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:

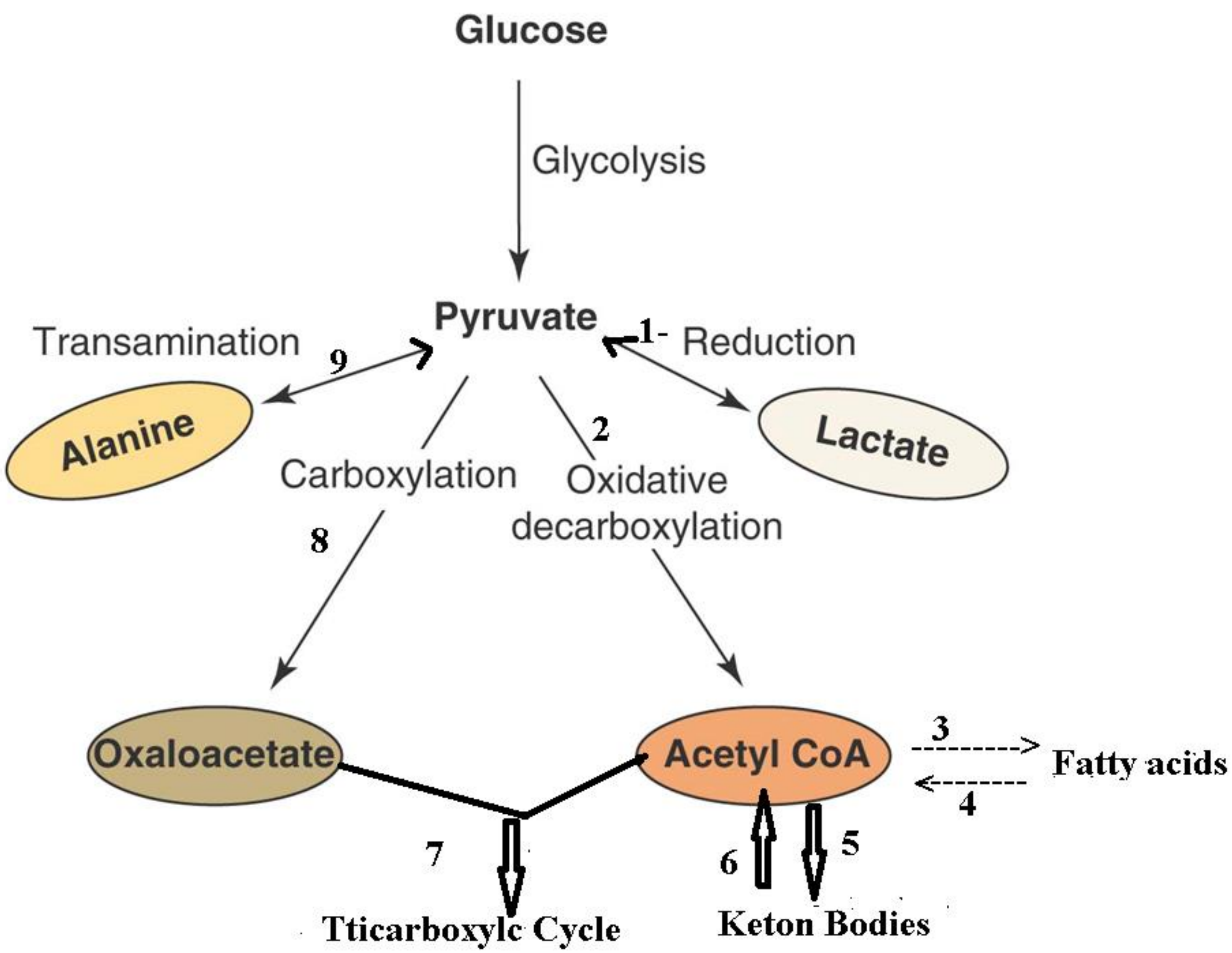
1. The reaction **mechanism** involved in the conversion of pyruvate to acetyl-CoA.
2. The organization of the **3 enzymes-E1, E2, E3-** of the multienzyme complex.
3. The **5 coenzymes** involved in the reaction and the 5 B vitamins from which they derived.
4. The **allosteric and covalent modification**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

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

- 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), and NAD (niacin)-are required.

E1 : Pyruvate dehydrogenase

E2 : Dihydrolipoyl transacetylase

E3 : Dihydrolipoyl dehydrogenase

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 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.**

