_3. (Aconitase)

- The next step is oxidation, decarboxylation of Isocitrate. what happening here is the decarboxylation of one of the carboxilic group of TCA cycle which is not one carbon of the acetyl CoA that enter oxidation, And the other thing is production of NADH because it's an oxidation reduction reaction this is the first site in which the NADH produced and CO_2 released

* The C atom that released as CO_2 is one of citric acid carboxylic group ← ٤ و ٥ من الاكسجين الزايدة.

- The next step is to convert α-ketoglutarate to succinyl CoA by α-ketoglutarate dehydrogenase, the step here oxidation and also decarboxylation And it's the second site of releasing carbon atom.

And because it's oxidation reduction reaction NADH will be form "the second site"

- succenyl CoA will be converted to succinate by succenyl CoA synthase (here the first GTP).
- succenate will be oxidized by another enzyme called succenate dehydrogenase And this enzyme use FAD, as a result $FADH_2$ will be produced (the 1st site).

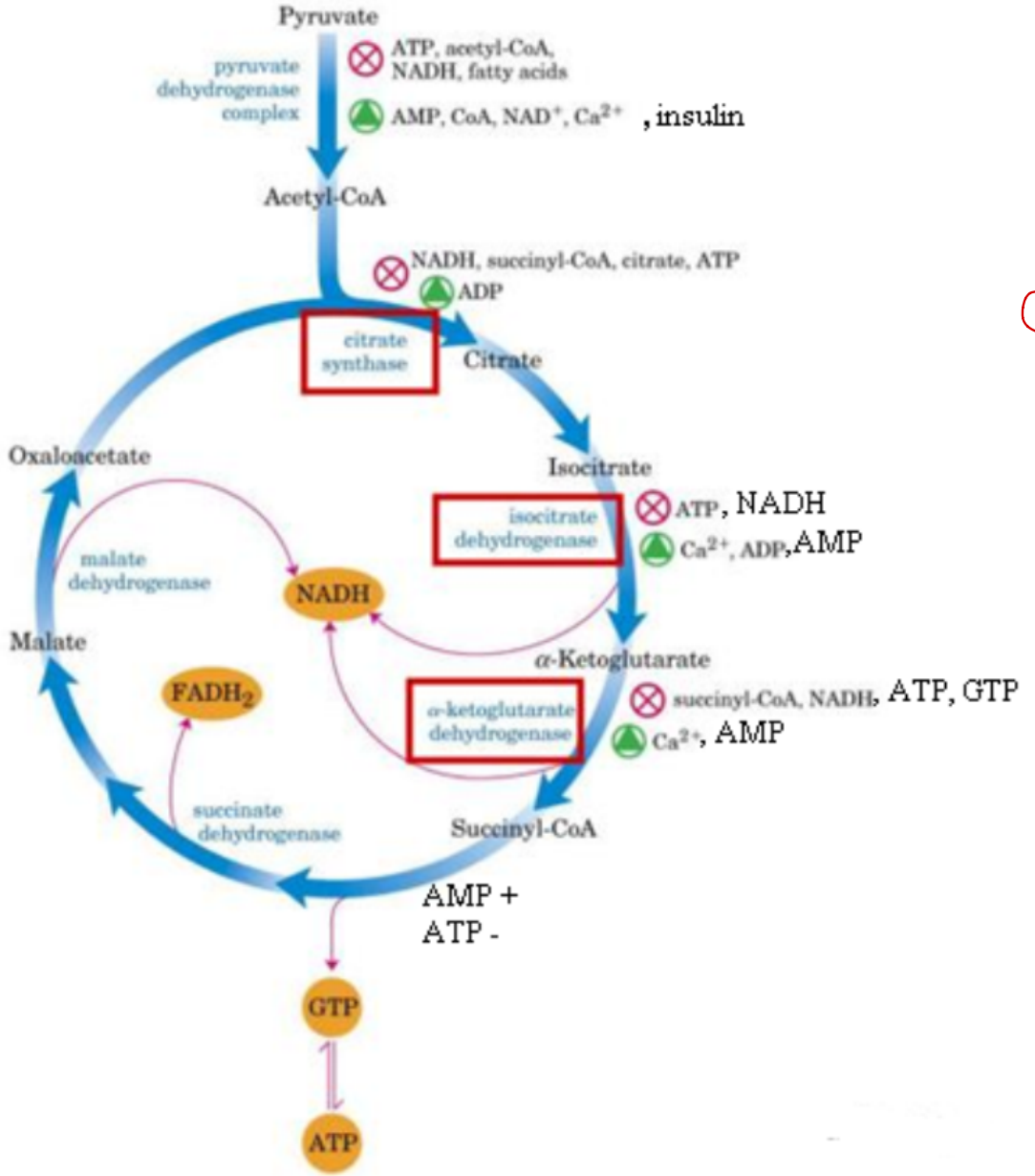
* All enzyme exite in the Matrix of mitochendrion except this enzyme which is in the innen mitochendrial membrane.

- Fumarate will be converted to Malate by fumarase by adding water molecule
- Malate converted to oxaloacerate by fourth redox reaction and using NAD to generate NADH by malate dehydrogenase enzyme.

- the last story we going to talk about it converted Malate to oxaloacerate if you look at the ΔG it's positive (not favorable reaction) but the cell need this reaction to take place so it's synthesysed in very low amount catalitecally and the next reaction will pull the whole reaction forward.

* The NADH and $FADH_2$ considered as sources of energy.

Regulation of Citric Acid Cycle



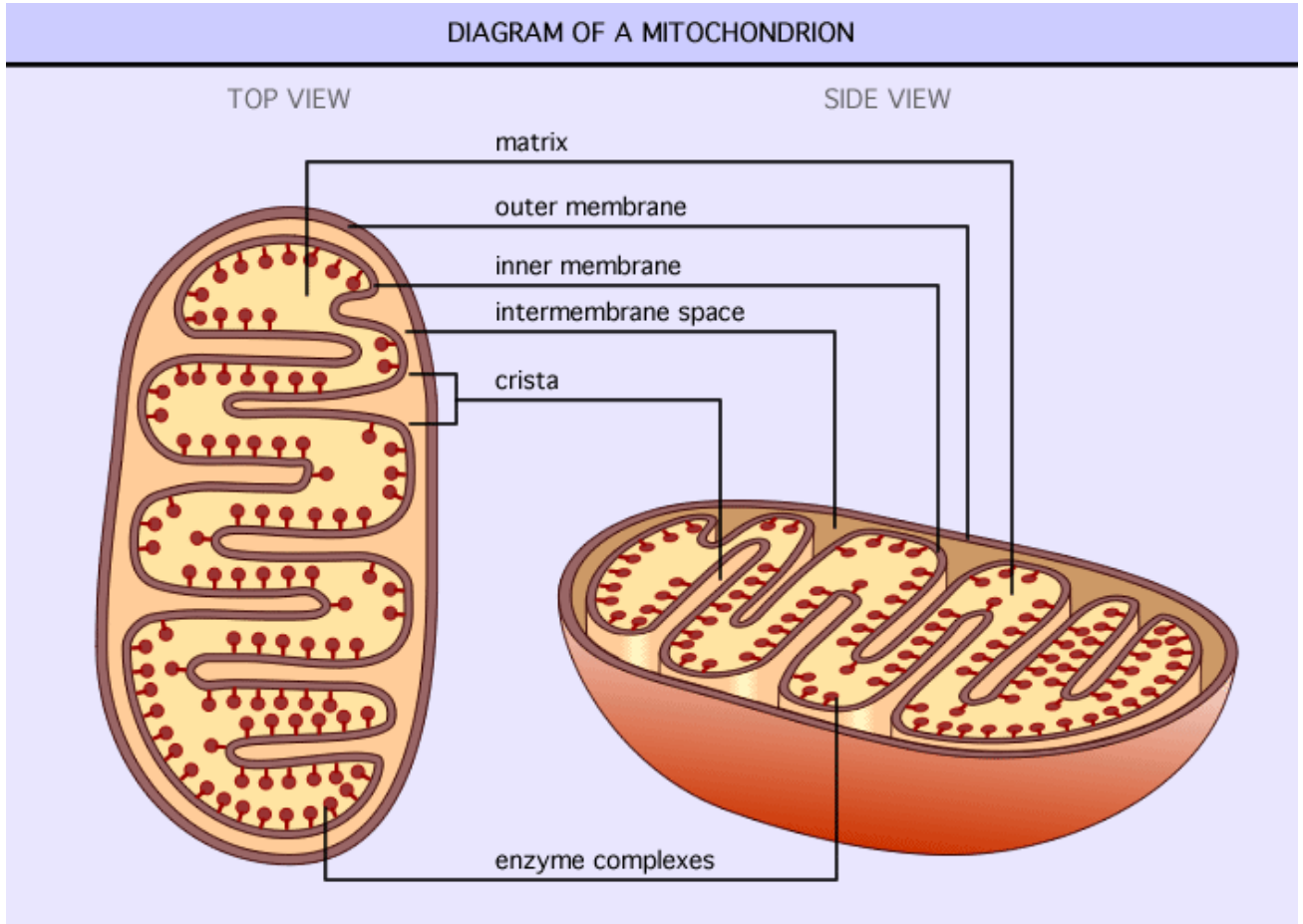
The citric acid cycle is regulated at its three exergonic steps

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.
- 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.
- 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.

- the purpose of understanding the electron transport chain is to see how those reducing power produced from TCA or any metabolic pathway are oxidized by a system in our cells called electron transport chain and oxidative phosphorylation to produce ATP

- 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
- The inner membrane space has many folds directed towards the mitochondrial matrix.



this table to tell us that each part of mitochondria have specific enzyme to do specific function

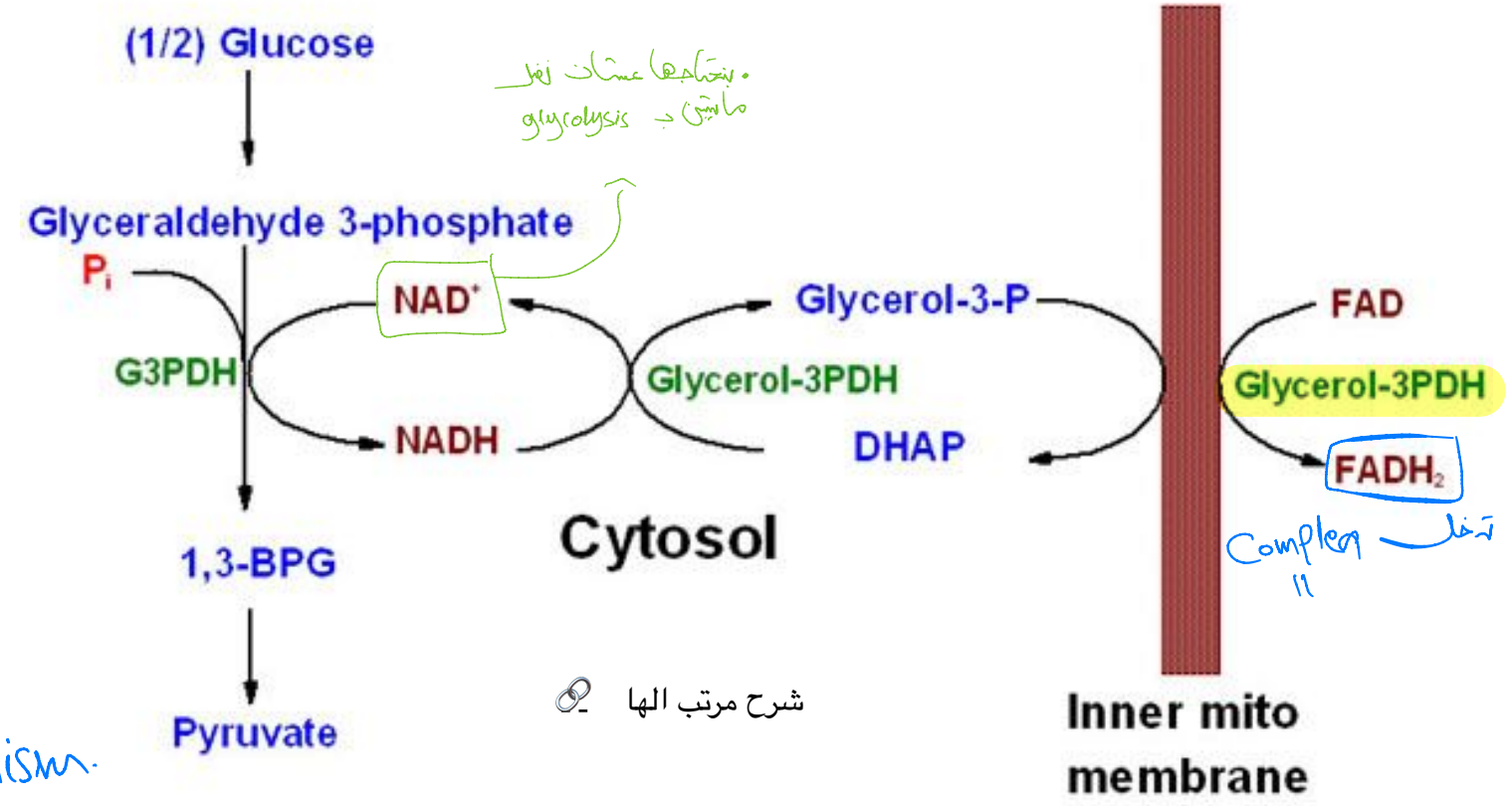
Location of the various mitochondrial enzymes in mitochondrial compartments.

Outer membrane	Intermembrane space	Inner membrane	Matrix
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-

shuttle ⇒ is vehicle that transport something from one place to other place and this exactly what happen inside our body → There are shuttle but not bus like structure There are a pathways that help to transport protein from one place to another.

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



- We should need a shuttle mechanism to transfer the NADH from the cytoplasm to inner mitochondrial membrane

- NADH if you remember is produced in one of the glycolysis reactions in which glyceraldehyde 3 phosphate dehydrogenase oxidize the glyceraldehyde 3 phosphate to 1-3 bisphosphoglycerate, it requires NAD and inorganic phosphate and produce NADH.

- NADH must be transferred to the electron transport chain for oxidation in order to do that there is an enzyme which called glycerole 3 phosphate dehydrogenase located in cytoplasm it will reduced DHAP in the precense of NADH to give glycerol 3-phosphate.

- In the inner mitochondrial membrane there is an enzyme like glycerol 3-P Dehydrogenase (one of them cytoplasmic and the other mitochondrial) and it will regenerate DHAP from glycerol 3 phosphate and requires FAD so $FADH_2$ will generated.

وتستمر العملية

- The purposes of this process is two things :-

1) Tranfer NADH from cytoplasm to mitochondria.

