*Why is pyruvate essential?

Because it is found at important metabolic crossroads

-Glucose → Glycolysis → pyruvate

-Pyruvate-→ carboxylation by pyruvate carboxylase→ oxaloacetate

-Oxaloacetate + acetyl CoA → citrate synthase → TCA CYCLE

Note: oxaloacetate is not consumed in the TCA cycle and is produced in catalytic amounts during the TCA cycle.

-Pyruvate-→ oxidative decarboxylation by pyruvate dehydrogenase→ Acetyl CoA

-Pyruvate←→ reduction by lactate dehydrogenase←→ lactate

Note: when lactate→ pyruvate this is called anaerobic glycolysis.

-Pyruvate←→ transamination ←→ alanine

-Acetyl CoA→ Fatty acid synthesis → FA

-FA → FA B- oxidation → Acetyl CoA

-Acetyl CoA → ketone body synthesis → ketone bodies

-ketone bodies → ketone bodies utilization → Acetyl CoA

---

*What is PYRUVATE DEHYDROGENASE?

1- Structure: Multienzyme COMPLEX made up of 3 enzymatic subunits:

E1- pyruvate dehydrogenase

E2-Dihydrolipoyl transacetylase

E3- Dihydrolipoyl dehydrogenase

2- Function: oxidative decarboxylation of PYRUVATE to ACETYL CoA

3-Site of function: Mitochondrial matrix

4-CoEnzymes: They are 5 coenzymes derived from vitamin B

1- Thiamine pyrophosphate (B1) 2-Lipoamide 3- Coenzyme A (B5)
4- Flavine amide dinucleotide (B2) 5-NAD (B3) (Niacin)

5- How is E1 controlled: By allosteric and covalent modification

6-Enzymes that help in controlling E1: They are 2 enzymes that work on E1

1-Kinase (phosphorylation to inactivate it)

2-Phosphatase (dephosphorylation to activate it)

The kinase and phosphatase are affected by several key metabolites: NADH, Acetyl CoA, CoASH.

7- Explain how does the regulation work:

In the fed state in the liver, the reaction turns on, glucose→ fatty acids in the liver.

In the fasted state, the reaction turns off, so pyruvate will be driven into gluconeogenic pathway.

Allosteric method: Inhibition of the enzyme when we have high levels of Acetyl CoA, NADH and ATP

Activation of the enzyme when we have high levels of Pyruvate, ADP and NAD

Covalent modification:

Kinase

stimulated -→ and adds a phosphate group to inactivate the pyruvate dehydrogenase when we have high levels of ATP NADH ACETYL COA

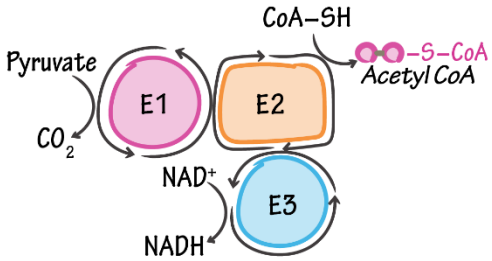
inactivated →by high levels of NAD+ ADP PYRUVATE

Phosphatase

stimulated -→ and removes a phosphate group to activate the pyruvate dehydrogenase when we have high levels of Mg+2, Ca+2, insulin /adipocyte, catecholamines/ cardiac.

7-Reversible or Irreversible? This enzyme's reaction is irreversible, meaning that we can NEVER synthesize PYRUVATE from Acetyl CoA. As a result, we conclude that its not possible to synthesize carbohydrates from fatty acid catabolism. (in mammals)

Pyruvate Dehydrogenase Complex Enzymes



Decarboxylase
Transacetylase
Dehydrogenase

Questions;

*What are the steps of the reaction of pyruvate dehydrogenase?

E1 uses TPP and bind it with pyruvate to remove CO2 then E2 uses it (pyruvate-tpp) to transfer the acetyl group from tpp to the lipoic acid (acetyl- lipoic acid). Then acetyl lipoic acid has its acetyl group removed by E2 and added to the CoA. E3 uses the energy conducted from the transfer of acetyl group from lipoamide to CoA to reduce FADH2 and then FADH2 reduces 2NAD+ to 2NADH and 2H+

*What are the products from this process (decarboxylation of pyruvate)?