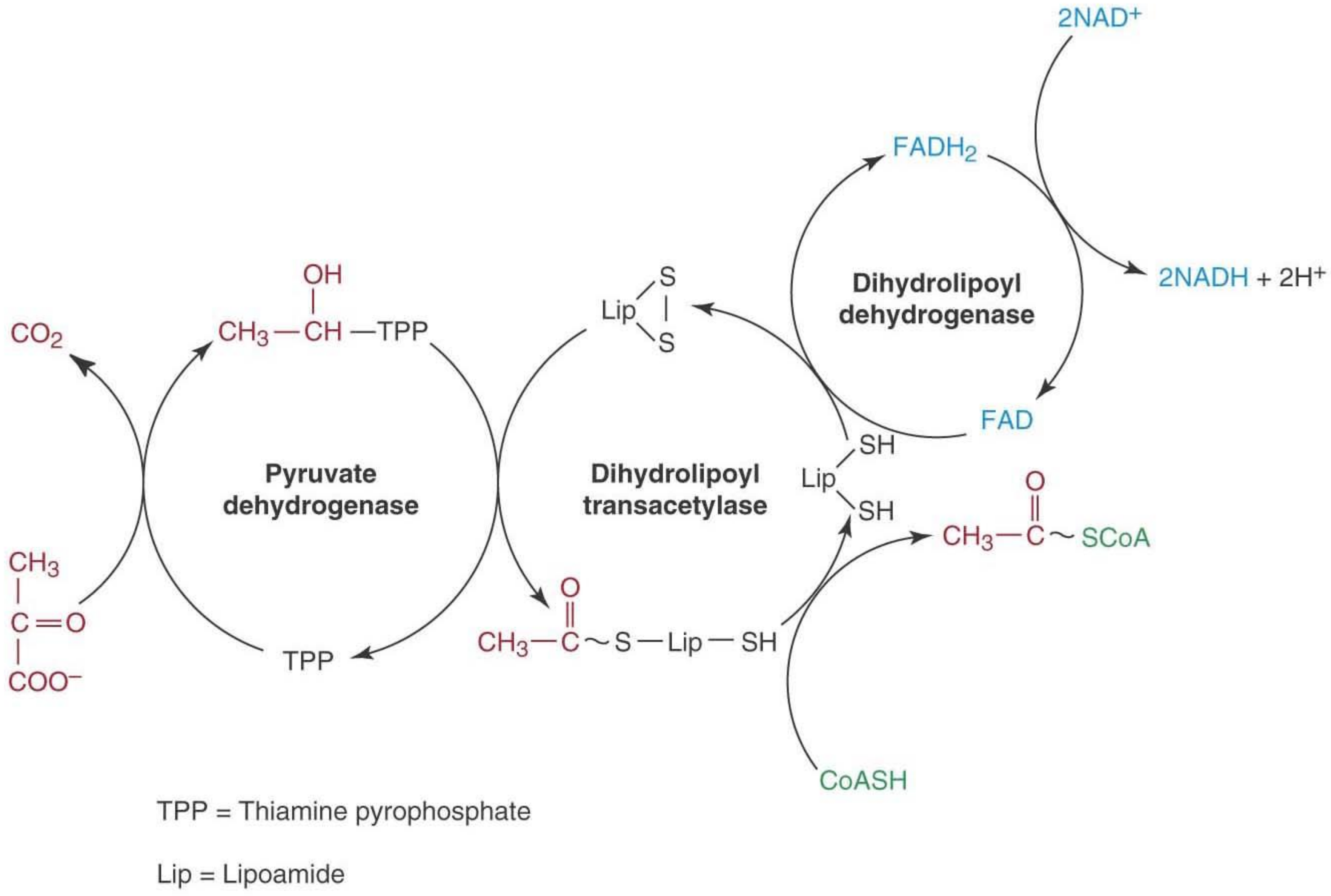
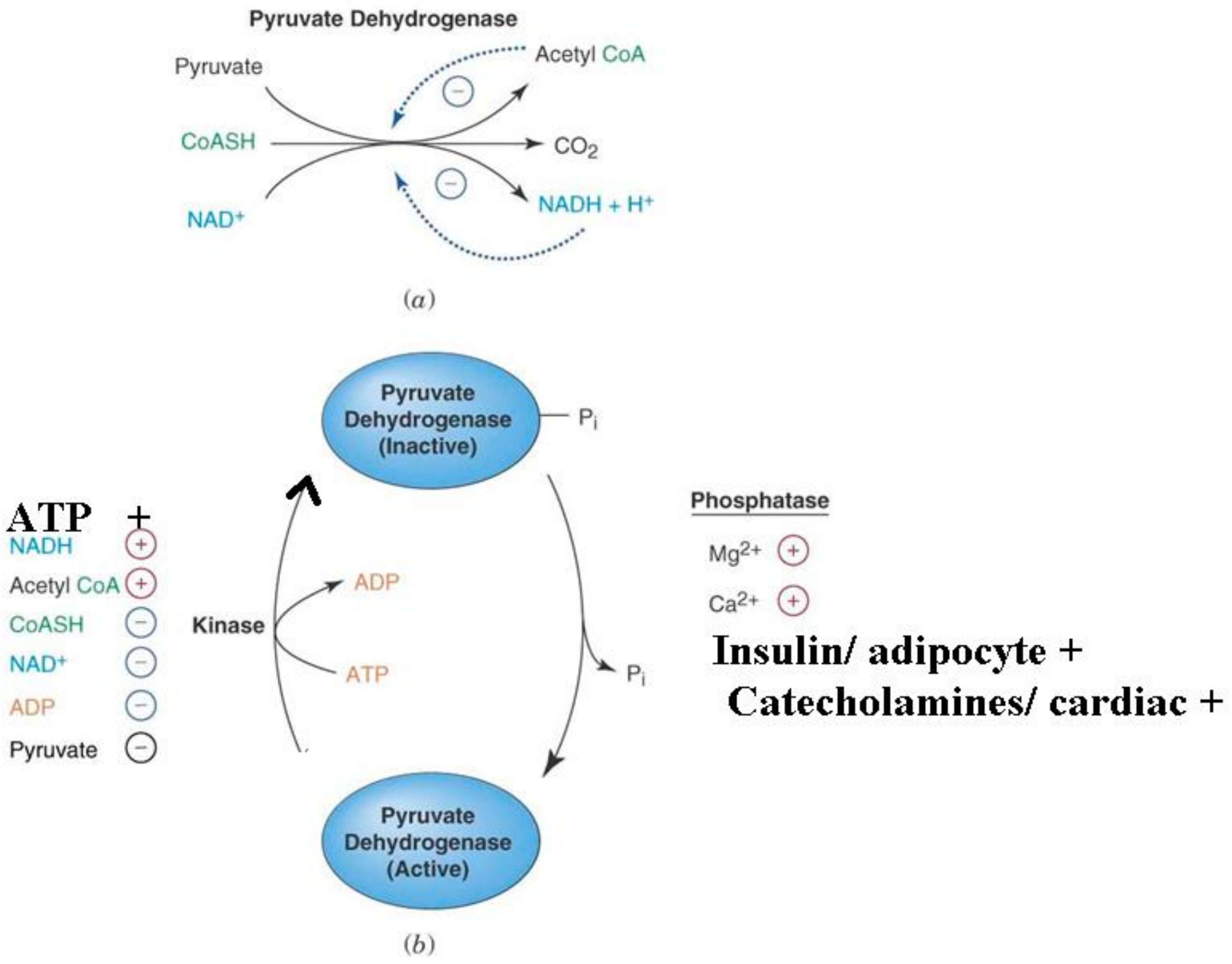


Figure 14.14 Mechanism of the pyruvate dehydrogenase multienzyme complex.