2) To regenerate NAD^+ in order to continue Glycolaric reaction. (glycolysis)

- يعني بنصنع NAD^+ مشان عملية glycolysis تكمل وبنقل الالكترونات via this shuttle على NADH form أو $FADH_2$ form

Malate-Aspartate Shuttle

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

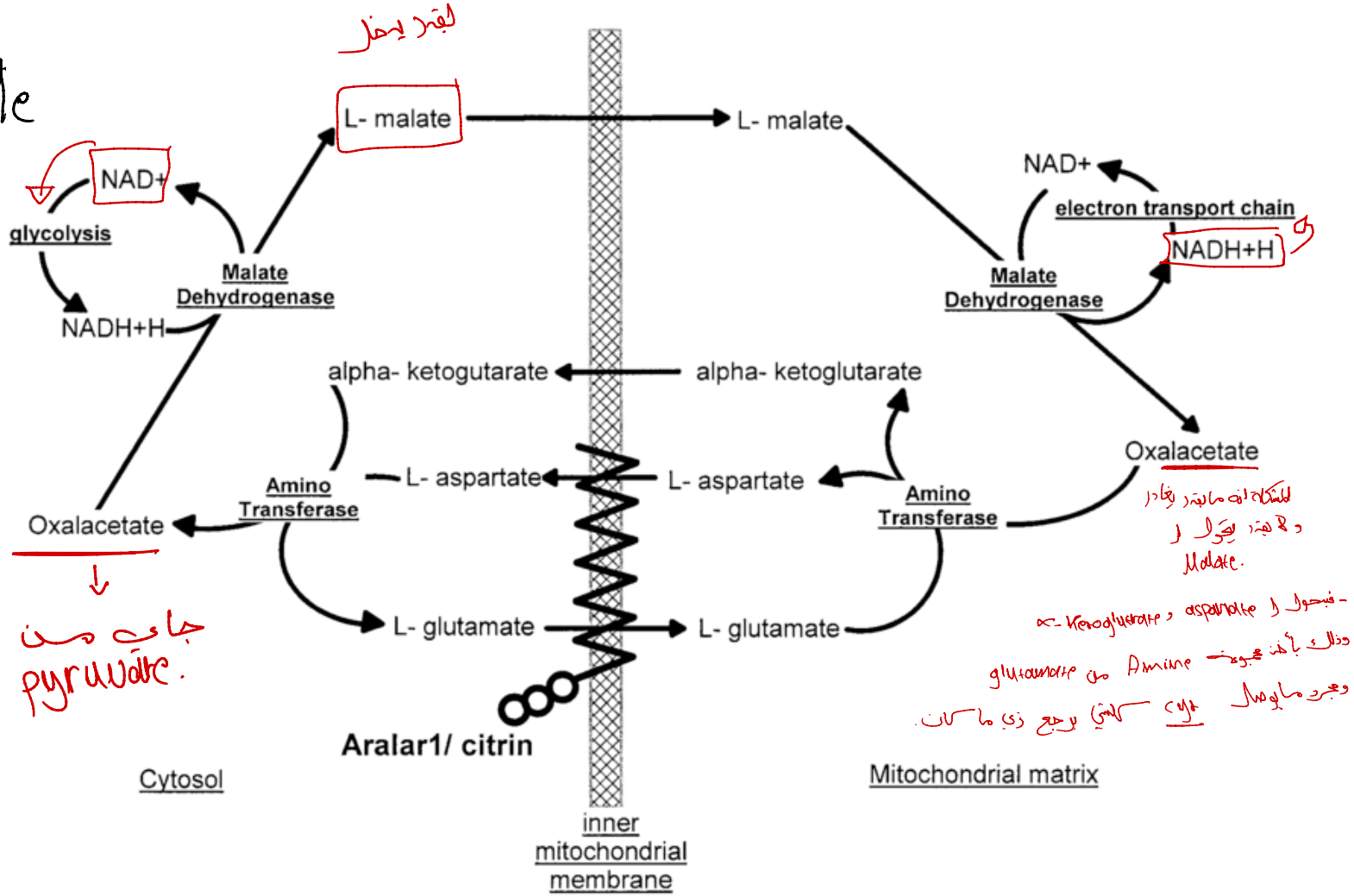
Malate is transported to mitochondria where NAD is reduced to NADH+H+ and OAA is regenerated.

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



شوفوا الشرح

*Now the cell is ready to oxidize NADH and $FADH_2$.

Carriers of Electron Transport Chain

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

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

- Those carriers will accept electrons from $FADH_2$ or NADH and donate in to next carrier, so we have redox reactions.

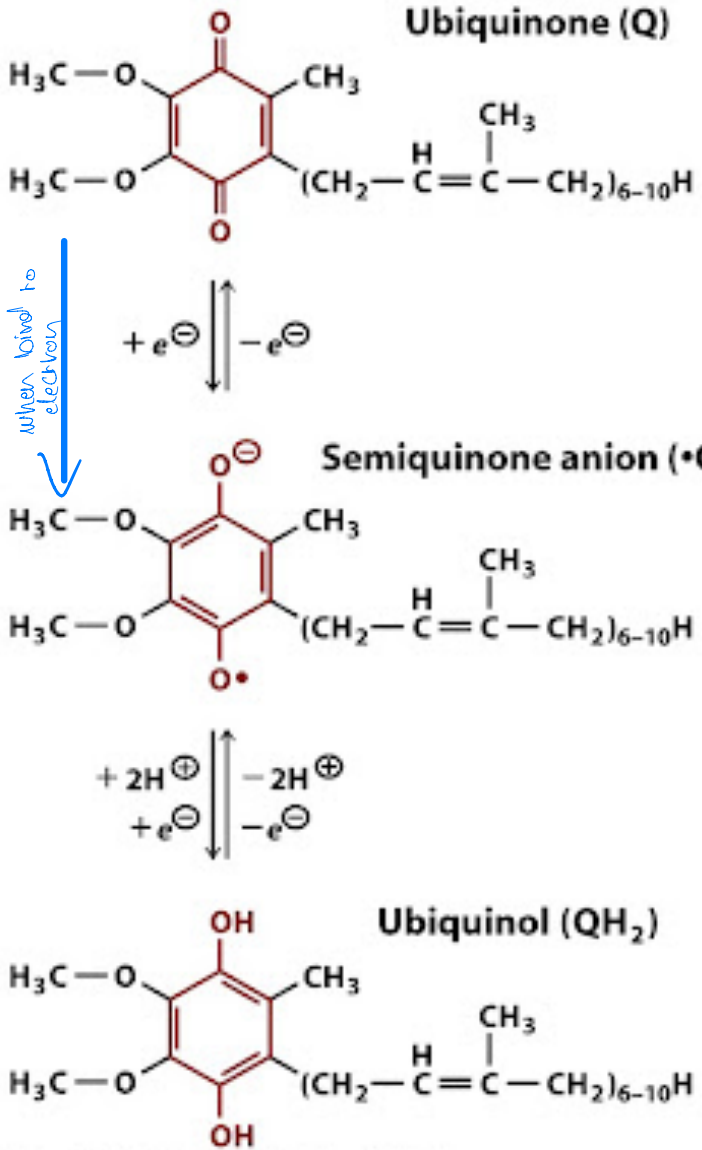
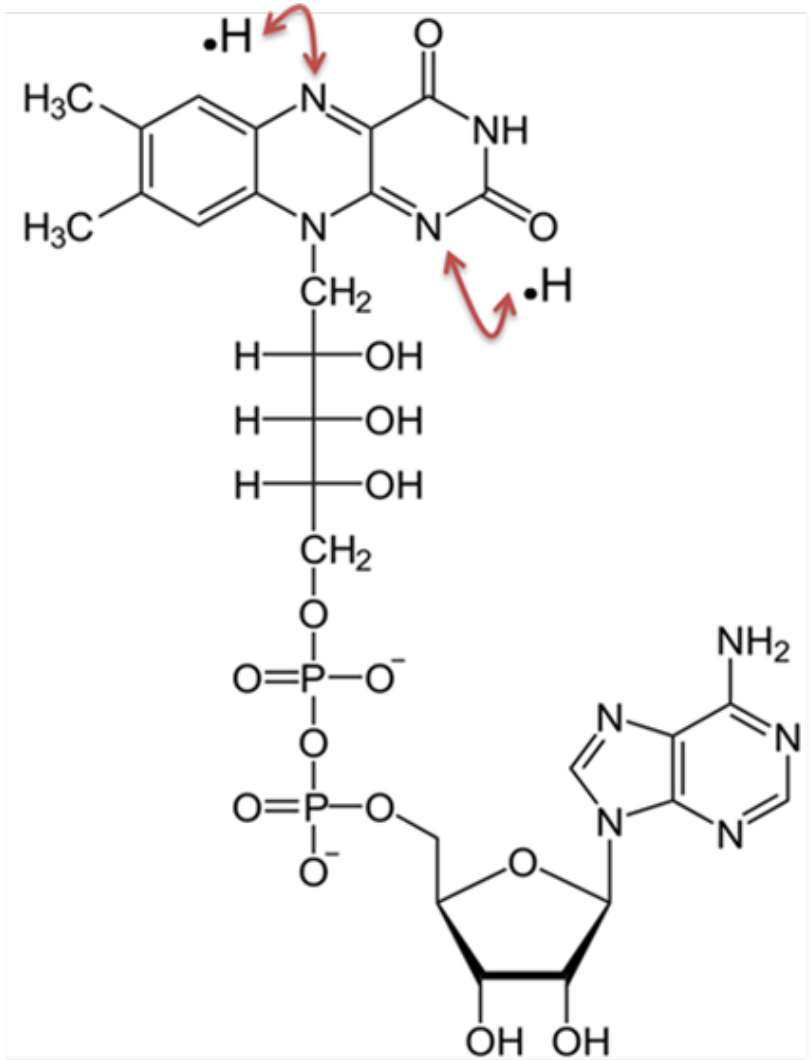
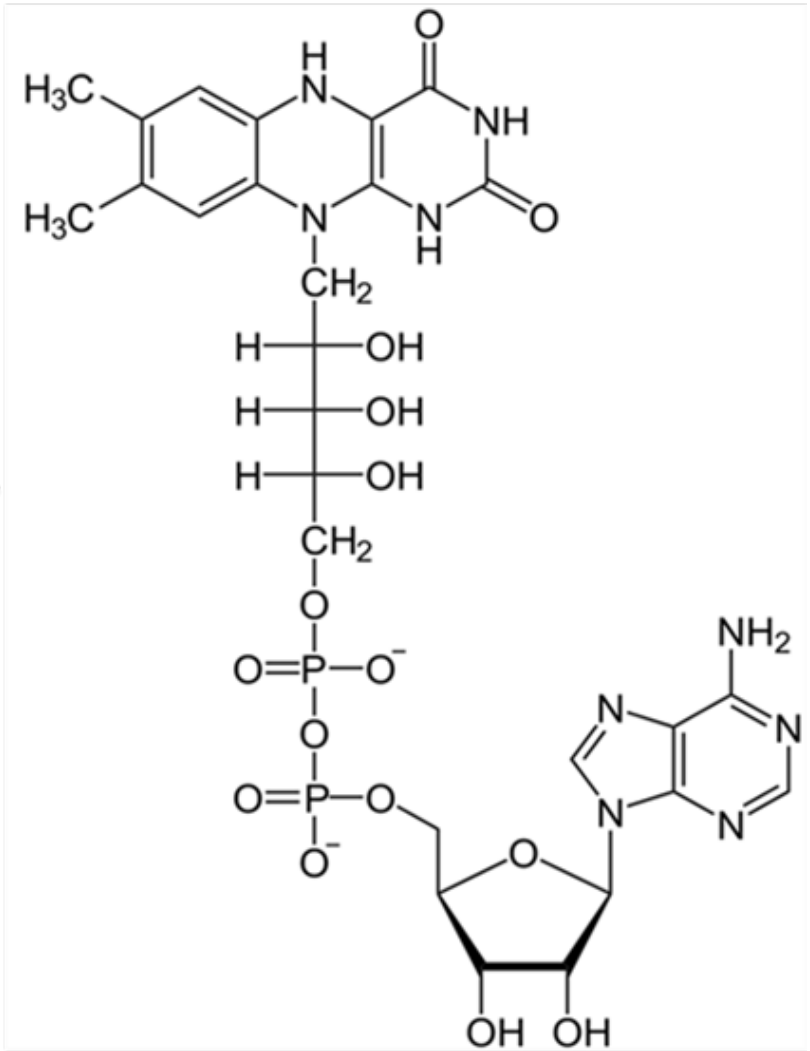


Figure 7-31 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.

- the electrons will flow from the smaller to larger redox potential.
- oxygen have the highest positive redox potential high affinity for electron (reduced)

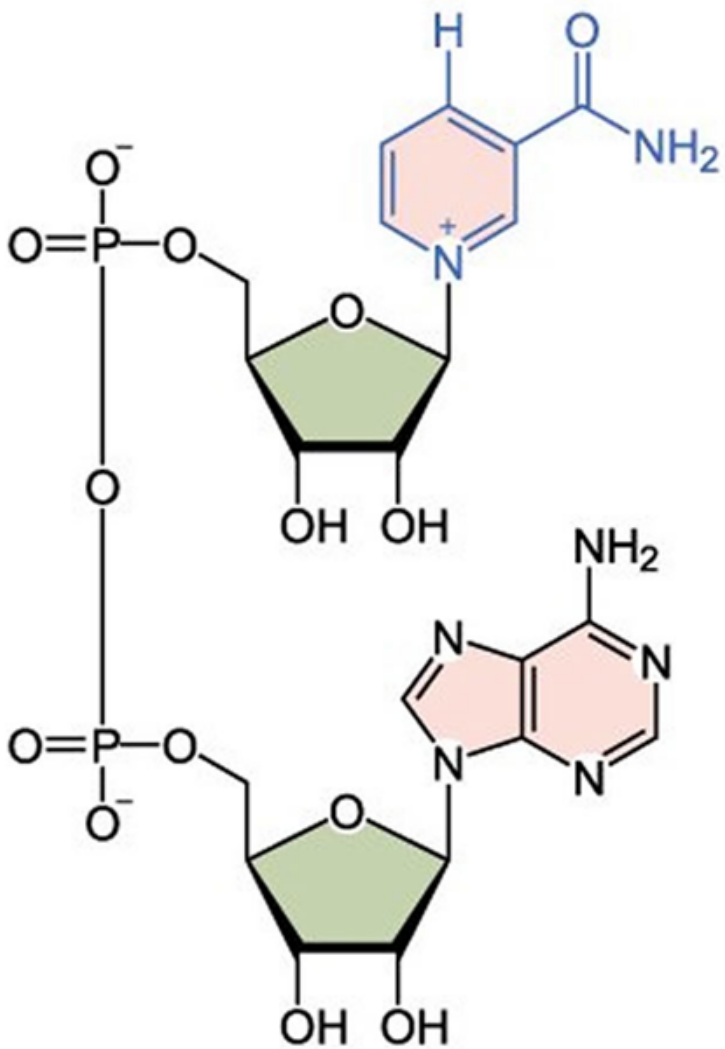
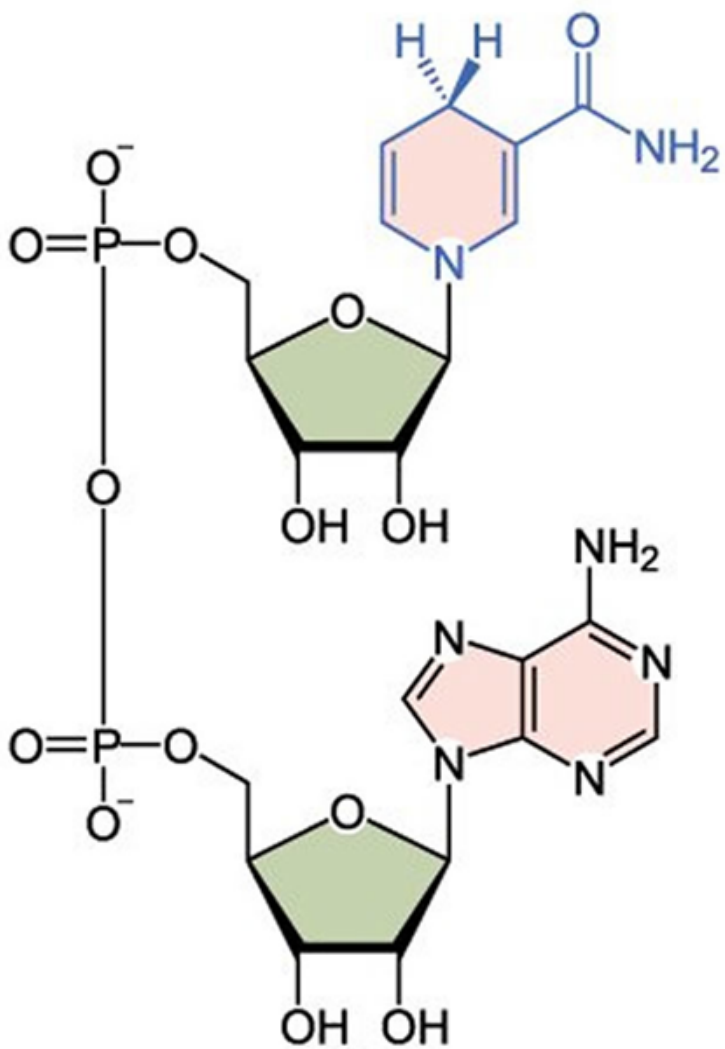
- each complex in electron transport chain have one or more prosthetic groups helping in accept and donate electrons.





FAD it's covalently bonded to flavo protein and when it's reduced it will carry 2 $\bar{e}$ and 2H.