CO2, Acetyl CoA, 2NADH + 2H+

*Where are the Cofactors used?

E1 used TPP, E2 used lipoic amide and CoA, E3 used Flavin, NAD was also used.

*In the first step pyruvate changed from keto to…

To alcohol

Fill in the blanks:

-All major nutrients can be converted to acetyl Co A in the first (**2 stages of metabolism**).

-The acetyl group of the acetyl Coa is fully oxidized to (**CO2 and water**) at the (**third stage of metabolism**).

- The Krebs cycle is a vital pathway of metabolism in all (**aerobics**) and occupies (**central position in metabolism**) because it is the common pathway for the oxidation of all the major nutrients like carbohydrates lipids and proteins.

-The major and the most common pathway for nutrient oxidation in aerobics is (**TCA cycle**).

-The TCA cycle is (**amphibolic**) which means it provides intermediates for the synthesis of biomolecules (gives intermediates used for both anabolism and catabolism).

- The oxidation of Acetyl CoA results in (**reduction of NAD and FAD to NADH+ + H+ AND FADH2**)

-The hydrogens or electrons of the reduced cofactors are then transferred to (**oxygen in the ETC**) to form (**water**)

-As the electrons are transferred to oxygen (**ATP is generated**).

-Glycolysis occurs in the cytoplasm because it is (**anaerobic)**. While TCA occurs in the (**mitochondrial matrix)** because Its (**aerobic)** and all its enzymes are present there except for (**succinyl dehydrogenase**).

- Oxaloacetate is synthesized in a very low amount **(catalytic amount**) which means an amount that only suffice to react with the available acetyl Co A molecules and that's under the normal physiological condition.

-Oxaloacetate is produced by the (**glucogenic pathway**) which is the (**carboxylation)** of pyruvate.

TCA PRODUCTS IN RED INPUTS IN GREEN

1-Oxaloacetate+ Acetyl CoA+H20 → citrate synthase→ citrate + Co A
ΔG0=-9kcal/mol

Note: *This enzyme is candidate for regulation (stimulated by ADP and inhibited by HIGH energy charges like ATP and NADH, SUCCINYL-COA)

*This step is necessary for fatty acid synthesis

* This is a condensation reaction

2- citrate - H20 + H20 → Aconitase → isocitrate

ΔG0=+1.5kcal/mol

Note: * This is a dehydration followed by a hydration reaction, so water is not a product, water is used to rearrange the molecule by transferring the OH group from a carbon to another.

3- isocitrate +NAD → isocitrate dehydrogenase → a- ketoglutarate +CO2 + NADH + H+

ΔG0=-5kcal/mol

Note: *This is oxidative decarboxylation reaction

*This enzyme is candidate for regulation (inhibited by HIGH energy charges like ATP and NADH, the inhibition of it will result in INCREASE in citrate which can be transported out of the mitochondria as a substrate for fatty acid synthesis. Stimulated by AMP/ADP which will lower KM by 10 folds).

*The first dehydrogenase used in the cycle is isocitrate dehydrogenase and the first production of NADH

*The CO2 released is **not** FROM ACETYL CO2

*a-ketoglutarate is an intermediate for anabolic rxns

* This is rate limiting step of the rxn.

4- a- ketoglutarate +NAD + CoASH → a-ketoglutarate dehydrogenase →Succinyl CoA + CO2 + NADH + H+

ΔG0=-8kcal/mol

Note: *This is oxidative decarboxylation reaction.

*The second dehydrogenase used in the cycle is isocitrate dehydrogenase and the second production of NADH

* IMPORTANT! THIS ENZYME RESEMBLES PYRUVATE DEHYDROGENASE

* This enzyme is subjected to regulation, ATP, GTP, NADH, and succinyl CoA inhibit the complex, AMP, and Ca+2 are positive effectors. The complex consists of α-ketoglutarate dehydrogenase,