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



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

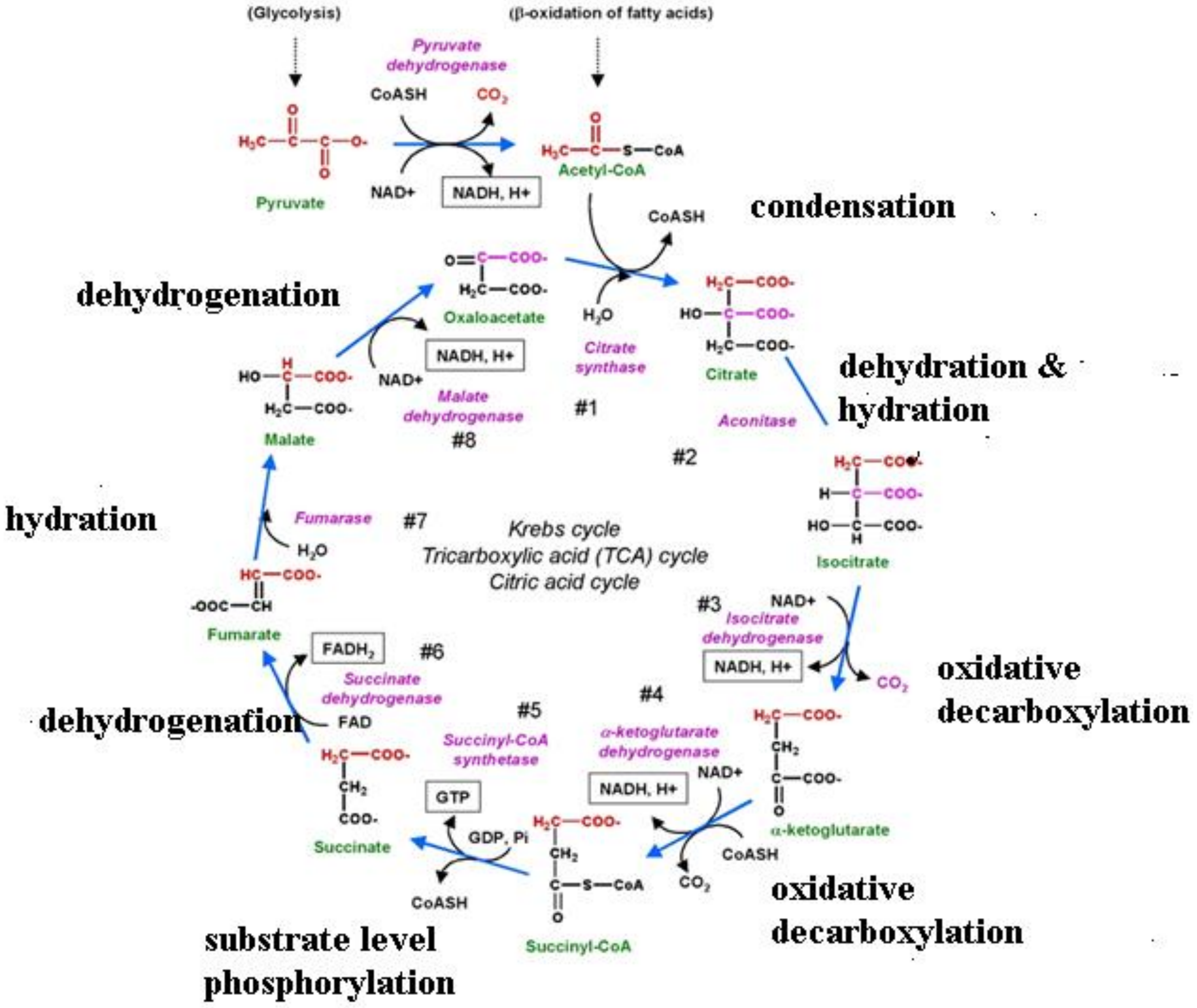
IMPORTANT FEATURES OF TCA CYCLE

- 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

1. **ACONITASE**: dehydration followed by hydration ΔG0`= +1.5 kcal/mol
2. **ISOCITRATE DEHYDROGENASE**: ΔG0`= -5 kcal/mol, oxidative decarboxylation of isocitrate to alpha-ketoglutarate; 1^{st} 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
3. **α-KETOGLUTARATE DEHYDROGENASE COMPLEX :** ΔG0`= -8 kcal/mol 2^{nd} molecule of CO2, and the 2^{nd} 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. The complex consists of α-ketoglutarate dehydrogenase, dihydrolipoyl transsuccinylase and dihydrolipoyldehydrogenase. 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



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)

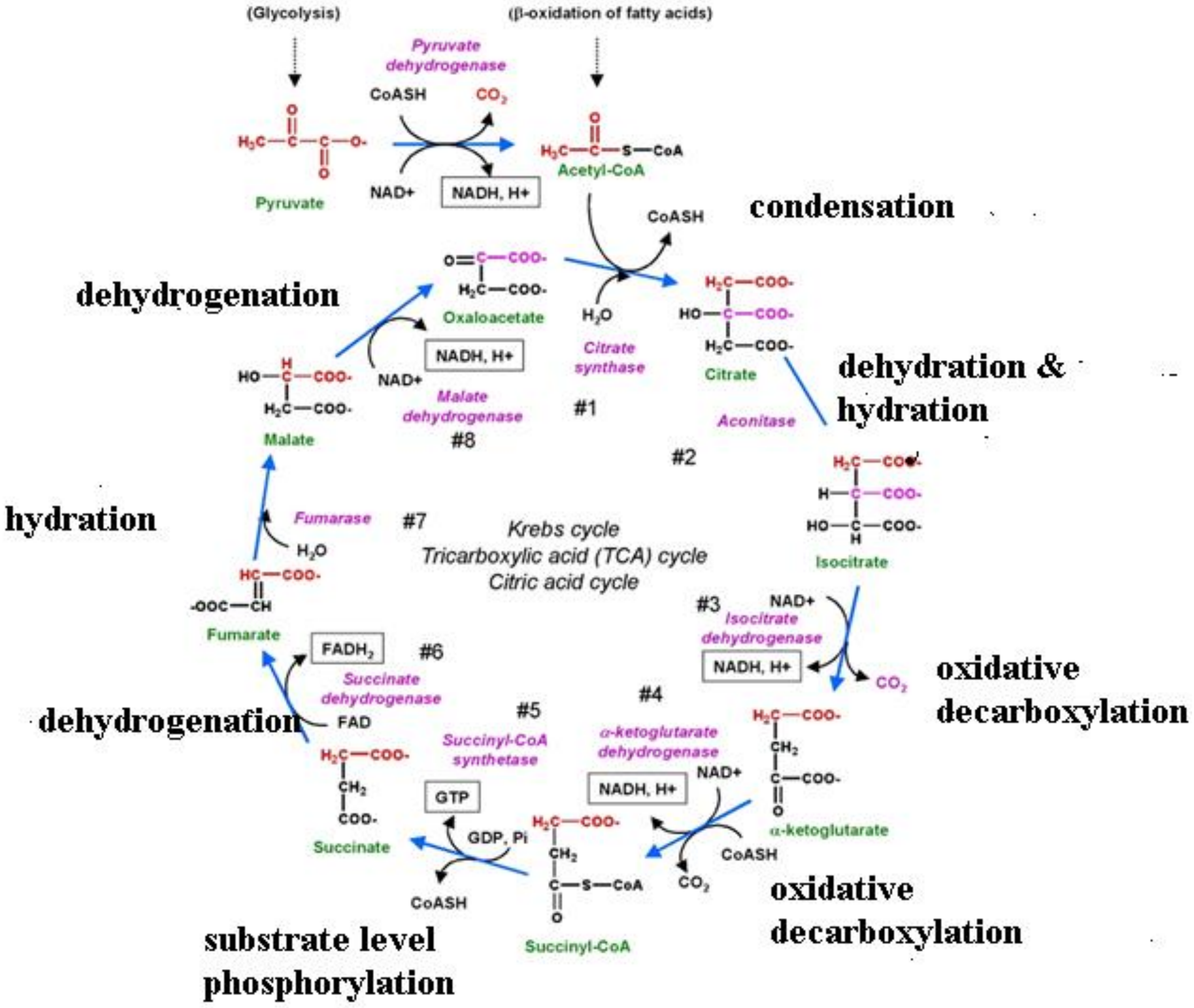
Adenylate kinase: AMP+ATP→2ADP…(2)

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

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8. MALATE DEHYDROGENASE:

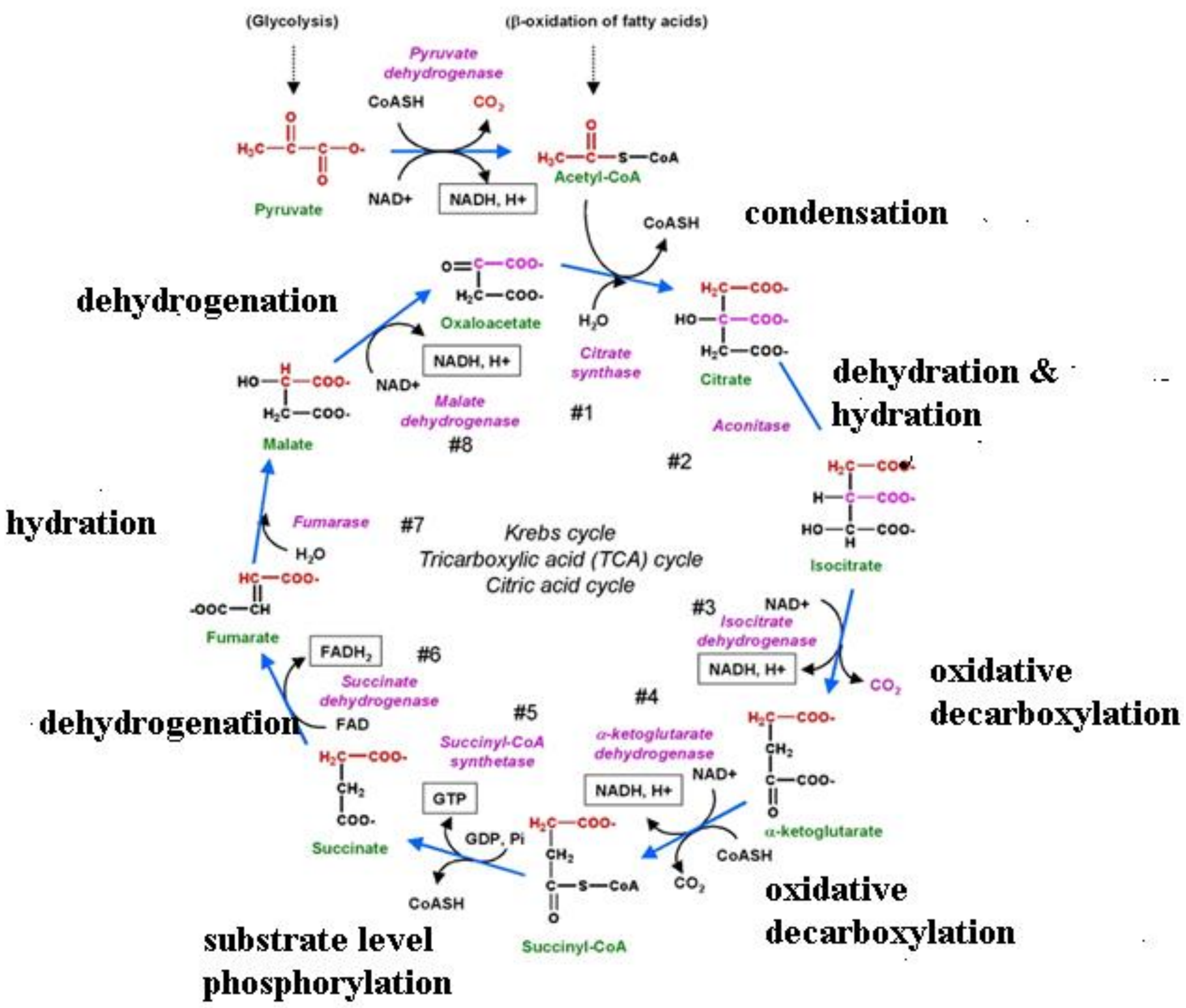
ΔG0`=+7.1. completes the cycle by regenerating OAA-A REGENERATING SUBSTRATE. It is the final of three reactions in which NADH+H is produced.

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

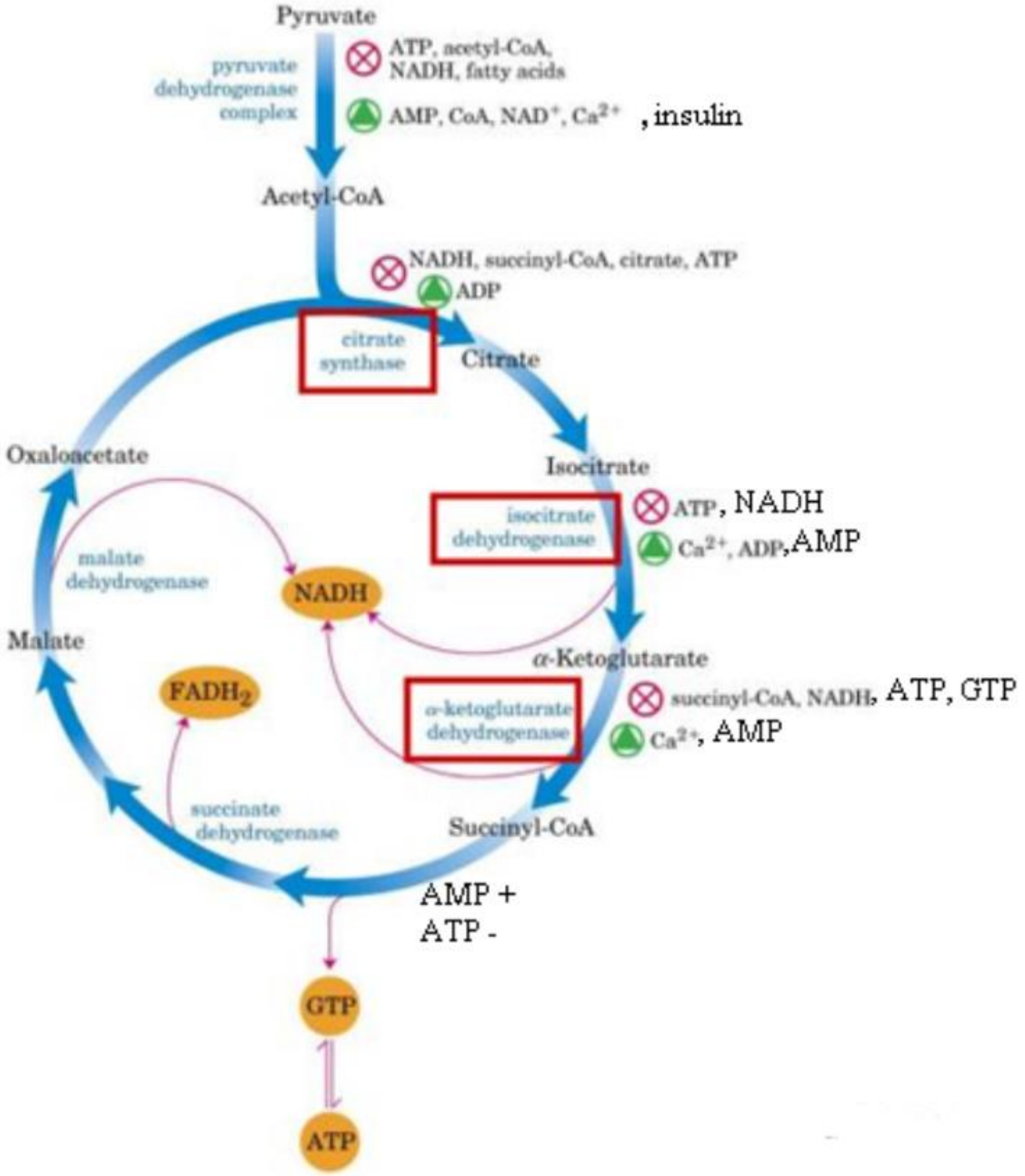
SUM:

AcetylCoA+3NAD+FAD+GDP+Pi+2H2O→

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



Regulation of Citric Acid Cycle

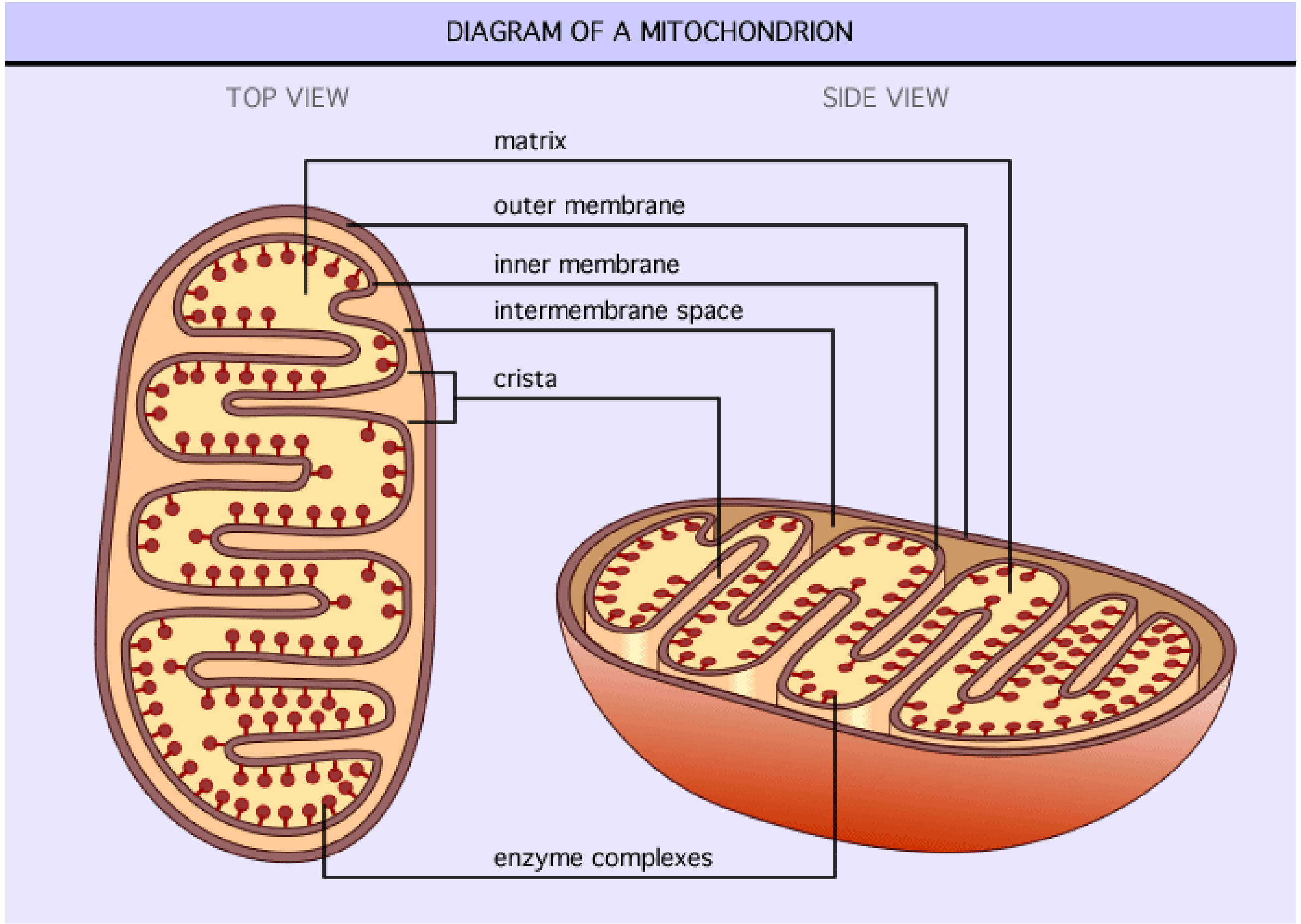


The citric acid cycle is regulated at its three exergonic steps

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.

- 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.

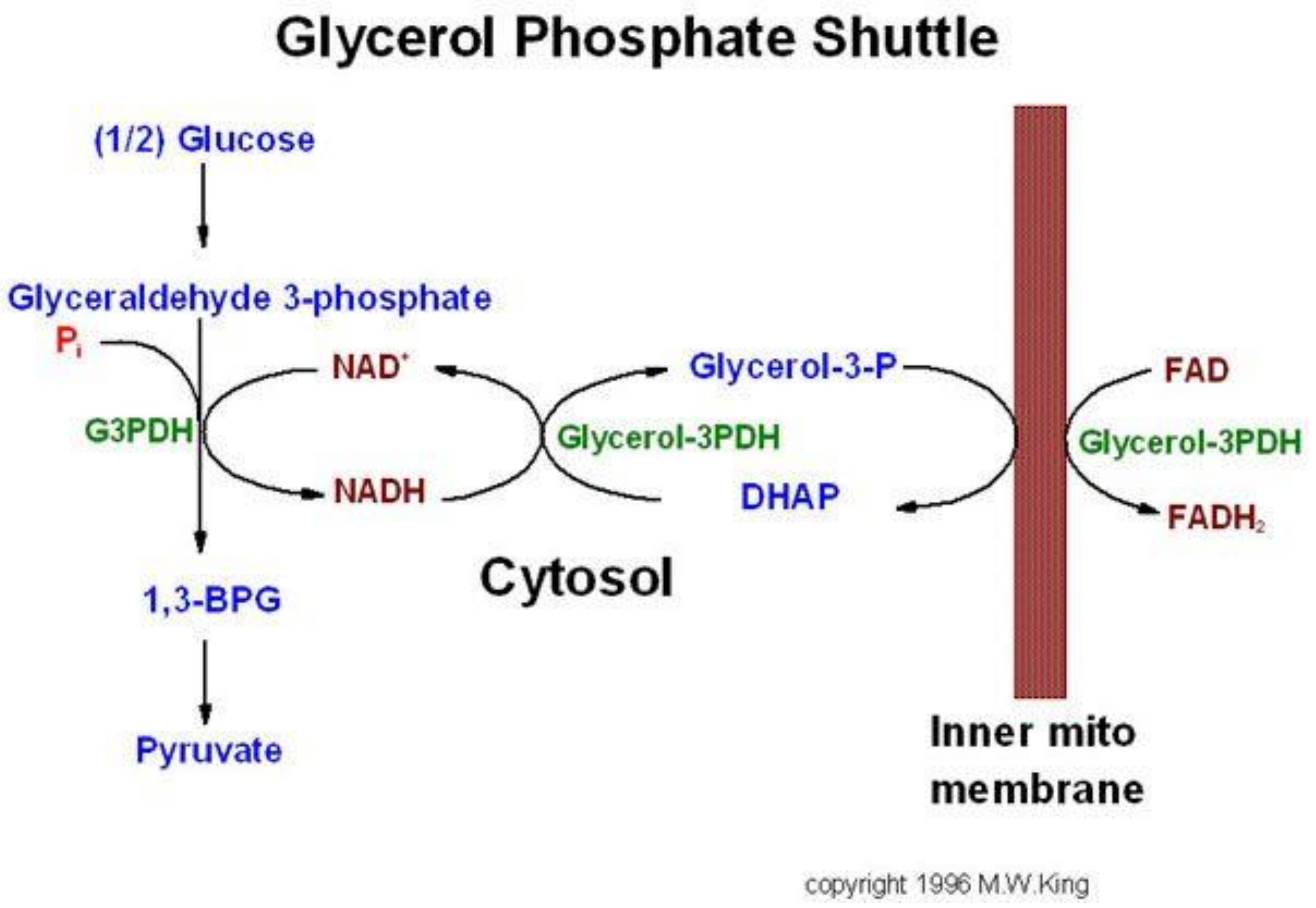


Location of the various mitochondrial enzymes in mitochondrial compartments.