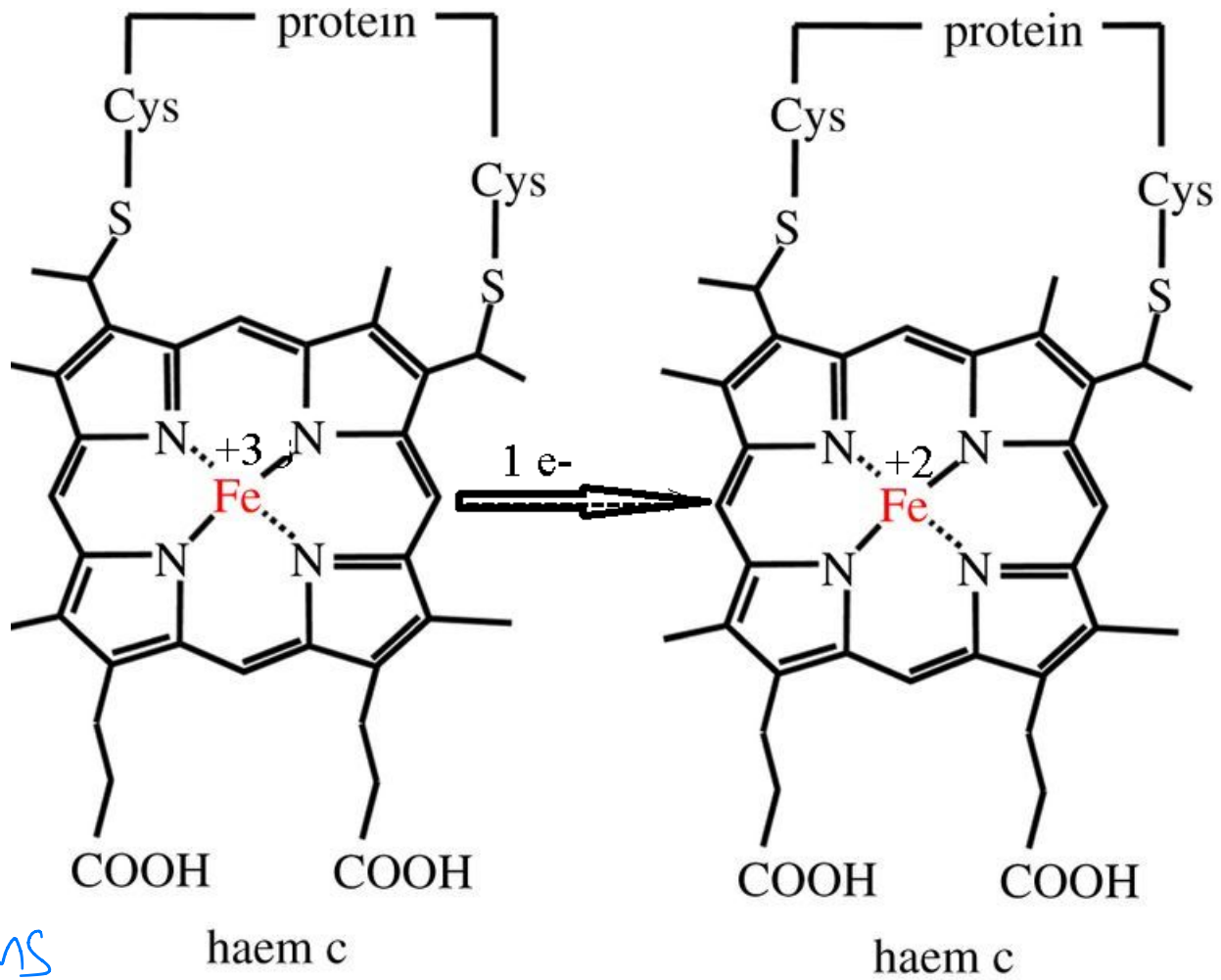
$FADH_2$ attached to complex 2 (Succenate dehydrogenase)

NAD^+	NADH
	

* This heme protein is prosthetic group in the some of the ETC.

Cytochromes (heme proteins)

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.



haem c haem c

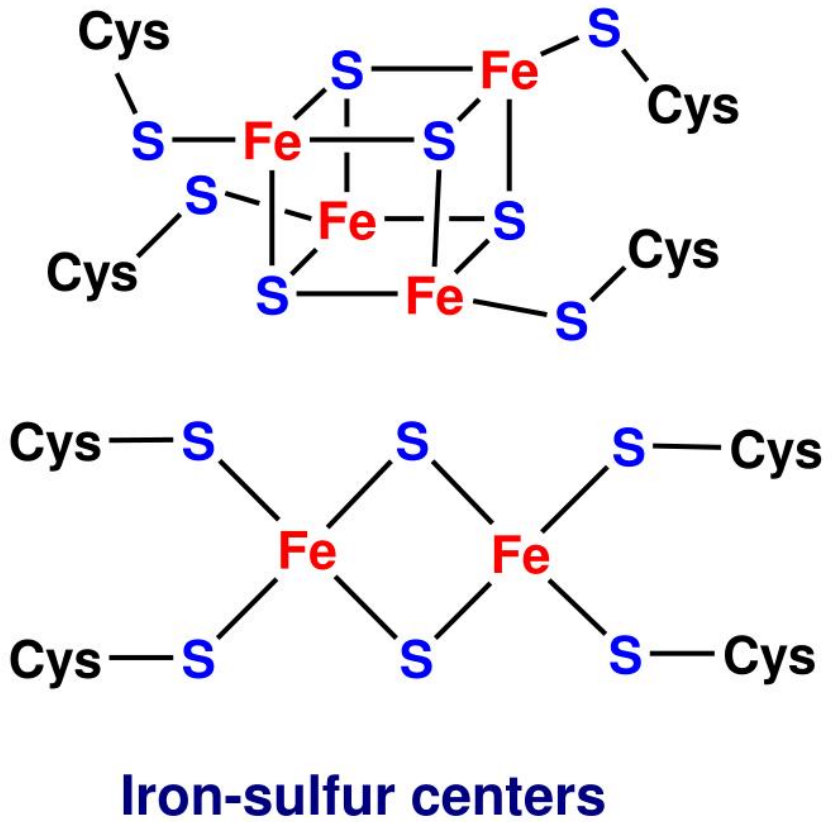
- There are different forms of cytochromes and the differences are two things :-
1. the arrangement of those conjegated bond on the pyrol rings
2. the type of side chain attached to them.

cytochromes found in complex 3 and 4

Iron Sulfur Centers

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.



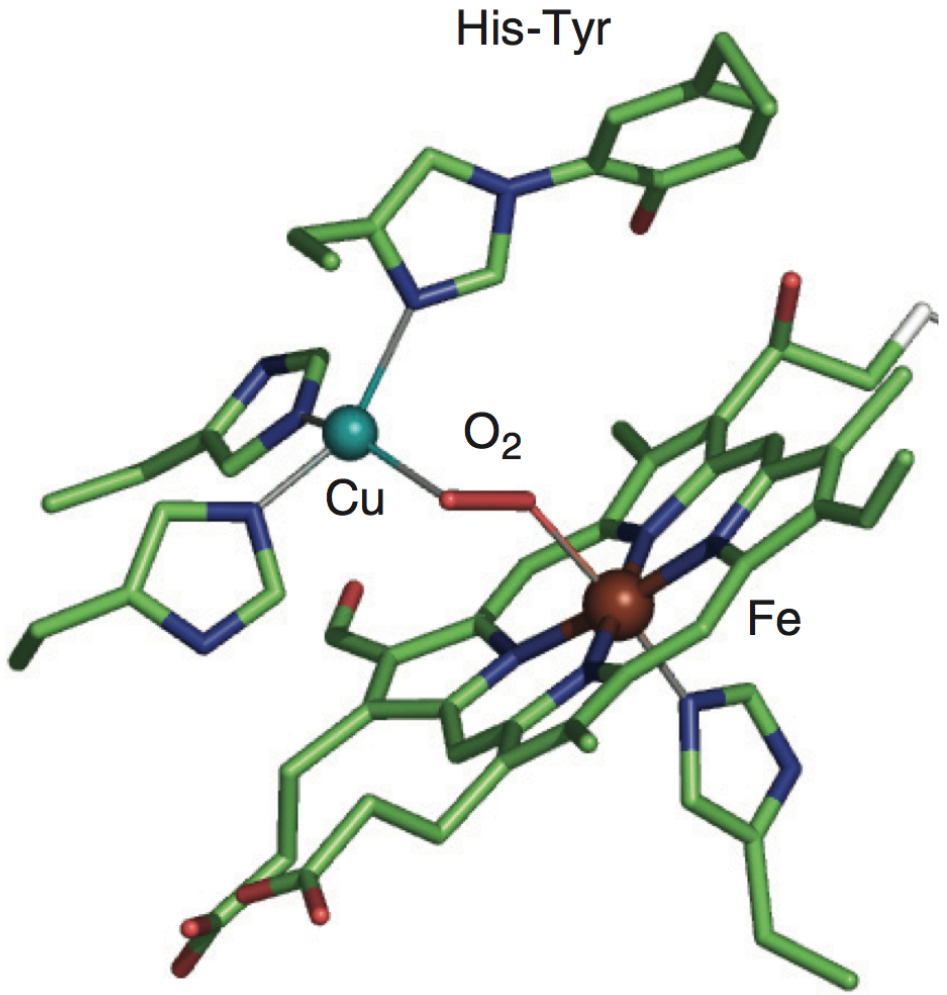
Iron-sulfur centers

Copper Containing Proteins

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

1 e-

Cu2+----------□Cu1+



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+ NADH + H+ E0'=-0.32 volts.
- The energy of the transferred electrons under standard conditions is expressed as ΔE0'
- 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.

Requirements of OxPhos

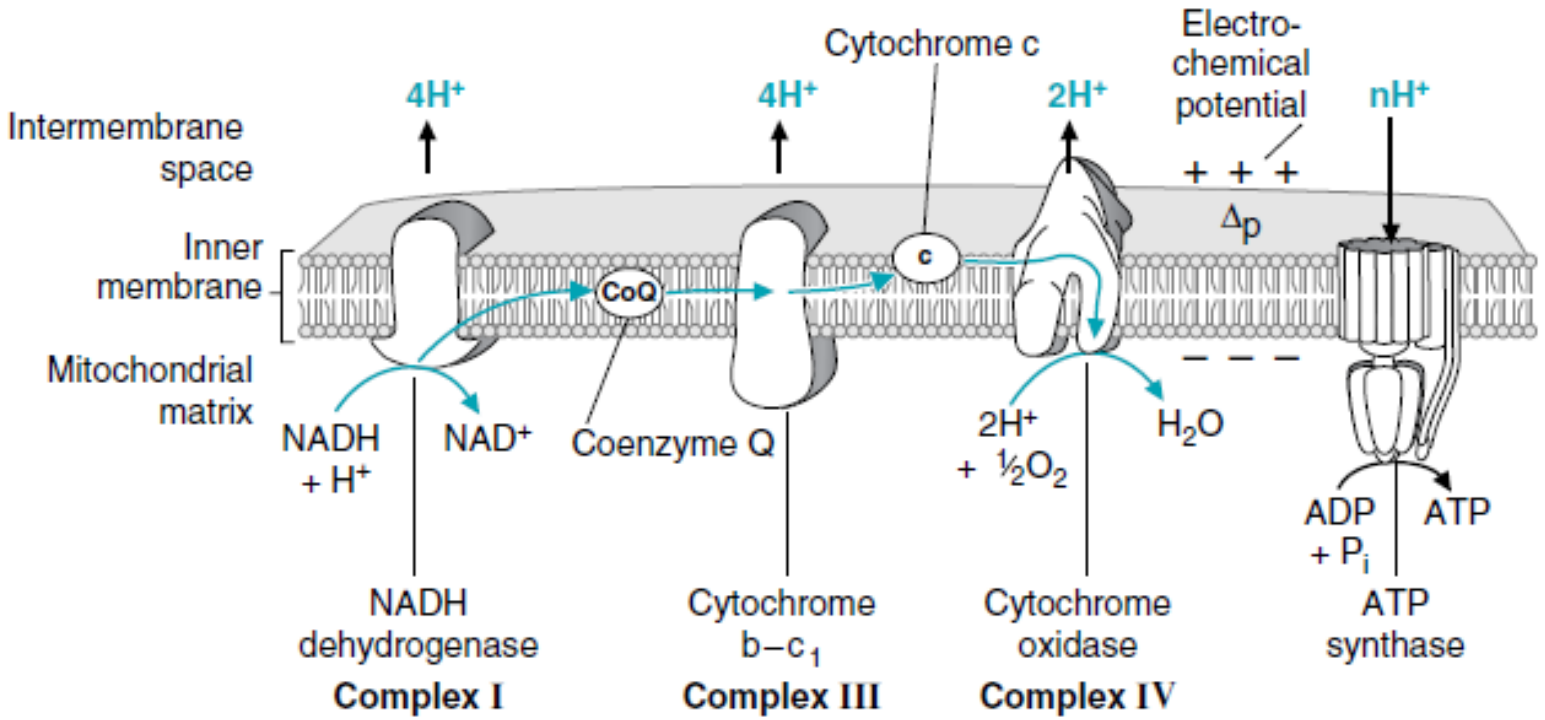
Redox reaction: electron donor (NADH or FADH2) & electron acceptor (O2)

An intact IMM

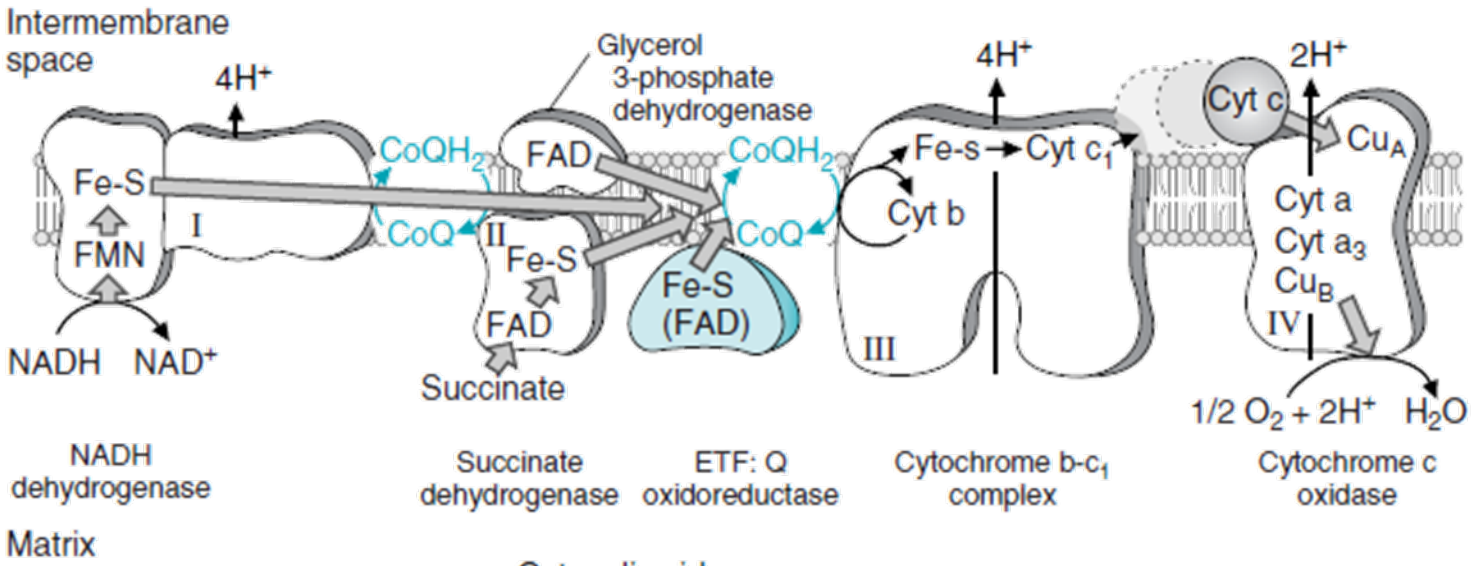
ETC of proteins: 4 complexes+1 soluble protein +CoQ