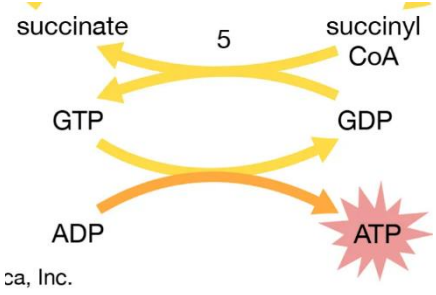
dihydrolipoyl transsuccinylase and dihydrolipoyldehydrogenase. A-*A-ketoglutarate represents a significant point of convergence in metabolism. Several aa are converted to glutamate which if trans-aminated or oxidatively deaminated yields alpha ketoglutarate. (this means anabolic pathway of ketoglutarate is transamination of glutamate to ketoglutarate and the catabolic pathway of a-keto gluterate is giving succinyl-Coa)

5- Succinyl CoA + GDP + P → succinyl Coa A synthase (thiokinase) →Succinate + CO A +GTP

ΔG0= -8kcal/ mol

Note: * This reaction is substrate level phosphorylation

- The energy resulted in the cleavage of the thioester bond is coupled with the phosphorylation of GDP to GTP



* Nucleoside diphosphate kinase:

GTP + ADP → GDP +ATP

*Adenylate kinase:

AMP+ATP→ 2ADP

SUM= GTP+AMP→ GDP+ATP

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

6- Succinate + FAD → succinate dehydrogenase→ Fumarate + FADH2

ΔG0= 0kcal/ mol

Note :* This reaction is dehydrogenation

*This reaction is the only dehydrogenation in the TCA cycle that is not an NAD linked so that's why this enzyme is also called a flavoenzyme

* Malonate is a competitive inhibitor.

6- Fumarate + H20 → fumarase → L- Malate

ΔG0= 0.9kcal/ mol

Note :* This reaction is REVERSIBLE hydration by adding a hydroxyl group

* This enzyme is specific for the trans and L isomers of the unsaturated and hydroxy acids respectively.

6- Malate + NAD → malate dehydrogenase → Oxaloacetate + NADH+ H+

ΔG0= 7.1 kcal/ mol

Note :* This reaction is dehydrogenation

* It completes the cycle by regenerating oxaloacetate

*The final reaction that produces an NADH + H+

* The equilibrium greatly favors the reverse reaction which is the reduction of the oxaloacetate however 1- citrate

synthesis is closely associated with the dehydrogenase 2- removal of oxaloacetate assist in pulling the

malate dehydrogenase reaction toward the formation of oxaloacetate.

*Oxaloacetate can be reversibly transaminated to aspartate.

General notes:

1- Oxaloacetate acts catalytically which means there is no net synthesis or degradation of the four carbon intermediates.
2- Each turn of the TCA cycle involves the uptake of two carbon atoms in the form of acetyl COA and the release of two carbon atoms as CO2 but not the same carbons that were taken upon condensation.
3- Each turn of the TCA cycle results in:

the transfer of three **pairs** of electrons (6e) in the form of hydride ions form NADH

transfer one **pair** of electrons(2e) in the form of two hydrogen atoms to reduce FAD to FADH2

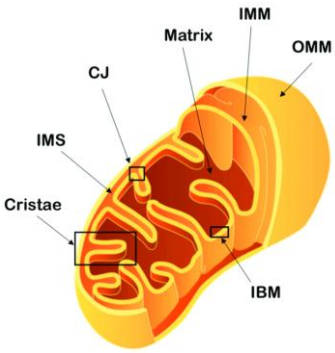
NADH and FADH2 are energy rich molecules because they contain a pair of **electrons high transfer potential.** These electrons are transferred to oxygen through a series of carriers results in the release of a large amount of energy which can be used to generate ATP.

OXIDATIVE PHOSPHORELATION

The process where ATP is formed, as we know forming ATP requires energy, so what is the source of energy that drives this reaction?

Energy released as the e- are transferred by reducing powers like NADH and FADH2 to oxygen in the mitochondrial membrane.

What is mitochondria?



Mitochondria is an organelle that consists of two membranes the outer membrane and inner membranes.

1- outer membrane: freely permeable to molecules less than 10 kilodalton molecular weight.
2- Inner membrane: inner membrane space has many folds directed towards the matrix.

Between the outer membrane and the inner membrane we have INTERMEMBRANE SPACE which contains many enzymes that catalyzes the interconversions of adenine nucleotides.