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-COA DH
			BETA-KETOACYL-COA THIOLASE

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



Malate-Aspartate Shuttle

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

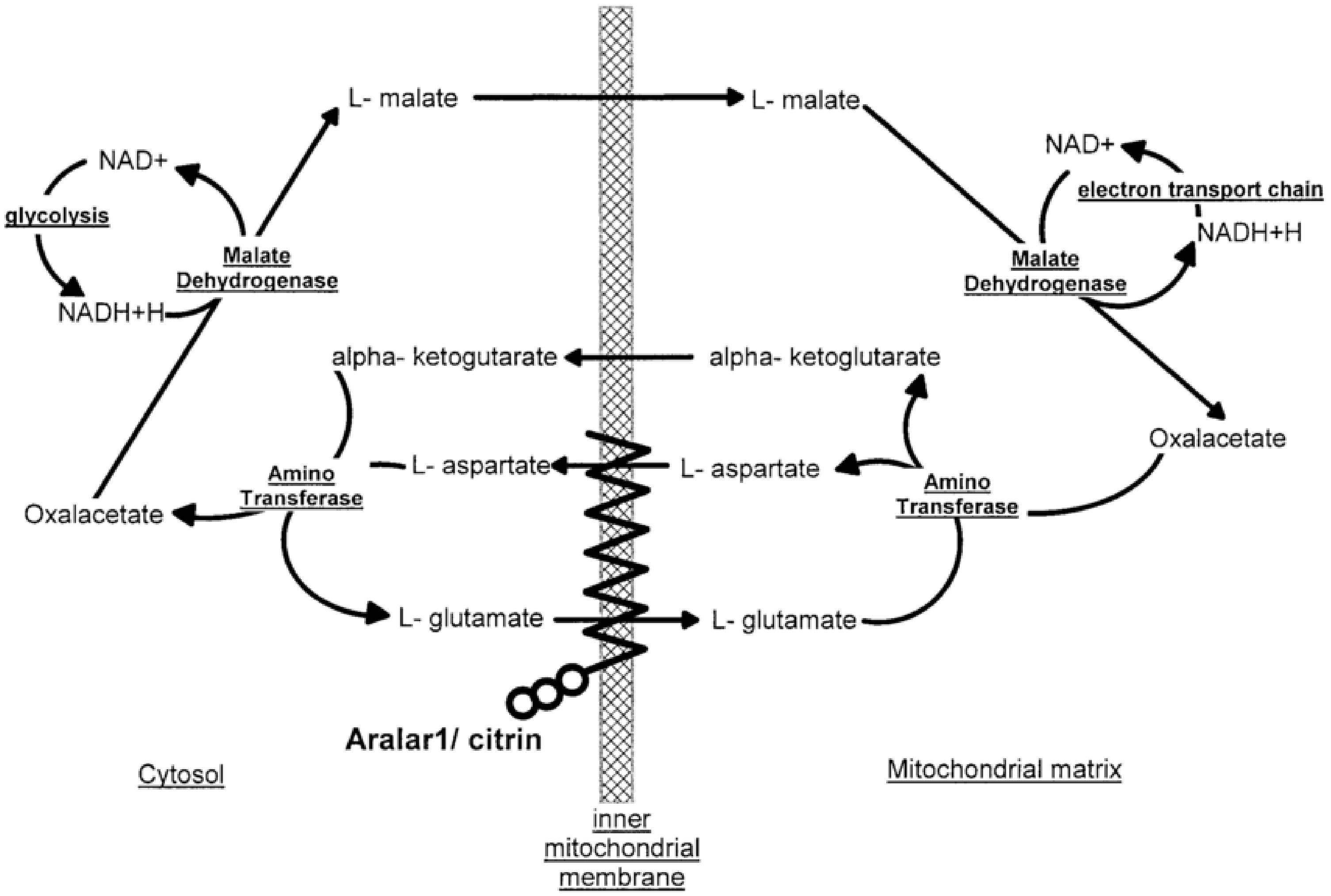
Malate is transported to mitochondria where NAD is reduced to NADH+H+ and OAA is regenerated.

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



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.