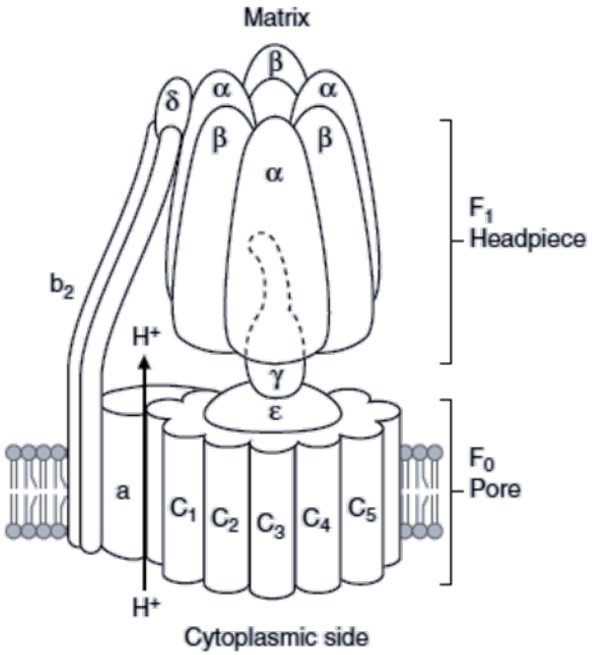
ATP synthase

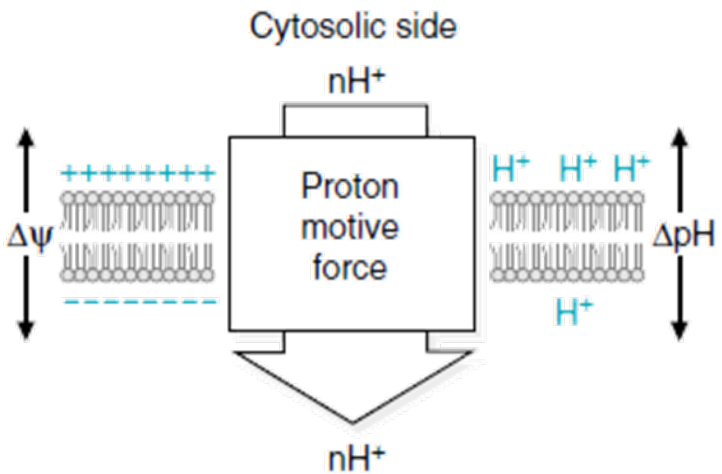
- electrons will flow regarding to redox potential



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







- During their passage of electrons in some of these complexes ⇒ electrical current will be produced and so the complexes will pump protons which will result in accumulation of H^+ outside the matrix

ANATOMY OF THE RESPIRATORY CHAIN

1. Complex I: NADH Reductase.

Dehydrogenase

NADH + e-> CoQ

2. Complex II: Succinate-CoQ Reductase,

Succinate+ e □CoQ.

3. Complex III, Cytochrome C Reductase,

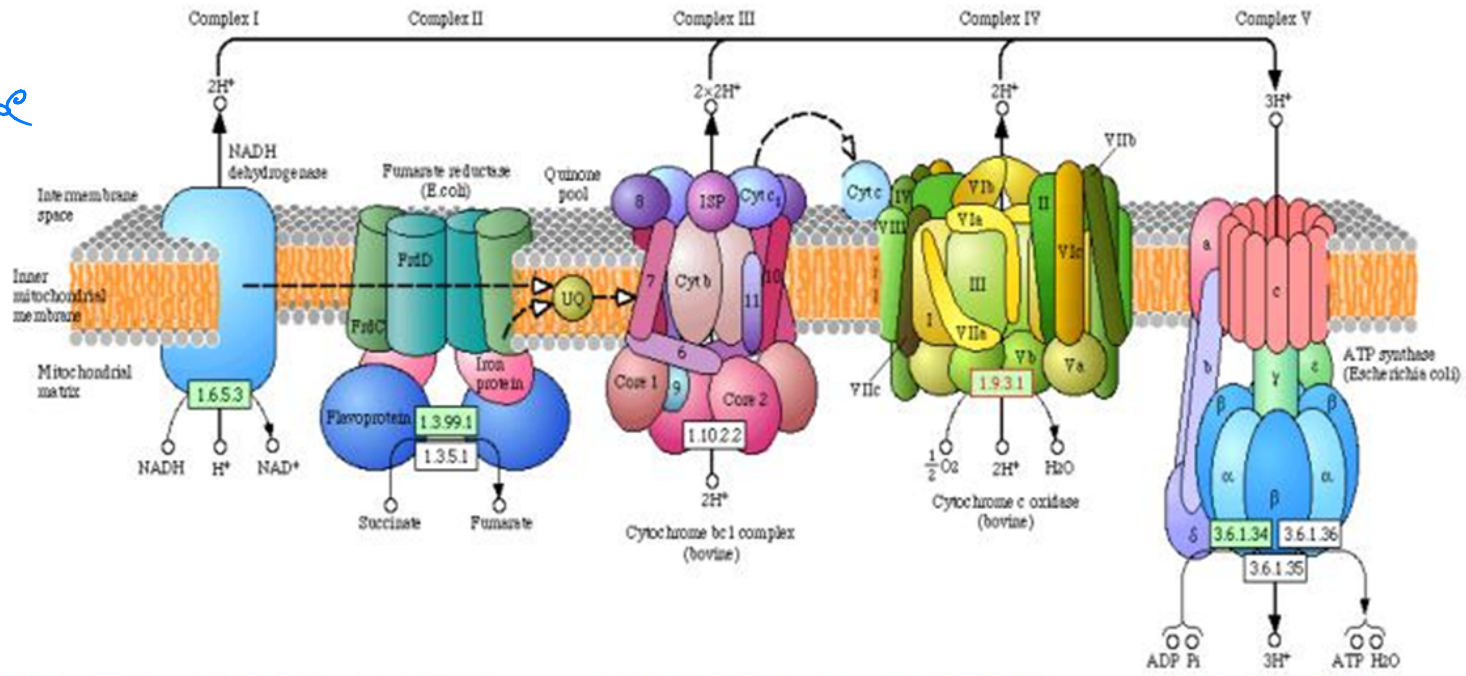
CoQ +e □Cyt c

4. Complex IV, Cyt Oxidase,

Cyt c+e □ Oxygen

5. Complex V: ATPase.

Respiratory chain subunits encoded by two genomes: Nuclear and Mitochondria



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

- co-enzyme Q could swim and flow in this hydrophobic region in order to bind to proper molecule or dehydrogenase

Oxi–Red Components of the ETC

"NADH Dehydrogenase" OR oxidase – Complex I

NADH-Q oxidoreductase

More than 25 polypeptide chain

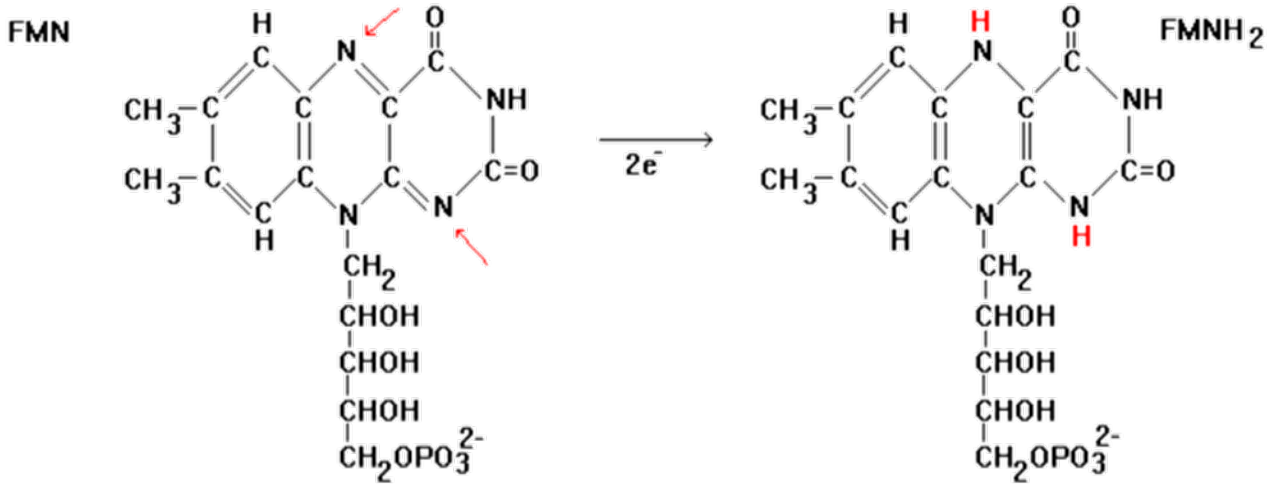
A huge flavoprotein membrane-spanning complex

The FMN is tightly bound

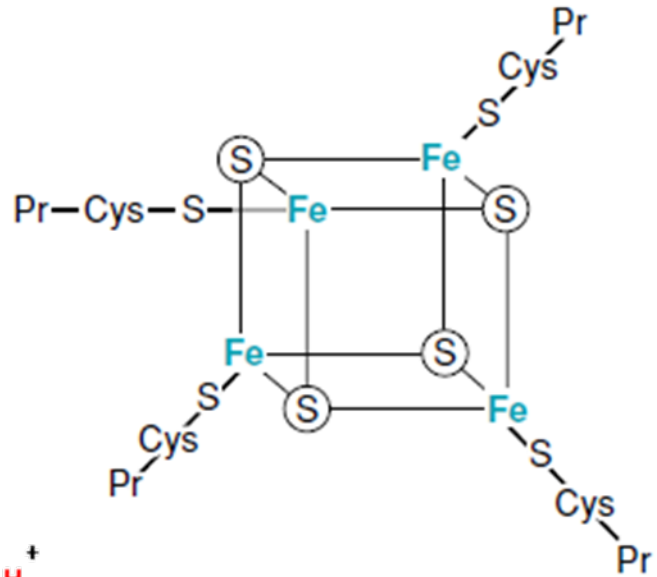
Seven Fe-S centers of at least two different types

Binds NADH & CoQ

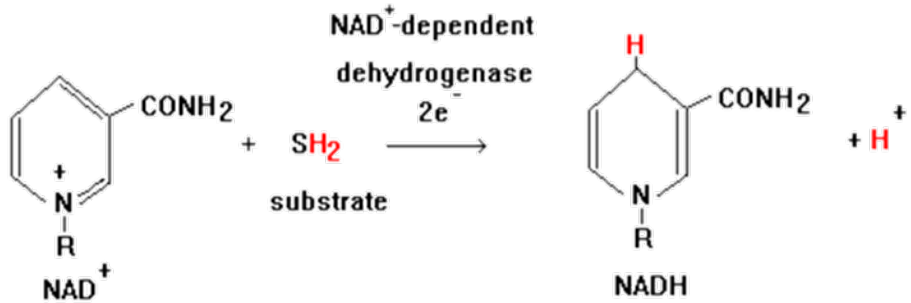
4 H+



- its substrate is NADH (coming from different sources) Donating its electron and FMN will take them. and the iron sulfur center will donate them to co-enzyme Q bind to complex I ← Gets. become reduced.

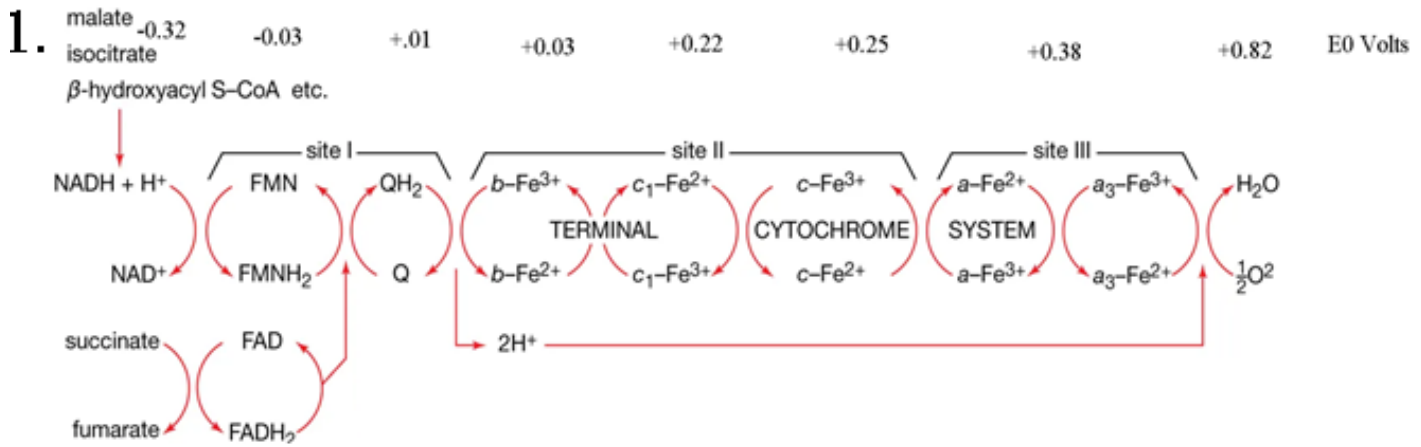


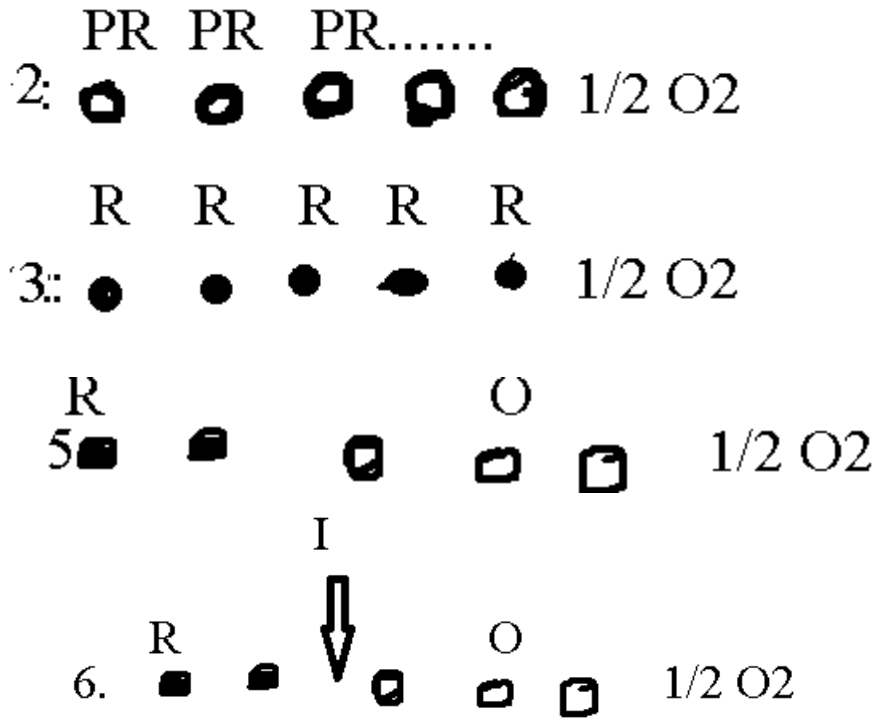
- and when is reduced it will be dissociated from complex I to go to the next dissociation which is complex 3



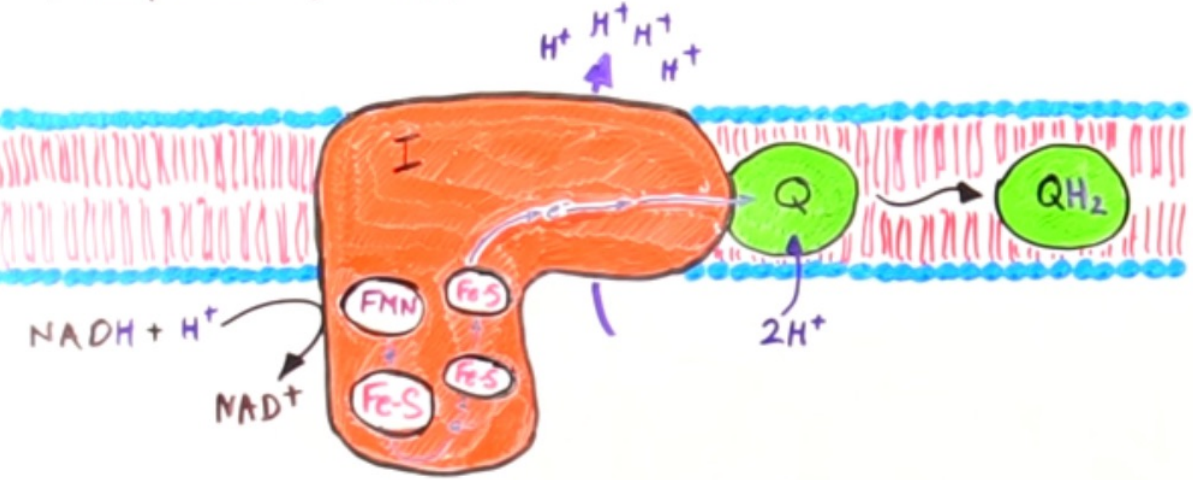
Sequence of carriers in ETC

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.