Each compartment in the mitochondria has various enzymes

Enzymes with the same function, reside in the same place

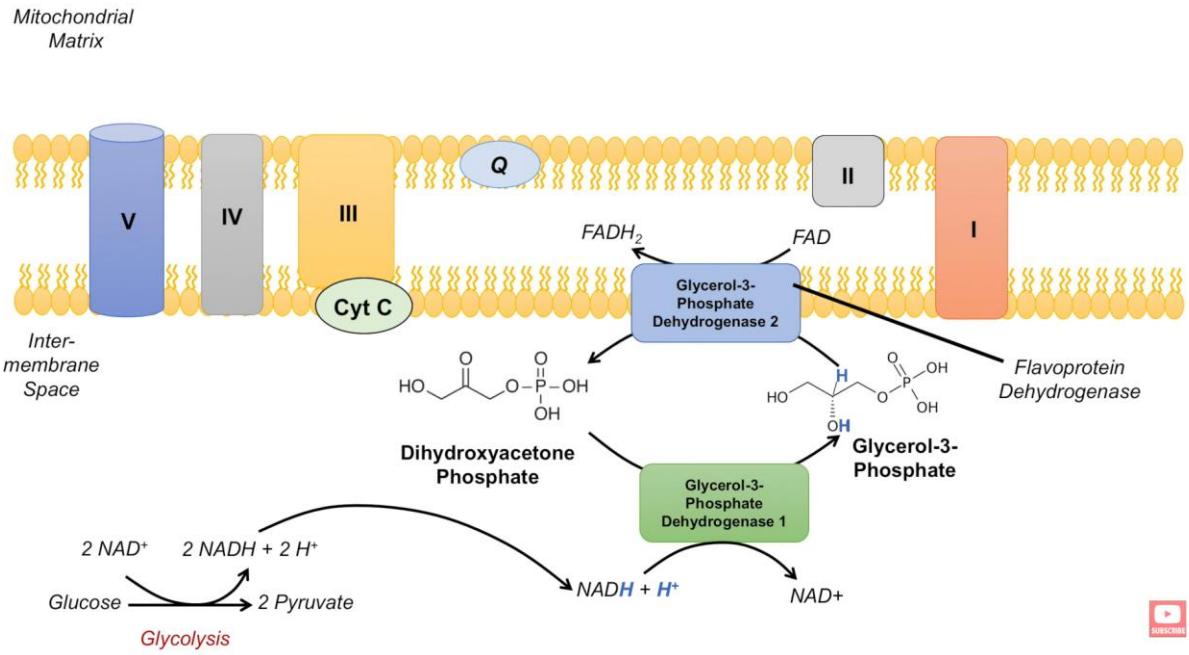
Outer membrane	Intermembrane space	Inner membrane	Matrix
NADH cytochrome b5 reductase	Adenylate kinase	NADH-Coenzyme Q reductase	PDH
Cytochrome b5	Nucleoside diphosphokinase	Succinate-Coenzyme Q	ALPHA-KG DH
Monamine oxidase	nucleosidemonophosphokinase	Coenzyme QH2-cytochrome c reductase	CITRATE SYNTHASE
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-COA DH
			BETA-KETOACYL-COA THIOLASE

Shuttles

1)a- glycerol phosphate-dihydroxyacetone phosphate shuttle

The concept is that, glycolysis occurs in the cytosol and produces NADH + H+ which is then oxidized to give out energy, but how is the NADH+ oxidized when the place of oxidation is in the inner mitochondrial membrane?

SHUTTLE= how can we transport NADH+ H+ to the mitochondrial inner membrane to get energy



*DHAP is reduced to glycerol 3 p (NADH+ + H+ is oxidized)

* glycerol 3 p is oxidized to DHAP by FAD dependent glycerol P dehydrogenase in the mitochondria

*NADH (CYT) + FAD (MIT) → NAD (CYT)+ FADH2 (MIT)

*operation in muscles

* we loose energy by using this method and that's because NAD has a higher energy value compared to FAD

2- Malate-aspartate shuttle:

Shuttling NADH+ (glycosis) in the cytoplasm to inner mitochondrial membrane.