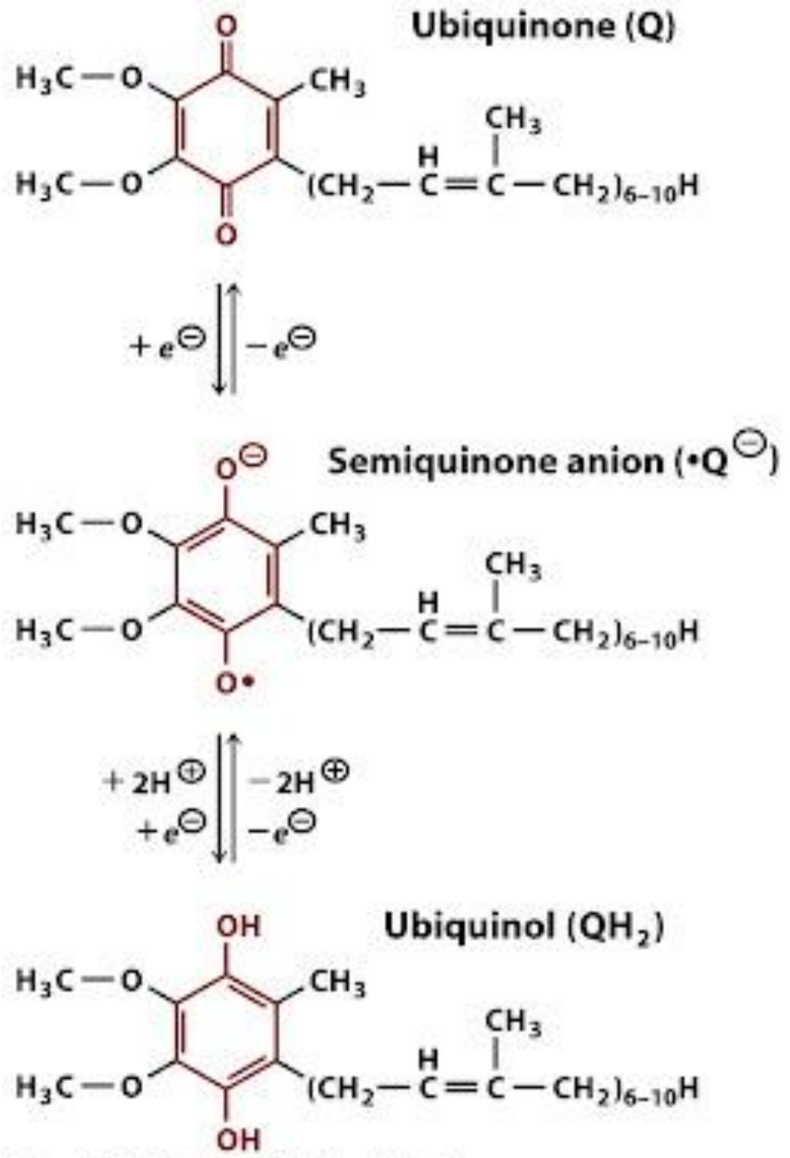
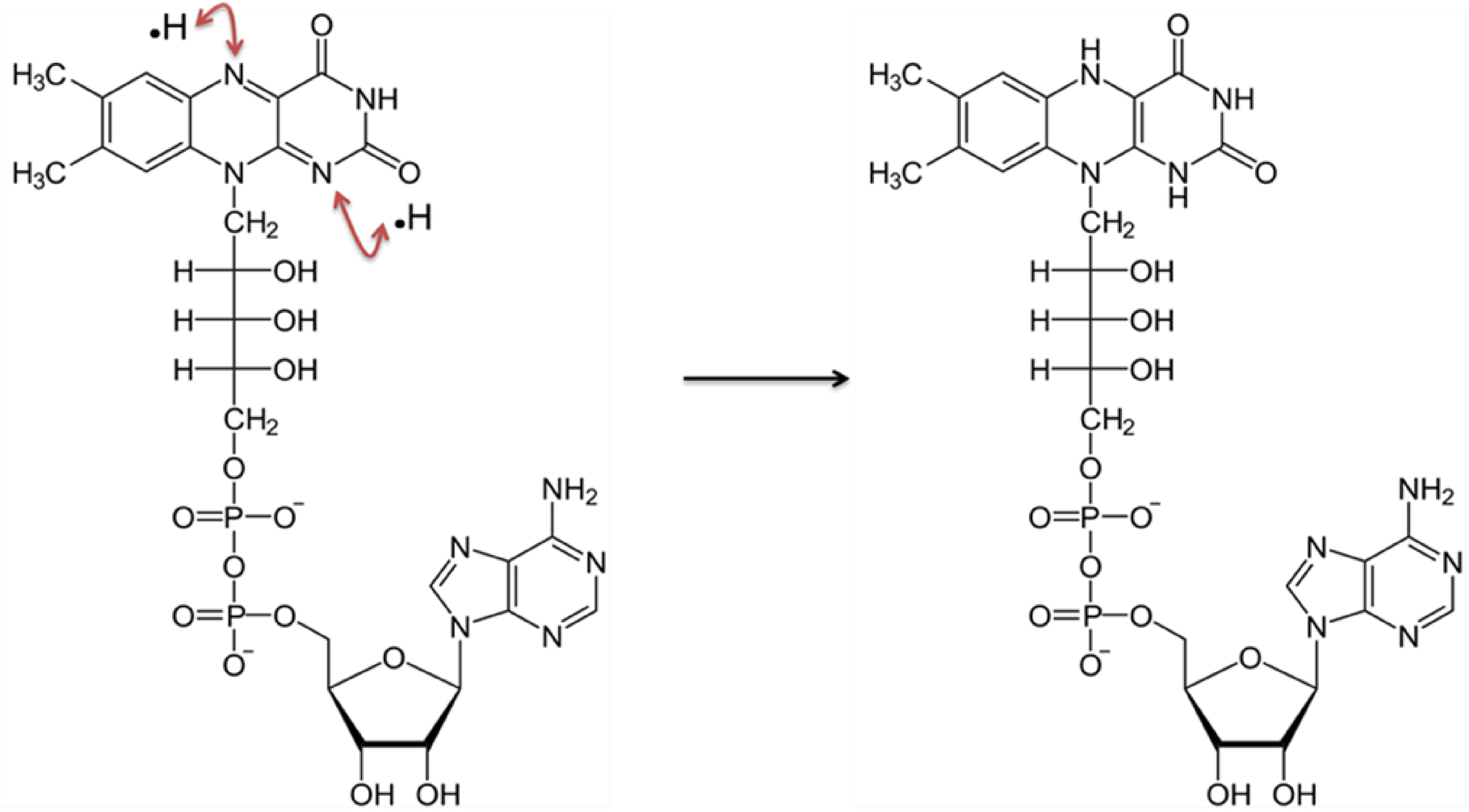
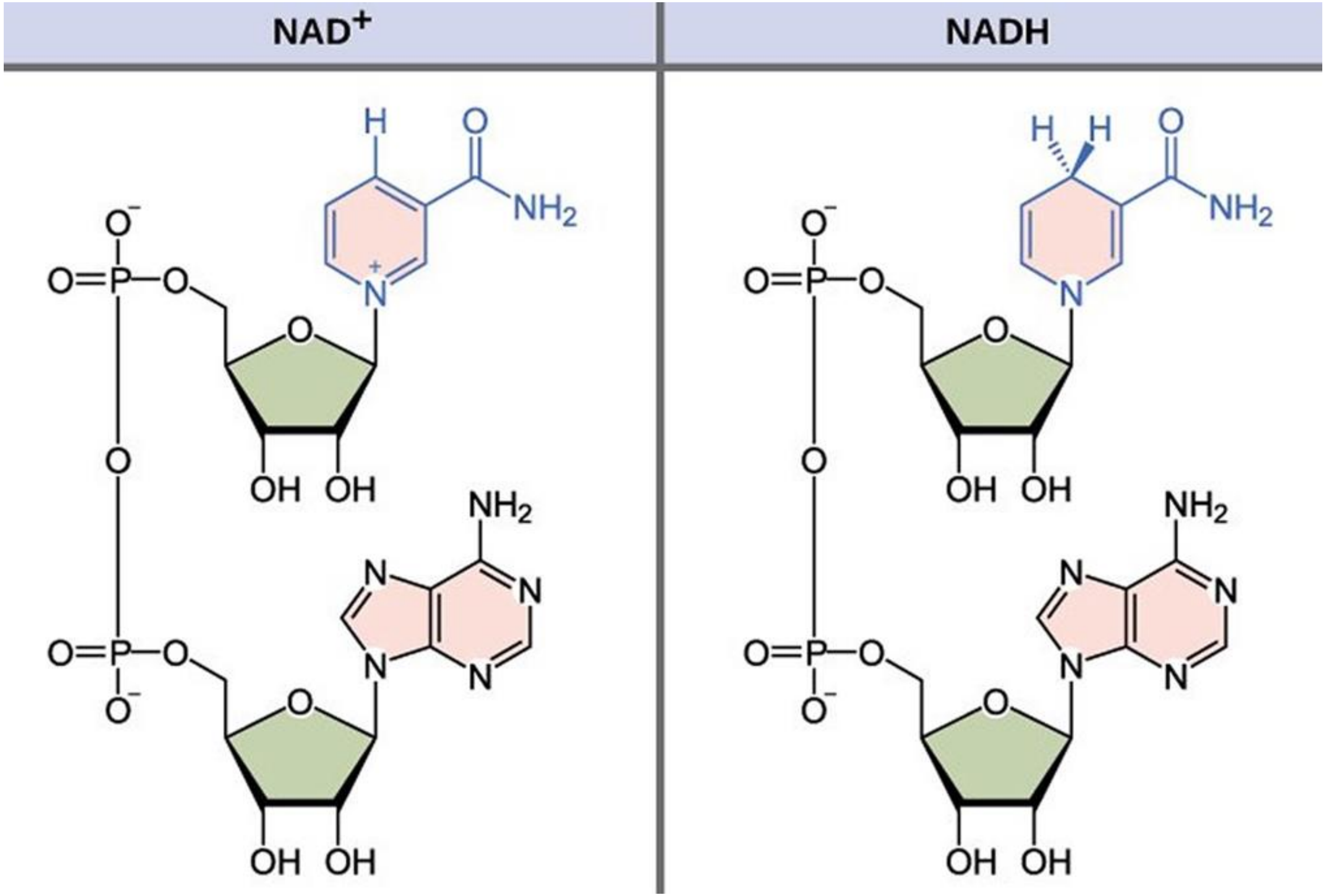