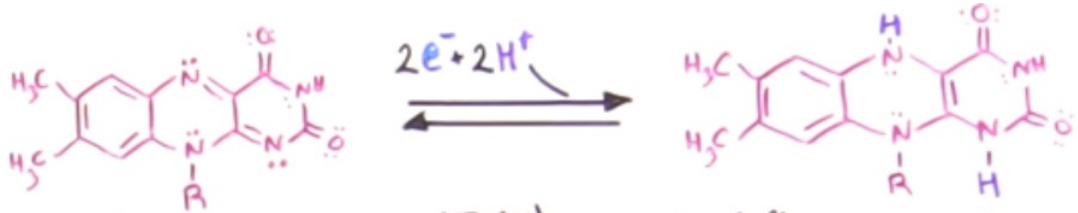




- **Complex I**, also known as NADH oxidoreductase or NADH dehydrogenase:
  1) is a large, L-shaped multisubunit protein complex located on the inner membrane of the mitochondrion.
  2) accepts the high-energy electrons from NADH molecules.

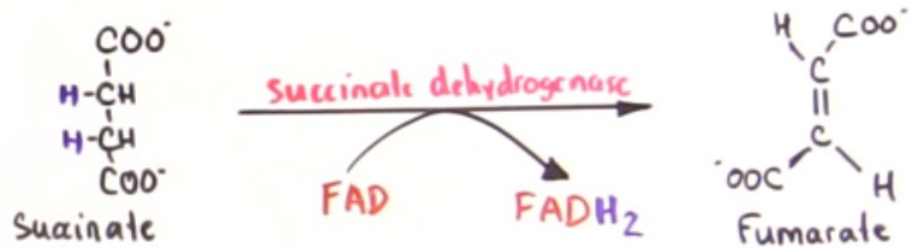


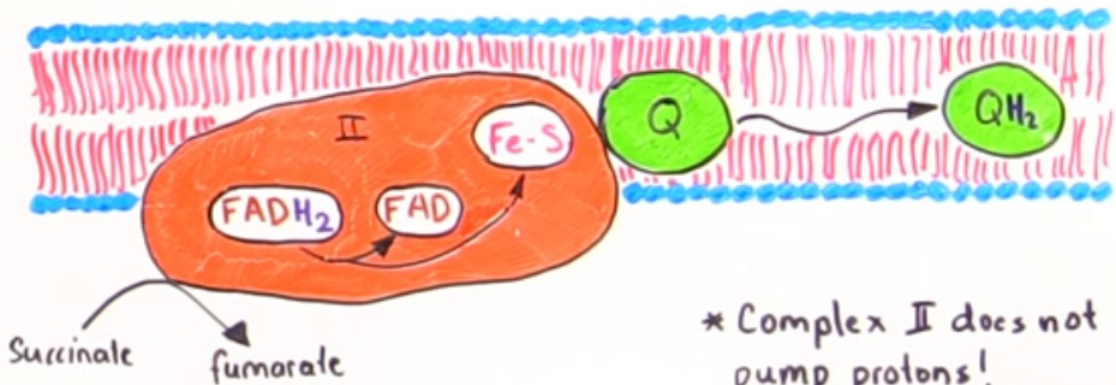
- The NADH molecule donates the two electrons onto an acceptor group found on the vertical component of complex I called flavin mononucleotide (FMN). The FMN is reduced into the $FMNH_2$ form. This prosthetic group contains the same isoalloxazine ring that is found on FAD.
- The electrons then move along a series of iron-sulfur groups and are ultimately transferred to coenzyme Q (ubiquinone). The ubiquinone also uptakes two protons from the matrix, thereby transforming into the fully reduced ubiquinol form (QH_2).
- As the electrons move through the series of Fe-S clusters, the complex uses this electrical work to pump 4 H^+ ions out of the matrix and into the intermembrane space.



oxidized flavin mononucleotide (FMN) — reduced flavin mononucleotide ($FMNH_2$)

- **Complex II**, also known as succinate reductase:
  1) is a protein complex that contains succinate dehydrogenase, which functions in the citric acid cycle.
  2) converts succinate into fumarate and generates the $FADH_2$. The $FADH_2$ remains attached to the complex and gives off the 2 electrons to a series of Fe-S clusters that ultimately transfer them to ubiquinone.





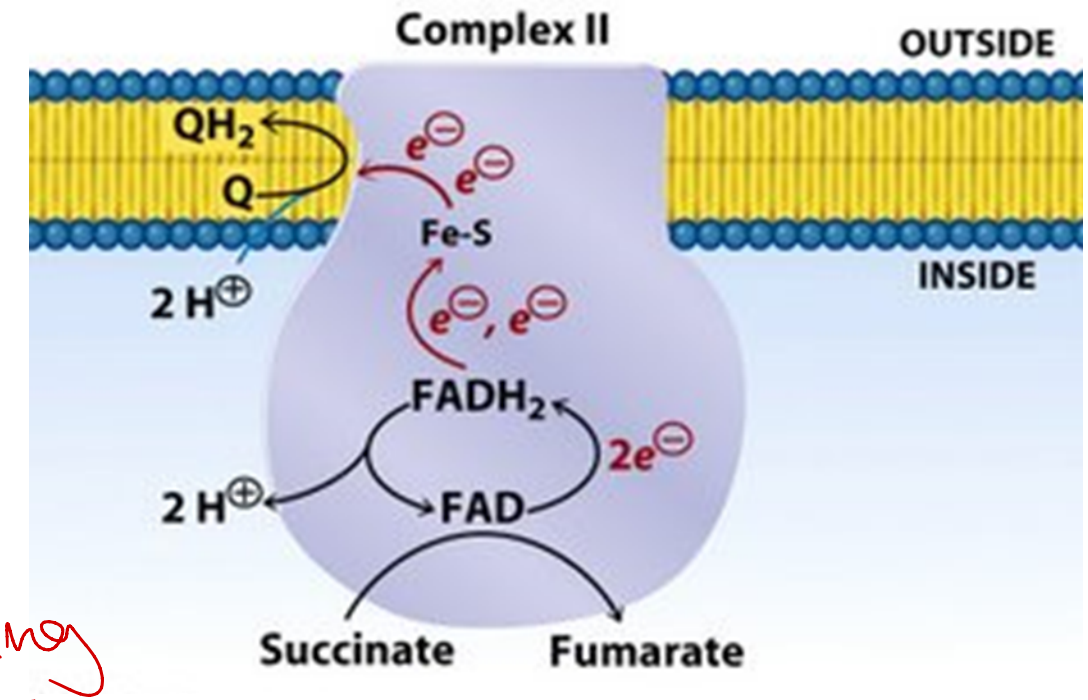
* Complex II does not pump protons!

Oxi–Red Components of the ETC

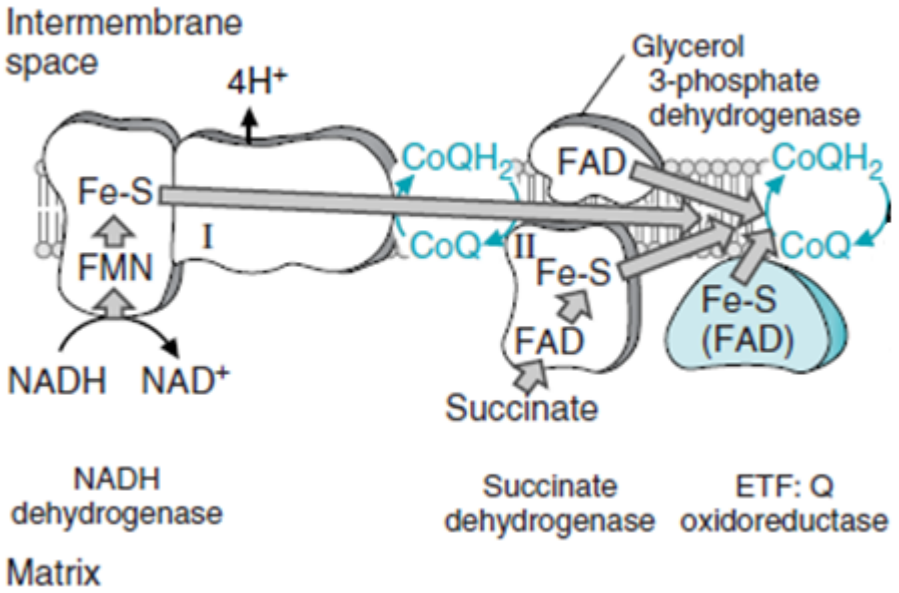
"Succinate Dehydrogenase" – Complex II

Flavo enzyme

- **Succinate Dehydrogenase & other flavoproteins**
- **TCA cycle**
- **ETF-CoQ oxidoreductase (ex. fatty acid oxidation)**
- **≈Substrates oxidized by FAD-linked enzymes bypass complex-I**
- **Three major enzyme systems:**
- **Succinate dehydrogenase**



• not pumping any protons.



- this enzyme which flavo enzyme require FAD.
- the protons are transported from succinate to FAD to make $FADH_2$
- $FADH_2$ will be oxidized by moving electron to iron sulfur center which will donate the electron for Co-enzyme Q and co-enzyme Q in order to be regenerated will take 2 protons from the matrix to be in reduced form. (In order to trunk electrons to complex 3).

- The reduced Co-enzyme Q go to complex 3.
- while the oxidized Q go to complex 1 and 2.

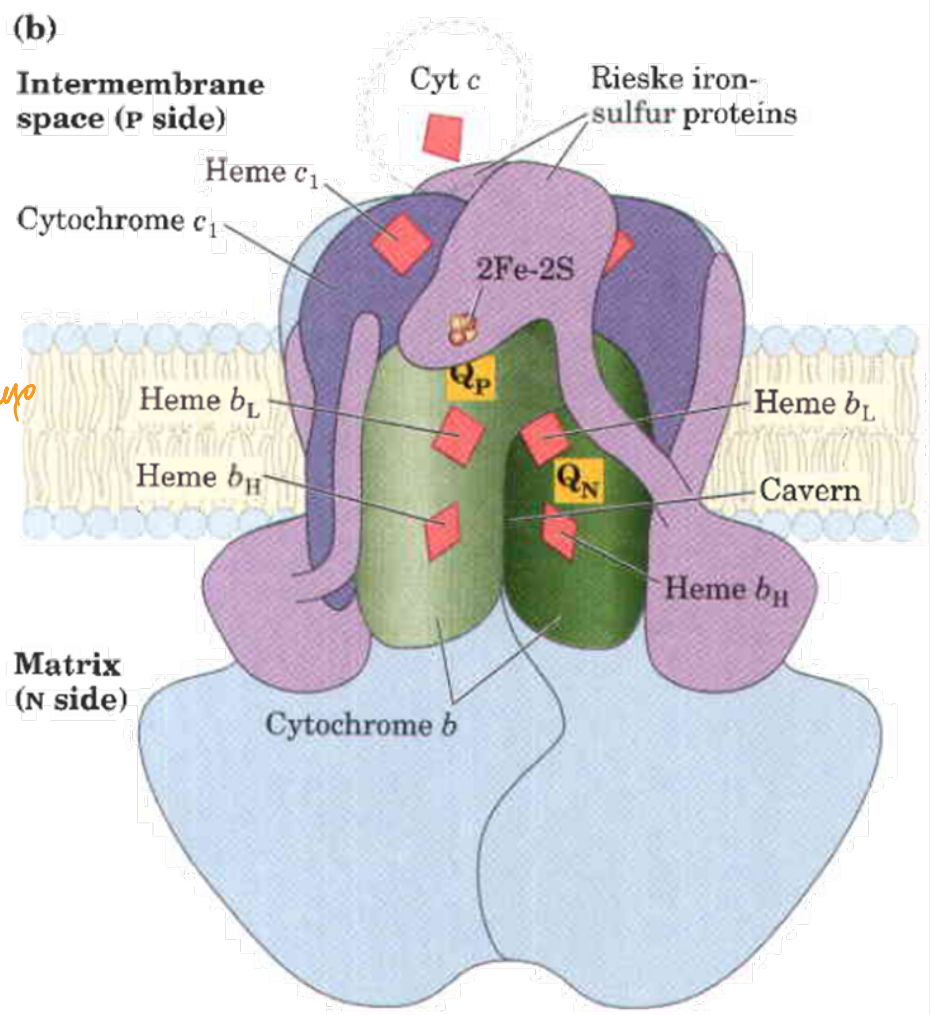
- السبب في ذلك الى ...

- In order to answer this Q which cyt carried one electron and Q is carried 2 electrons, So where is the second electron? Co-enzyme Q it will donate its two electrons and each electrons will seprate in different path within complex 3, one of them will go in the direction of cyt C_1 and the other in cyt b, that of cyt C_1 will be donated and reduce cyt C once its reduced it will be dissociated going to Complex 4

Oxi–Red Components of the ETC

"Cytochrome bc1" – Complex III

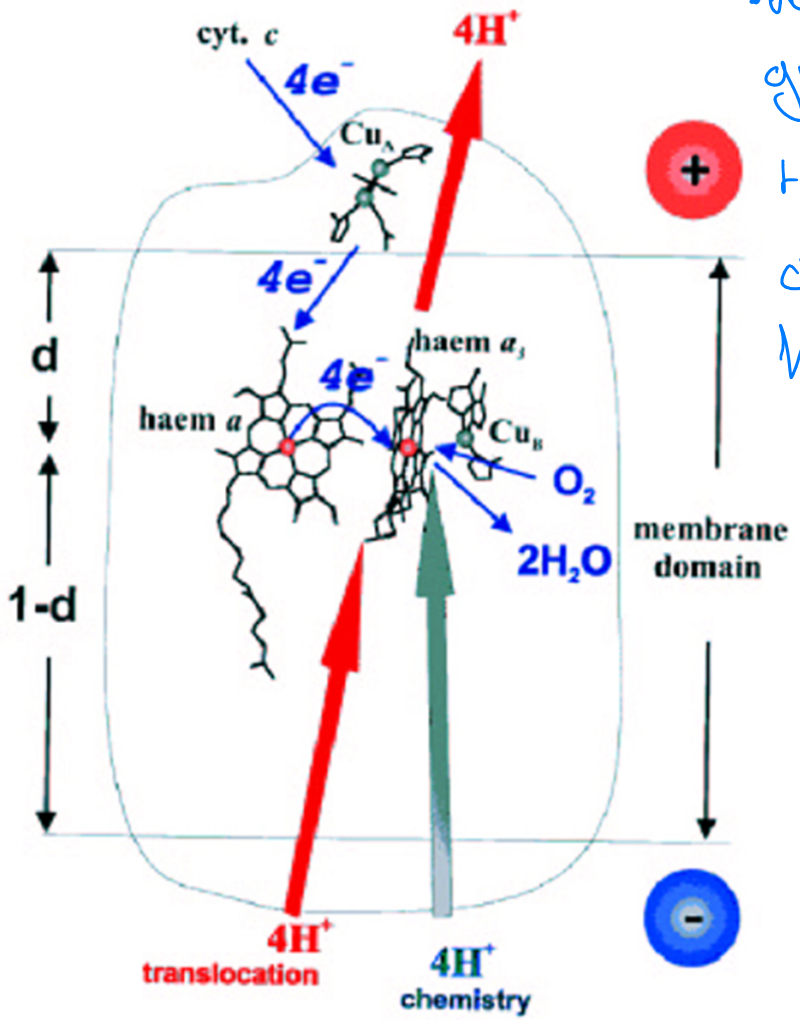
- **Also called: Q-cytochrome c Oxidoreductase**
- **Catalyzes the transfer of electrons from QH2 to cytochrome c**
- **11 subunits including two cytochrome subunits**
- **Contains iron sulfur center**
- **Contain three heme groups in two cytochrome subunits**