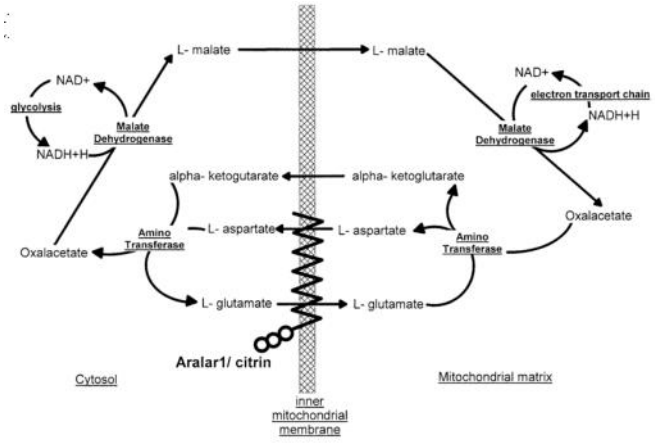
*OAA (cyt) is reduced to malate by NADH-dependent malate dehydrogenase.

*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).

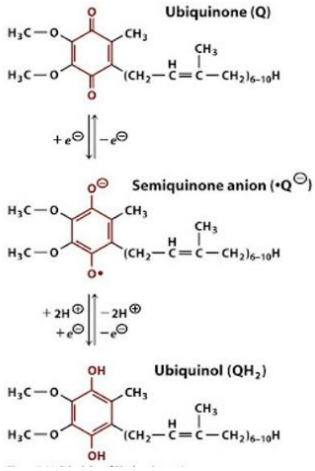
*Operational in liver and heart.



ELECTRON TRANSPORT CHAIN

The chain of carriers is called **the electron transport chain** or respiratory chain. Each carrier has a prosthetic group that will assist the protein complexes to accept and donate electrons. We have 4 carriers existing as integral protein complexes , 1 mobile lipid carrier, and one soluble protein.

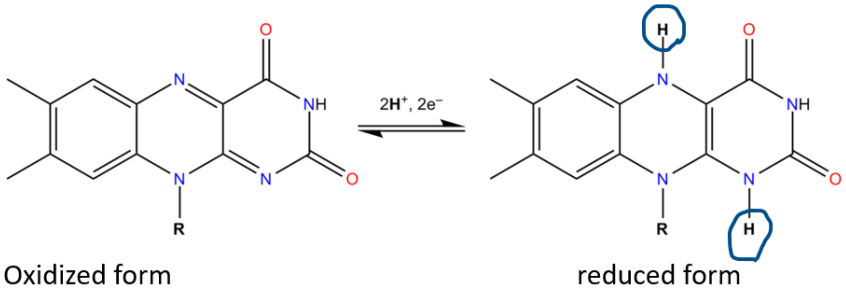
-(carrier) Co Enzyme (Ubiquinone): This is the only carrier which is NOT PROTEIN.It has a long isoprenoid tail which enables the molecule to diffuse rapidly in the hydrocarbon phase of the inner mitochondrial membrane. Due to its solubility, it can



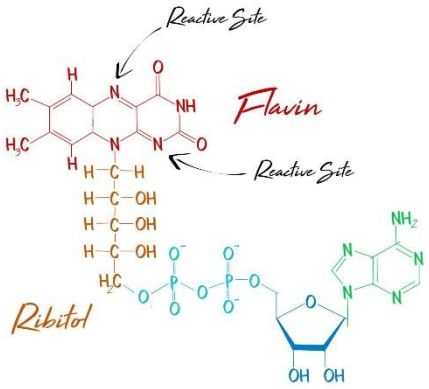
diffuse from one place to another depending on the complexes needed. It can carry 2e-, but not at once.

-(prosthetic) FAD : Flavine adenin dinucleutide.

Carry 2 H and is covalently bonded to complex 2 that contains succinate dehydrogenase.