Oxi–Red Components of the ETC "Cytochrome c oxidase" – Complex IV

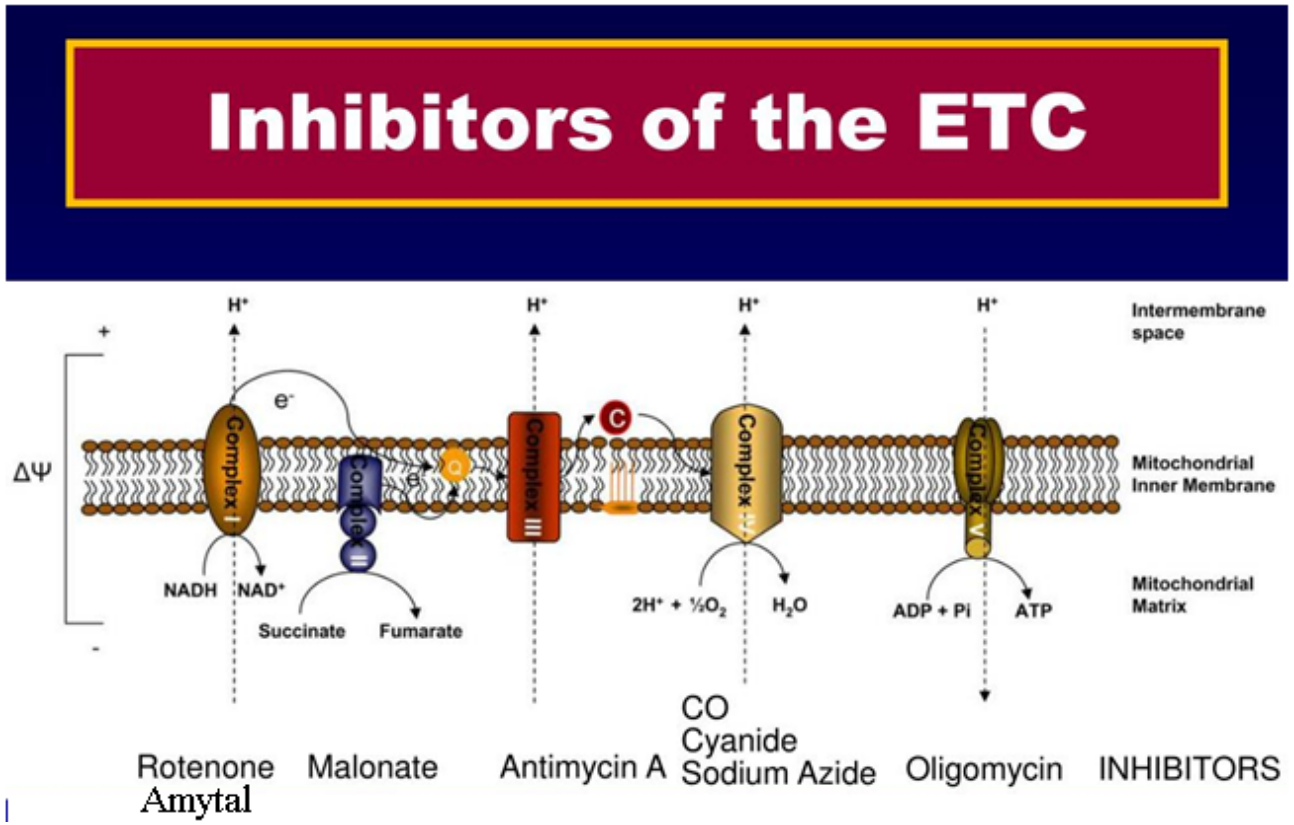
water molecule oxygen reduction

- Passes electrons from Cytocrome c to O2
- Contains cytochrome a and a3
- Contains two copper sites
- Contains oxygen binding sites
- O2 must accept 4 electrons to be reduced to 2 H2O (2H+/2e-)
- Cytochrome c is one electron carrier



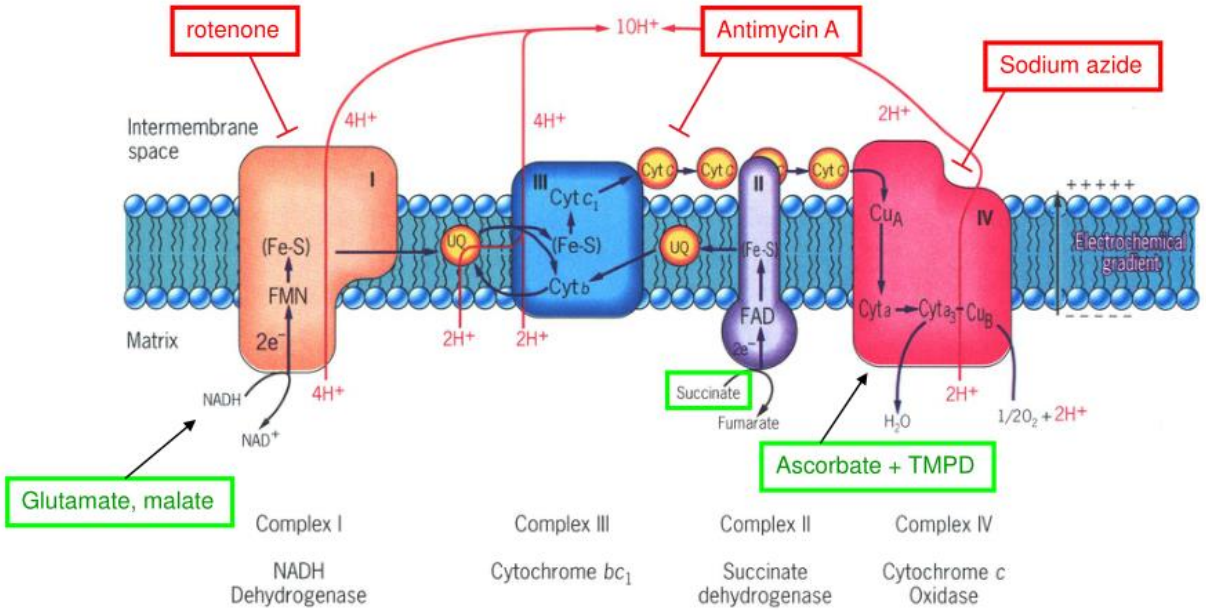
• have prosthetic group which 2 type of copper and 2 type of heme

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



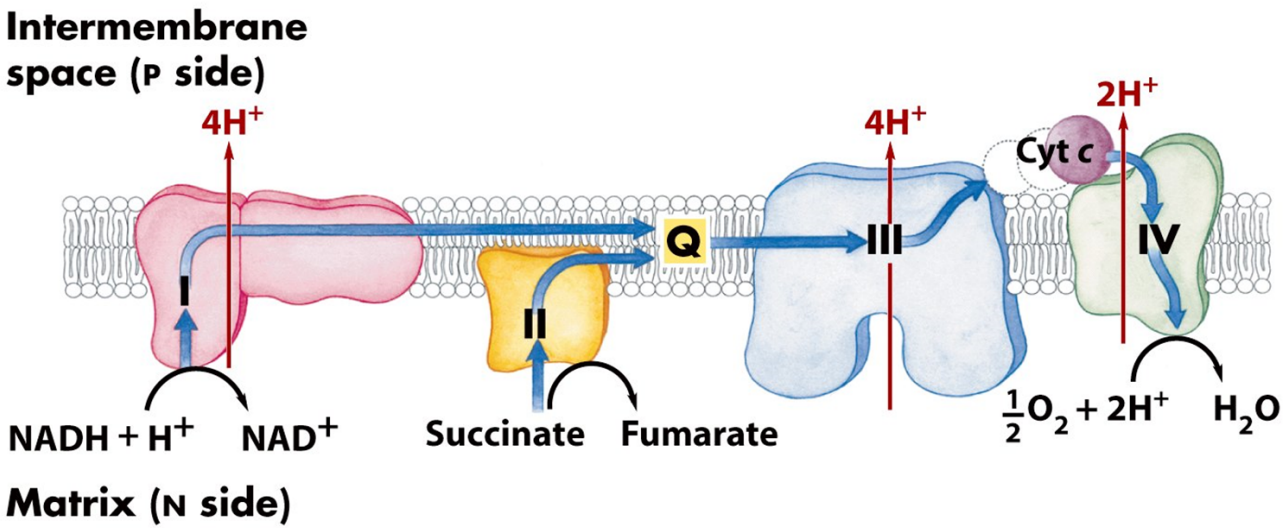
1. Complexex I, III, IV all have large enough E0 for the transfer of 2 electrons to support the synthesis of one ATP.
2. Complex I, III, IV are recognized as phosphorylation sites I, II, and III.
3. Oxidation of 1 molecule NADH+H+ or FADH2 corresponds to the synthesis of 3 or 2 molecules of ATP, respectively, 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



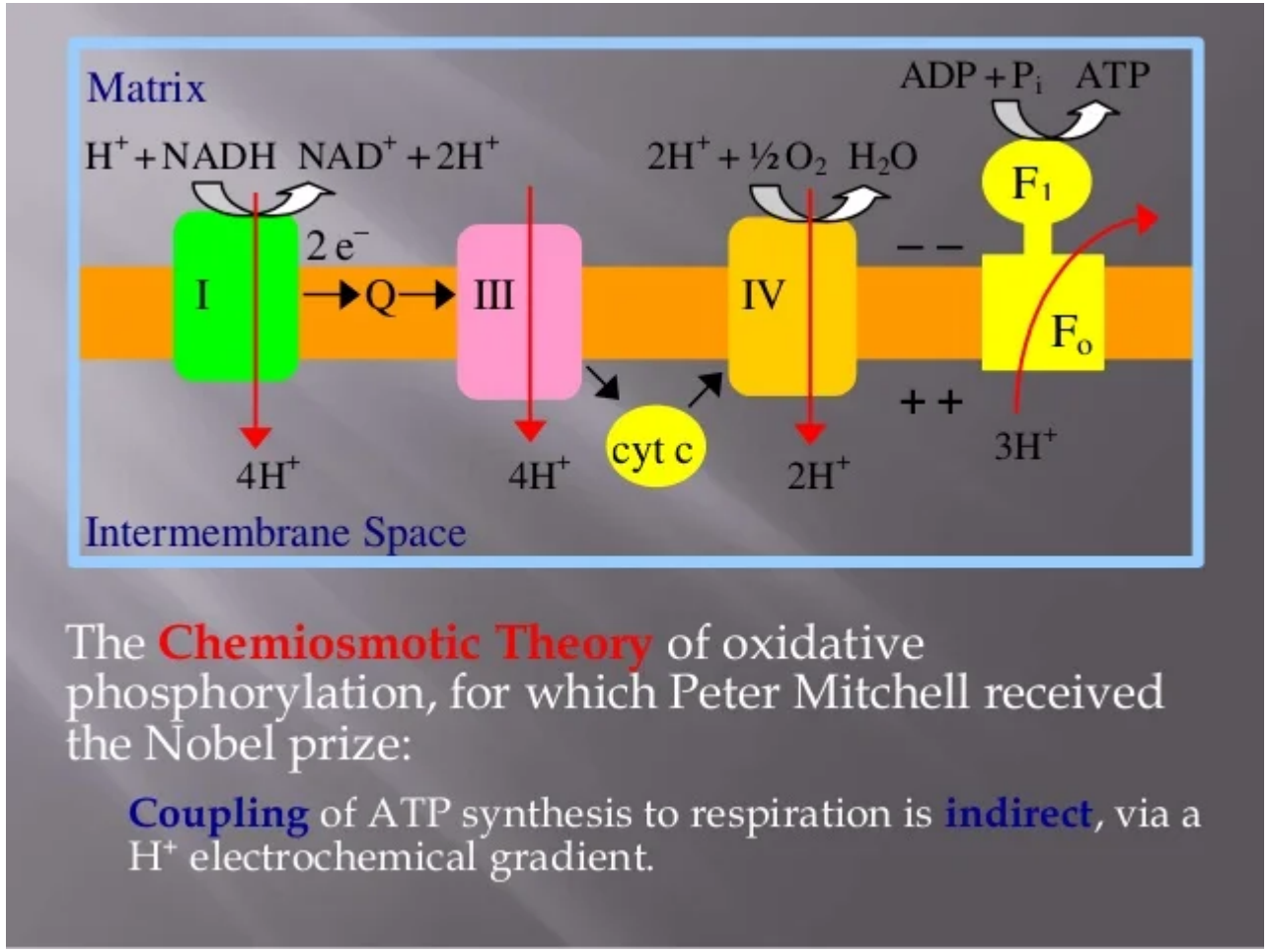
Pumping of Protons

- **For every 2 electrons passing:**
- **4H+ (complex I); 0H+ (complex II); 4H+ (complex III), 2H+ (complex IV**

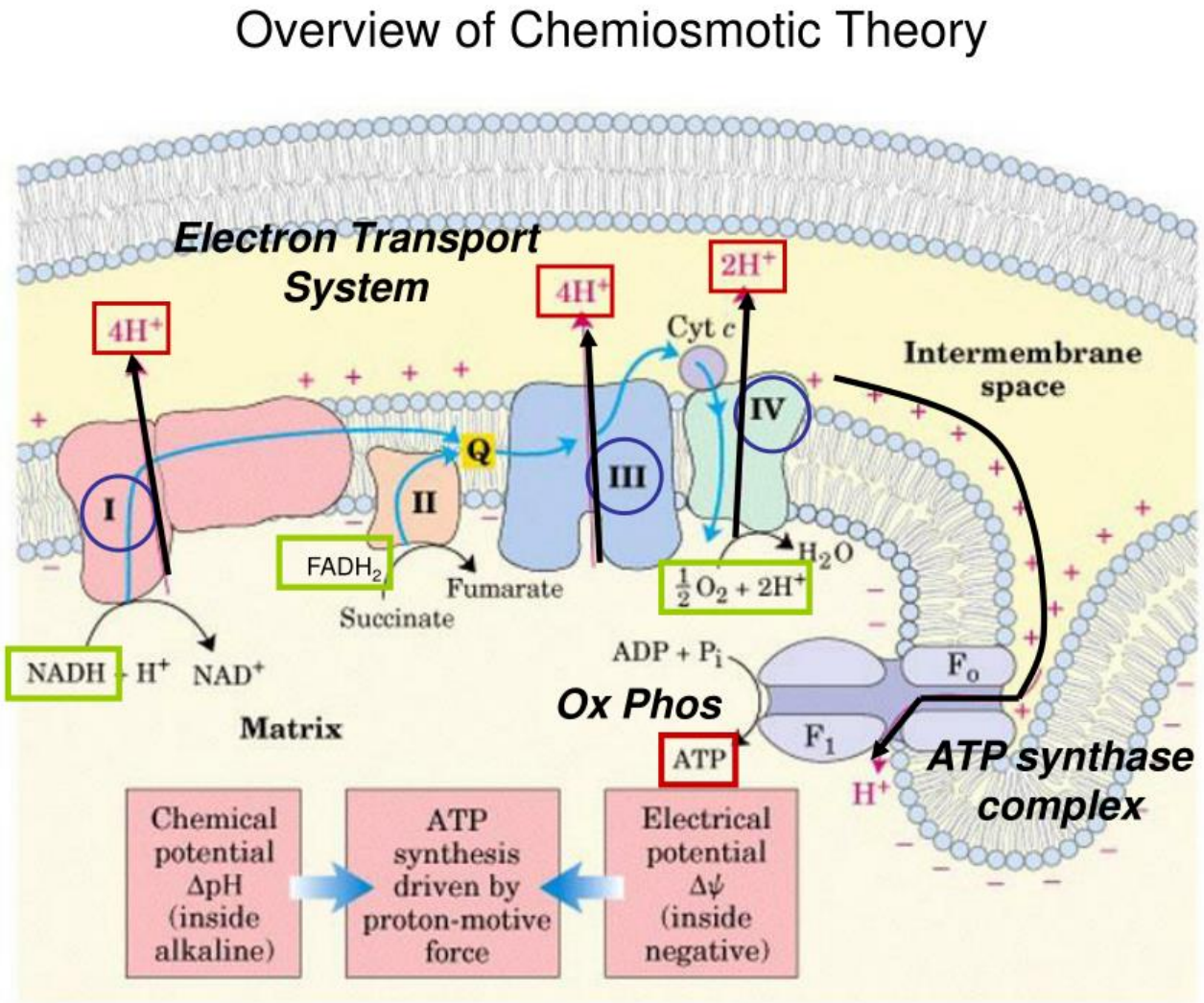


Chemiosmotic hypothesis:

- 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.



Chemiosmotic hypothesis: 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.

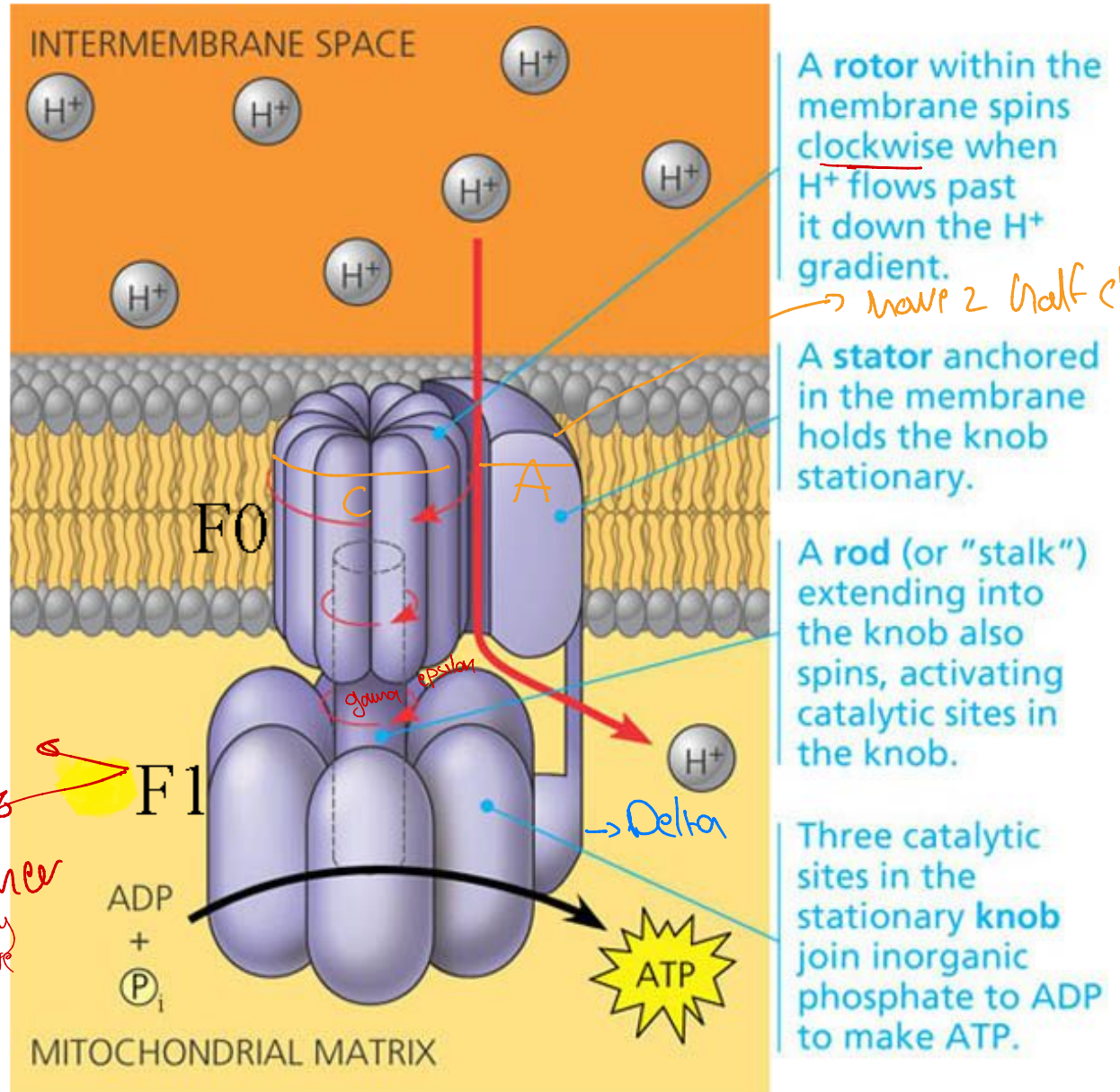


STRUCTURE AND MECHANISM OF ATP SYNTHASE-COMPLEX V

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:

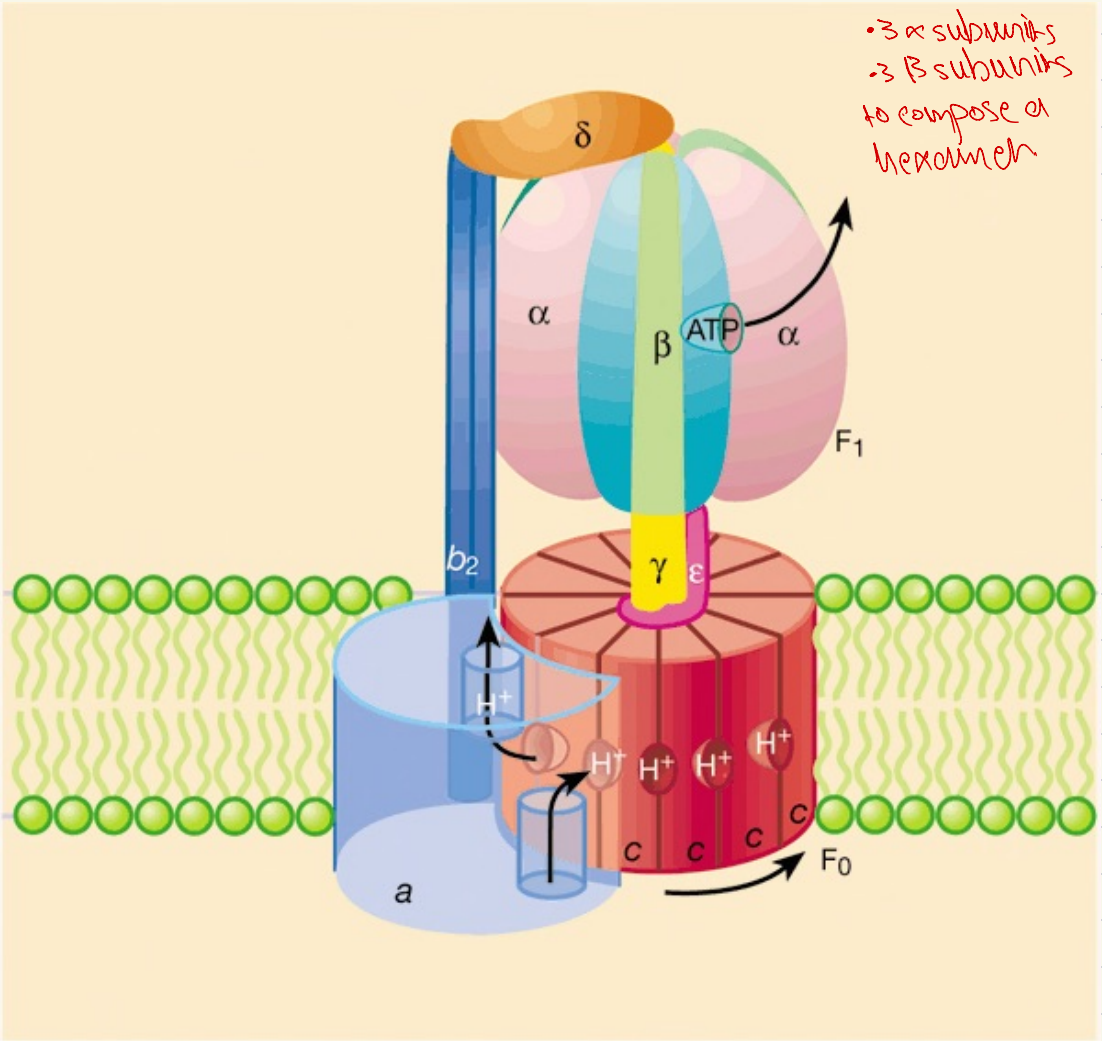
ADP +Pi---□ ATP +H2O

https://youtu.be/U26Jz3K1w2k



ATP synthase composed of two parts : one part is called Fo which located in inside the plasma membrane and the other part called F1 which located in the Matrix

- F1 composed of different subunits :- α, β, γ, delta and epsilon
- gamma associated with epsilon and it connects the two parts of enzyme together.
- the hexamer is not rotate while γ and ε rotate.
- The Fo called as this because it's inhibited by oligomycin. And this part of the enzyme is composed of two subunits, one part is called C subunit and the other is called A subunit.
- In C part there are about 10-14 c subunit which are form a ring like structure and that ring is called C ring



- Within the C subunit there are an important amino acid "Aspartate" a negative amino acid.

So when protons pass to this point they will associate with the negatively charge aspartate.

- A subunit contain 2 half channels.
- This A subunit does not move or rotate but the C ring will rotate.
- The function of C is to passage protons from the inner membrane space to the matrix
- The function of F_1 is to phospholyrate ADP to generate ATP
- It was found that the B subunit is the catalytic subunit, And it found in three different Conformational states.
- In the L state ADP and Pi are bound to the B subunit but they are far away from each other
- In the T state ADP and Pi are very close to each other (ATP in equilibrium with ADP, P_i)
(catalysis will done)
- In the O state the ATP will be released to the Matrix.
- What changes the conformation of B subunit from one to another?
- the rotation of gamma subunit will stimulate the changes from the L to T to O

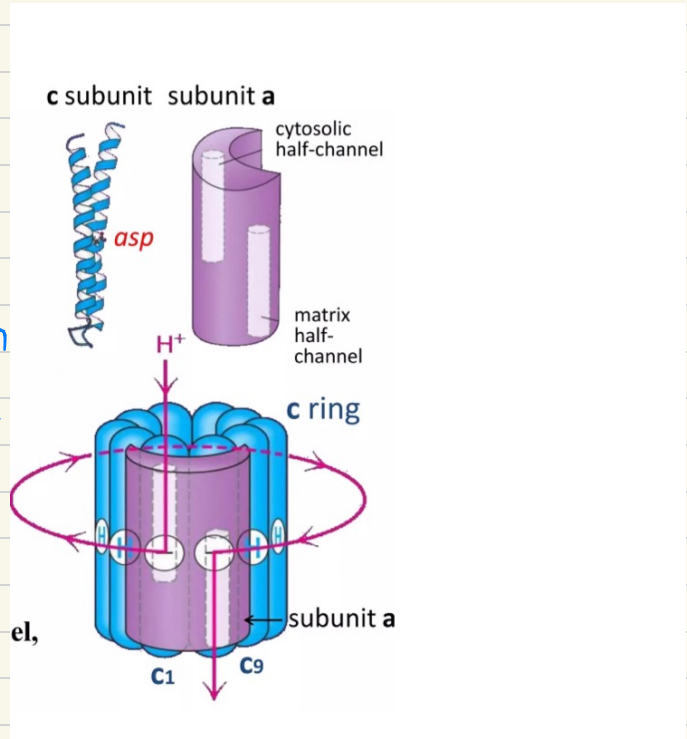
and what make Y subunit rotate is the C ring.

Now, what make C ring rotate ⟹ transport of protons.

- To sum up ⟹ proton transferring make C ring to rotate and c ring will rotate gamma, as a result the β subunit will change from one conformation to another

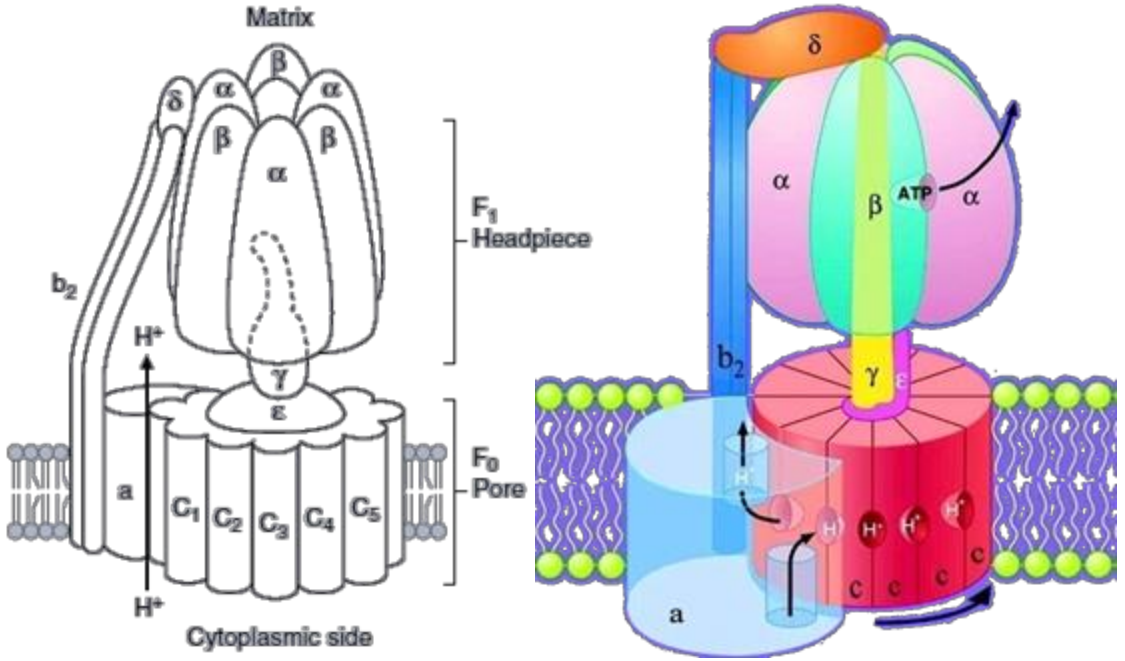
- Now, How C ring will rotate?

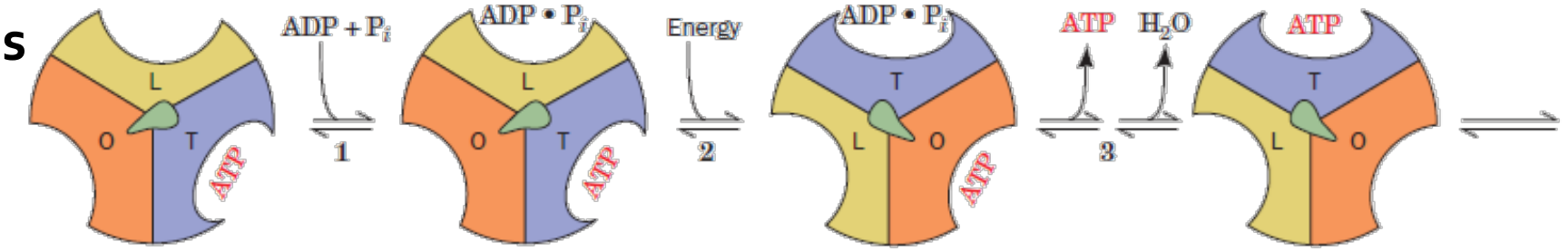
As we say C ring contains Aspartate which is negatively charged. and when protons enter to C ring they will bind to it make it Aspartic acid which is considered hydrophobic and as a result the aspartic acid will try to move to the hydrophobic region. Let's talk about A subunit which composed of two half channels as in photo, one of them inside of high H^+ concentration and the other in low H^+ concentration. Now during the movement of aspartate the C ring will rotate and the H^+ will pass from the two half channels to reach F_1, as well as the gamma will rotate resulting in β subunits change. Finally the ATP will released.