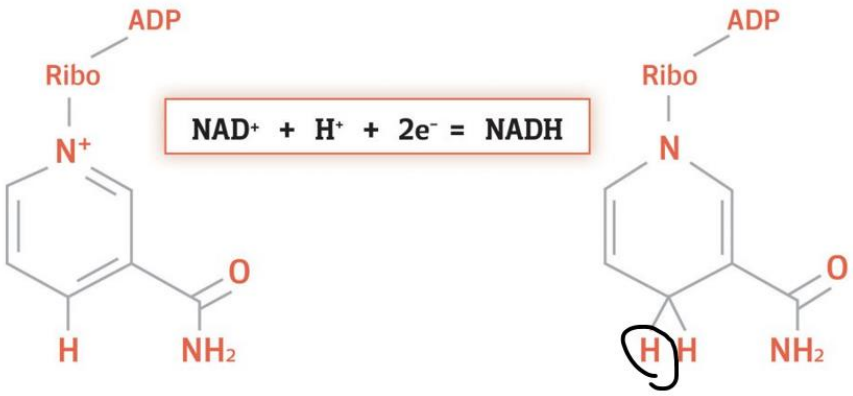


Oxidized form reduced form

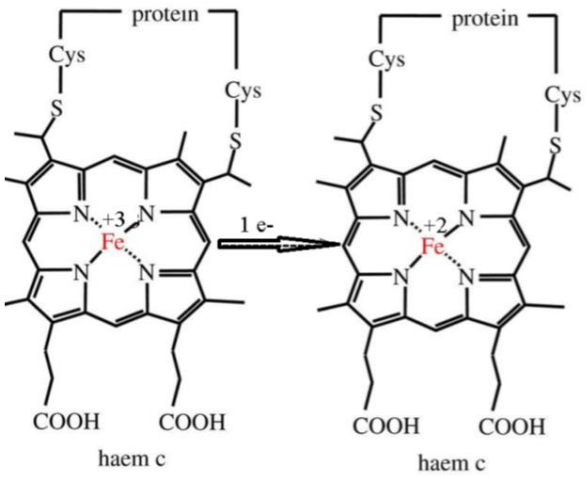


-(prosthetic) NAD: Nicotineamide adenine dinucleotide.

Bind H+ and 2e- .Carry 2 e-. Donate its electrons to complex 1.



-Cytochrome (heme proteins) : electron transfer proteins which contain heme group and accept a single electron in contrast to NAD and FAD and coenzyme Q which are two electron carriers.



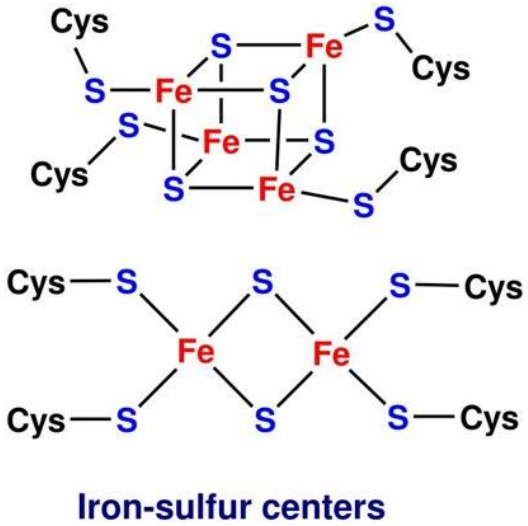
Present in complex 3 and 4. Mitocondria contains 3 classes of cytochrome (a,b,c).

Why do we have different cytochromes?

Because of the variation in conjugated double bonds and side chains.

- Iron sulfur centers :

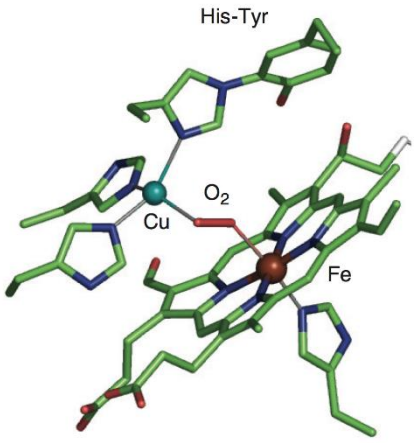
Iron sulfur proteins contain 2 or 4 iron atoms bound to an equal number of sulfur atoms and to cysteine side chains. One electron carriers. Found in complex 1 and 2.



Iron-sulfur centers

Copper containing proteins: in addition to the heme, they contain copper which participate in electron transfers. Found in complex 4.