ATP Synthase

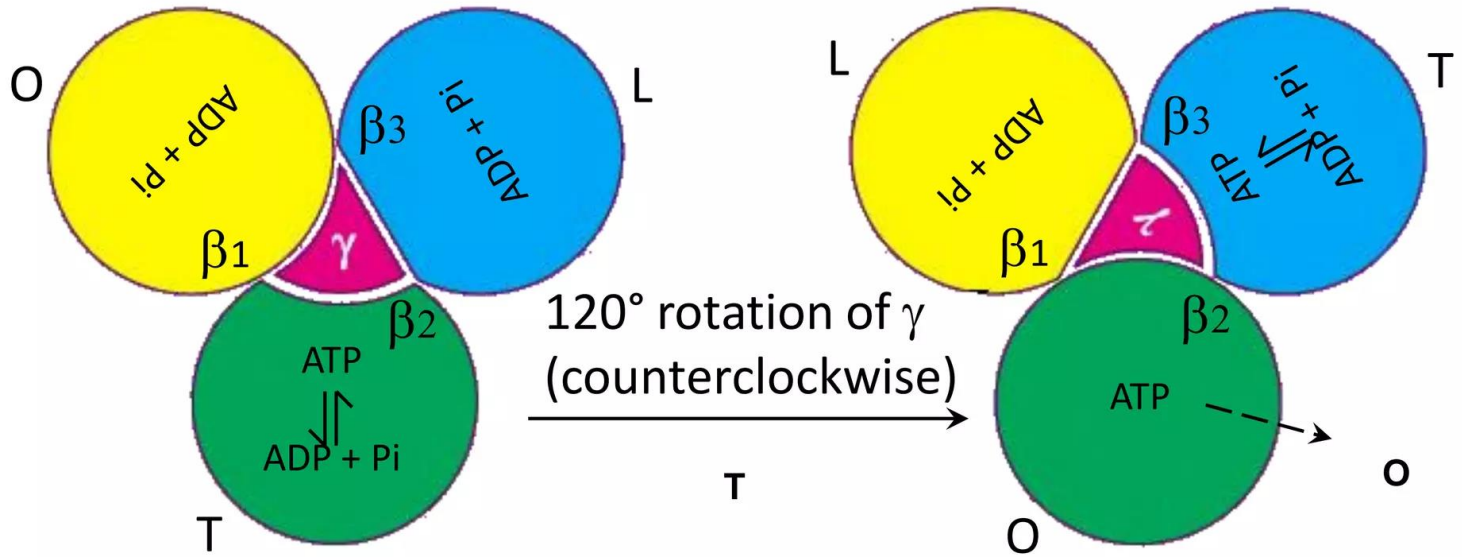
- F1:
  - "γ" subunit: rotates
  - "β" subunit: binds
  - "α" subunit: structural
  - 3 conformations: tight (T), loose (L), open (O)
- Fo:
  - "a" subunit: point of entry & exit
  - "c" subunit rotates
  - 4H+/ATP
- Can run backwards





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 + P_i → 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.



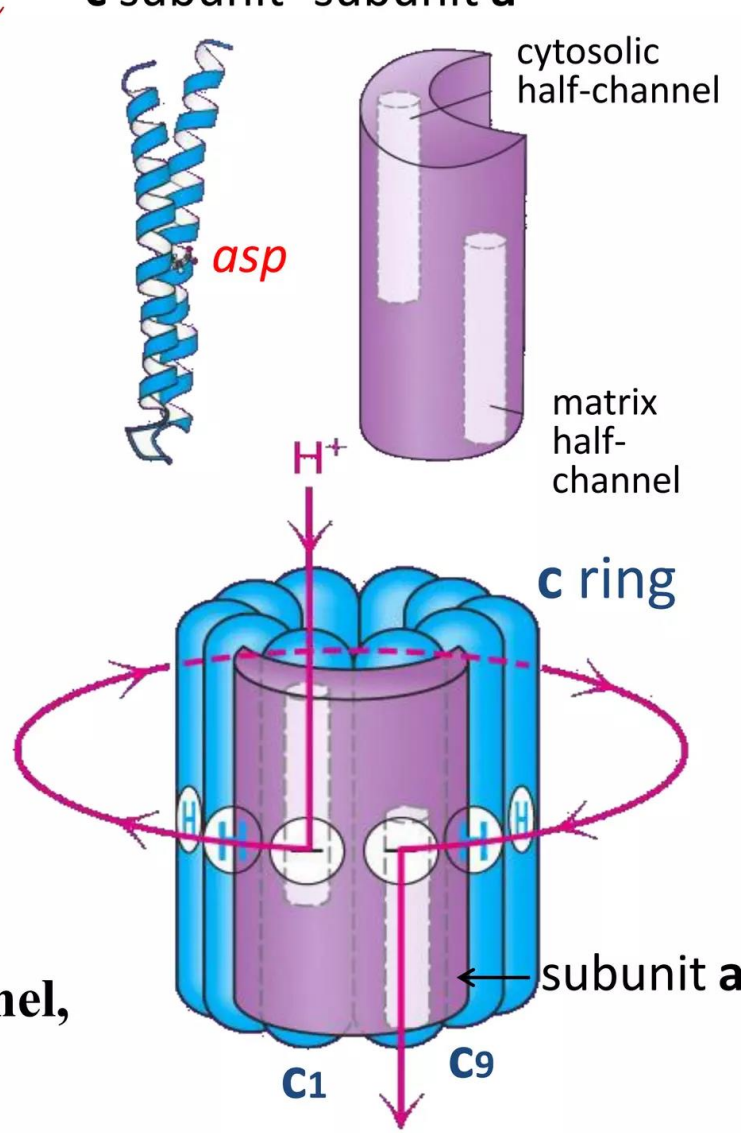
H^+ path through membrane

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**

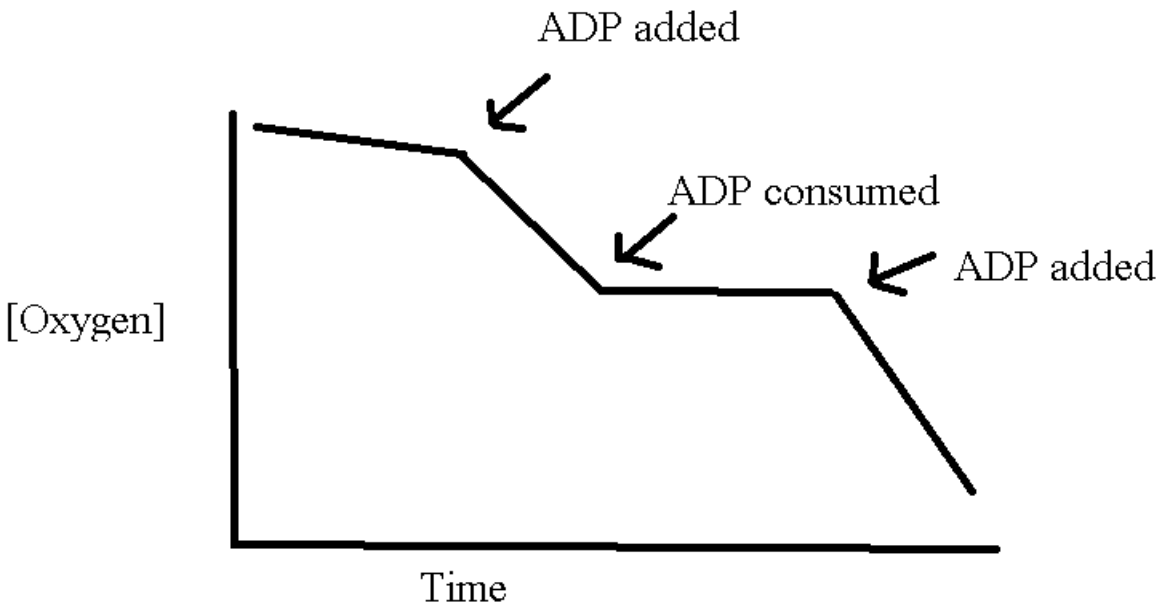
H^+ path

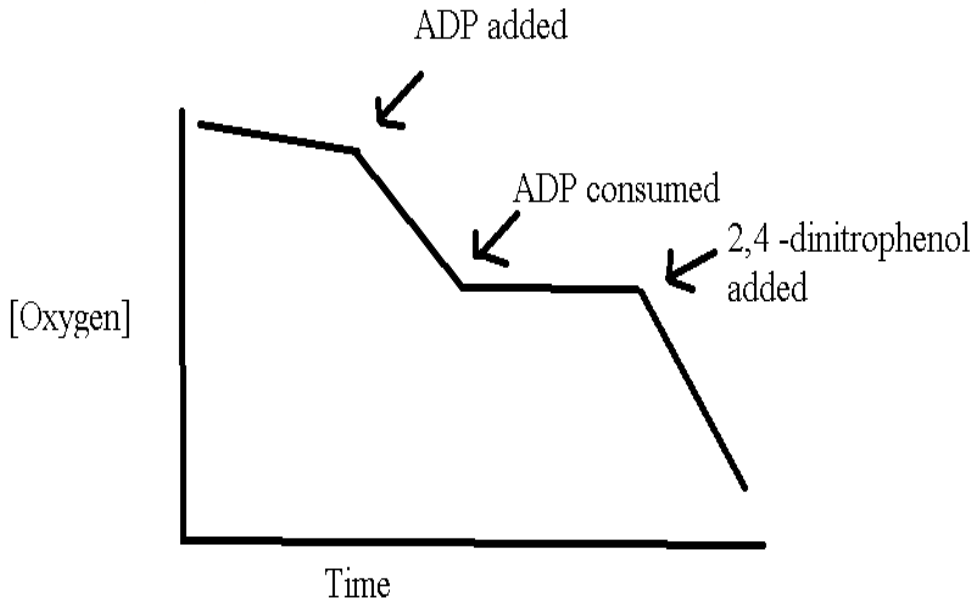
- **H^+ from cytosol diffuses via half-channel to *asp* on c ring subunit (c1)**
- **this subunit can now move to interface membrane, allowing c ring to rotate**
- **c9 now interfaces matrix half-channel, allowing H^+ to diffuse into matrix**



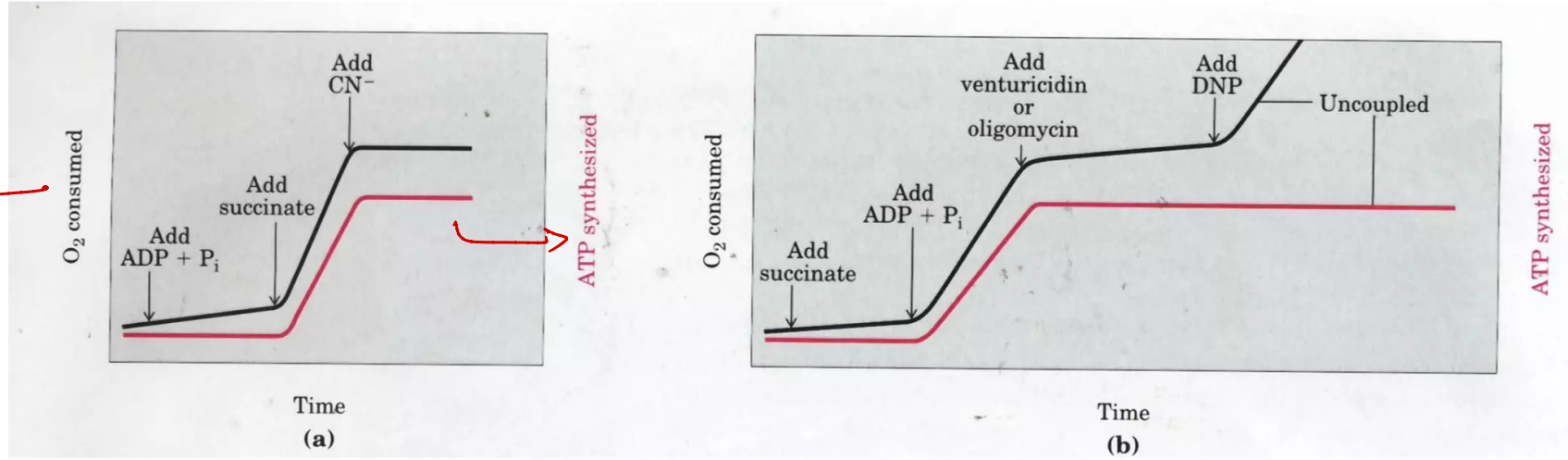
RECEPTOR OR ACCEPTOR CONTROL

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 can channel protons across the membrane. Under this condition, electrons transport runs unchecked at its maximal rate in the absence of the acceptor ADP.





How much O2 is reduced



Electron transfer was found to be obligatorily coupled to ATP Synthesis in isolated mitochondria suspensions:

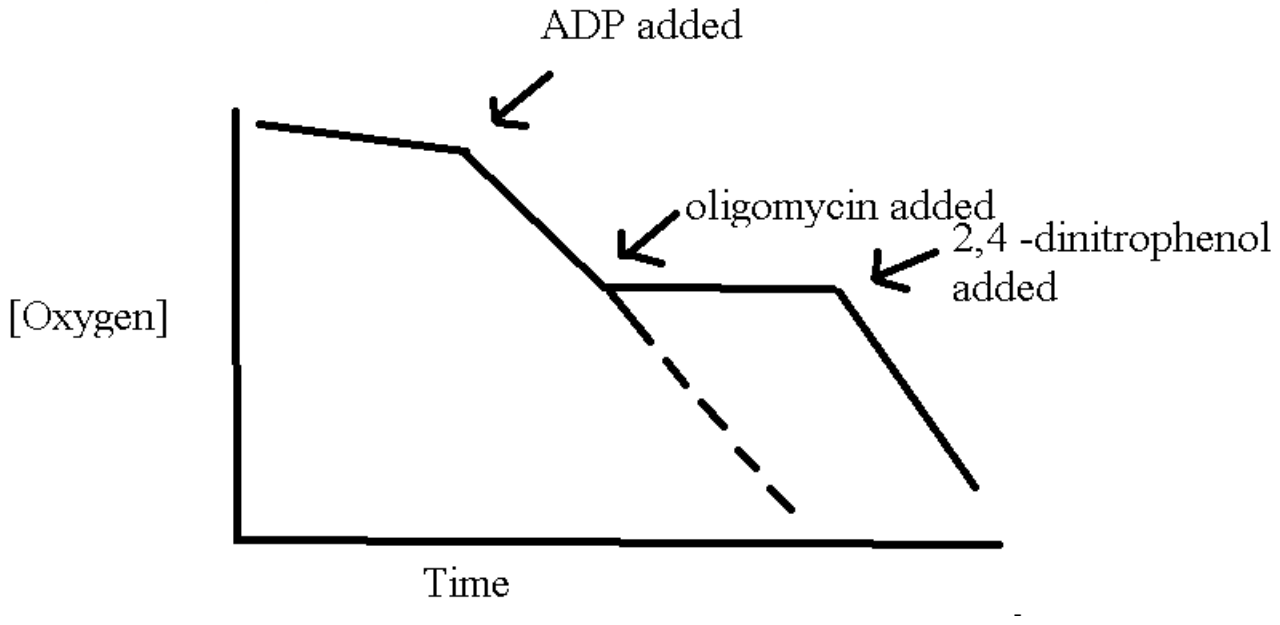
neither occurs without the other.

- without ADP nothing will happen

- In the first photo we add ADP but without substrate so there is no ATP synthesis and there is no oxygen Consumption because no electron flow and No substrate to be oxidized in order to form $FADH_2$. Now add succinate, you will see huge increase in oxygen consumption related to electron flow and you will see increase in ATP synthesis.
- If you add cyanide which inhibit cytochrom C oxidase (4) So oxygen Consumption inhibited
- This photo tell us that the phosphorylation oxidase is coupled with ATP synthesis, If it stops the ATP synthesis will stop.
- In the second photo, the same thing the oxygen Consumption will be stoped unnill the ADP is added
- If we add oligomycin which inhibit the ATP synthase by inhibiting F_o, the oxygen Consumption and electron flow will inhibited.
- If we add DNP we will see an increase in oxygen Consumption but no in increase in ATP synthesis Becuse DNP uncouples synthesis from oxygen Consumption and electron flow But How ?
  - It collect all hydrogen protons in the inner space take them and go to the Matrix with not through ATP synthase So it break the grandiant of H^+, and all energy of hydrogen passage from high to low conce. will convert to heat

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

⇒ tell us about the energy index of each of metabolites

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

$\frac{ATP}{O}$

- P:0 ratio varies with the substrate being oxidized:
- With NADH it is 3
- With succinate it is 2
- With ascorbate it is 1

for each NADH and $FADH_2$ we need one oxygen atom

- The overall equation for respiratory chain phosphorylation:

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

P:O ratio for pyruvate ⇒ $\frac{14}{5}$

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)**

لما نطلع ع رقبة الأطفال الصغار من الخلف
بنشوف لون بني وهو بسبب التركيز العالي للميتوكندريا ← which contains a lot of thermogenin uncoupler to keep the newborn baby warm.

بتحول الطاقة لـ heat بدل عملية صنع ATP.