Cu+2 → 1e- → Cu+1



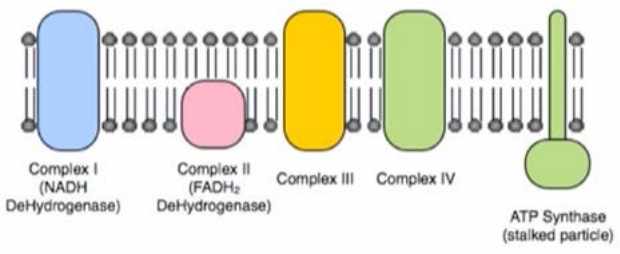
In order for the oxidative phosphorylation to occur we need an enzyme called ATP synthase (not of the electron transport chain), it requires redox reactions, intact IMM and electron donor/acceptor (the ultimate electron acceptor is oxygen) (NADH and FADH2 are electron donors) and the ETC complexes.

NADH+ + H+ → E- TRANSFER THROUGH MULTISTEP → OXYGEN

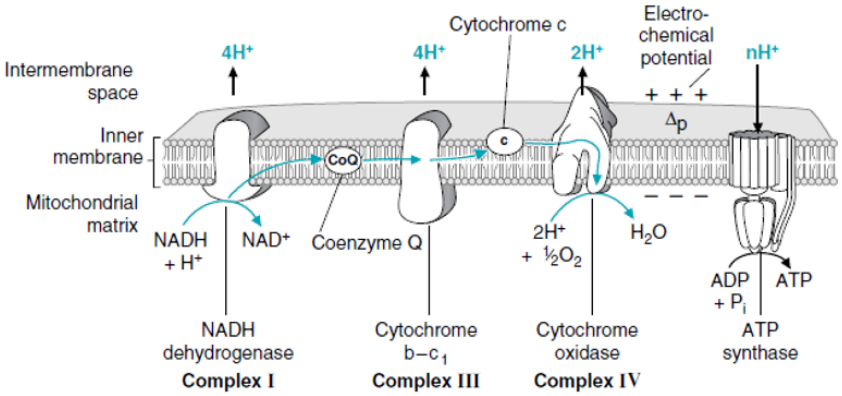
It can do it in one step because it'll produce the same amount of energy, but the cell is doing it step by step, because by doing so, some of the energy would be trapped by the cell enabling it to do work.

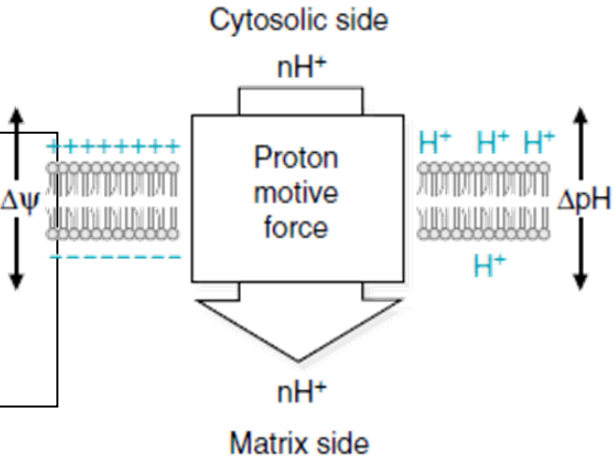
- Inner mitochondrial membrane is important it insulates two compartments and because during the transfer of electrons, electrical current will be formed, which will cause pumping of H+ to the inner mitochondrial membrane. This gradient will help the ATP synthase to phosphorylate the ADP and produce ATP.

WHAT IS THE CHEMO-OSMOTIC THEORY?



hydrogen ions (protons) are pumped from the mitochondrial matrix to the intermembrane space via the hydrogen carrier proteins while the electrons are transferred along the electron transport chain in the mitochondrial inner membrane. As the hydrogen ions accumulate in the intermembrane space, an energy-rich proton gradient is established. As the proton gradient becomes sufficiently intense the hydrogen ions tend to diffuse back to the matrix (where hydrogen ions are less) via the ATP synthase (a transport protein). As the hydrogen ions diffuse (through the ATP synthase) energy is released which is then used to drive the conversion of ADP to ATP (by phosphorylation).





This is delta psy, it refers to the electrochemical potential difference across the inner mitochondrial membrane

This is delta pH, refers to the difference in H+ gradient

They are both considered the motive forces that moves the protons.