Figure 7-31 Principles of Biochemistry, 4/e



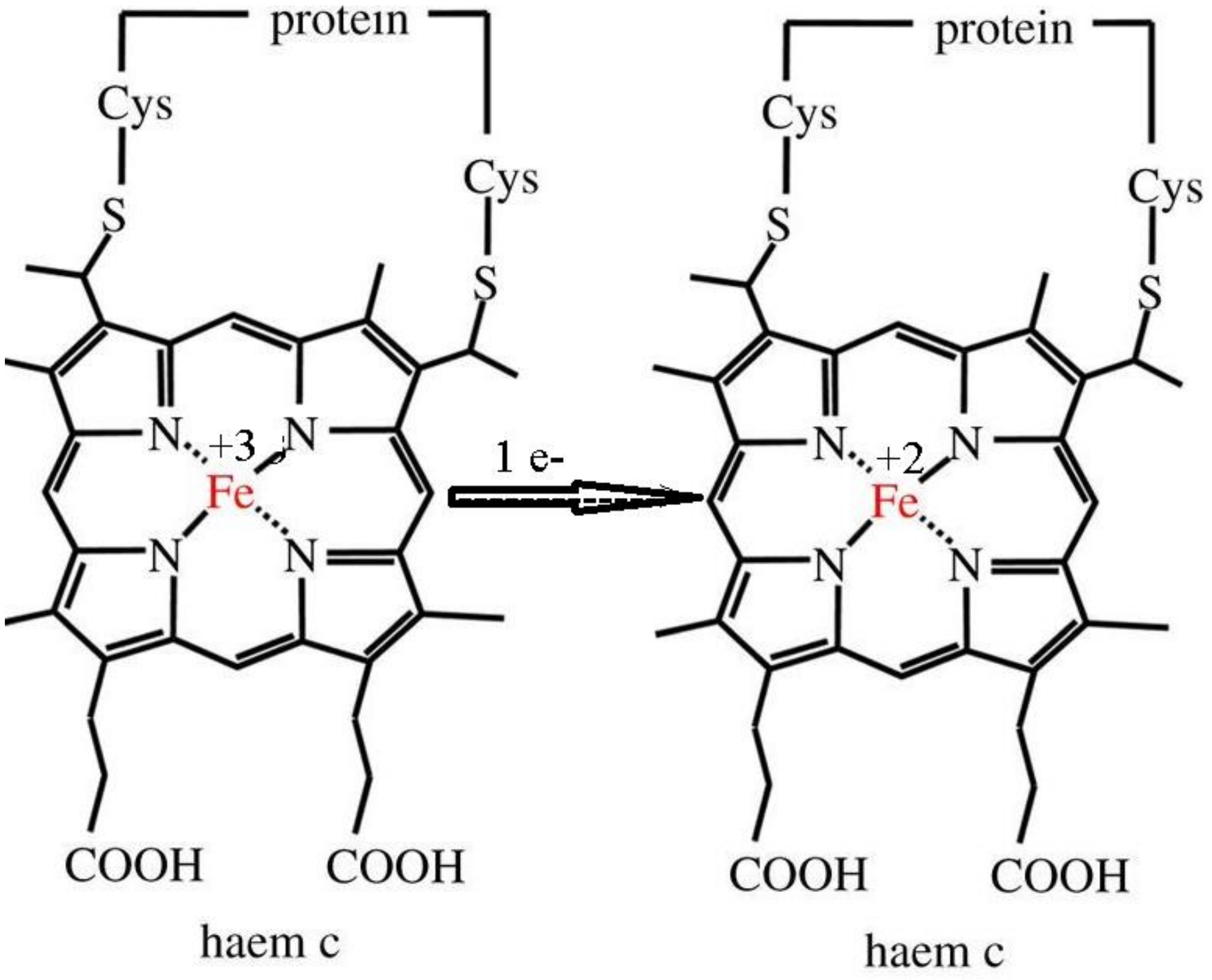
FAD $\qquad$ $FADH_2$


NAD+
NADH

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.

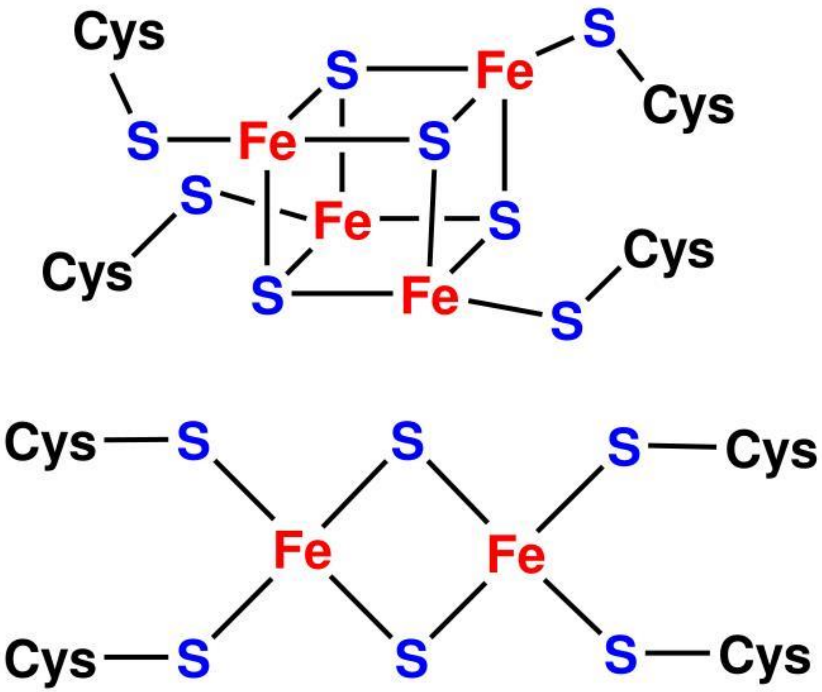
Mitochondria contain three classes o f cytochromes (a , b, & c)



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.



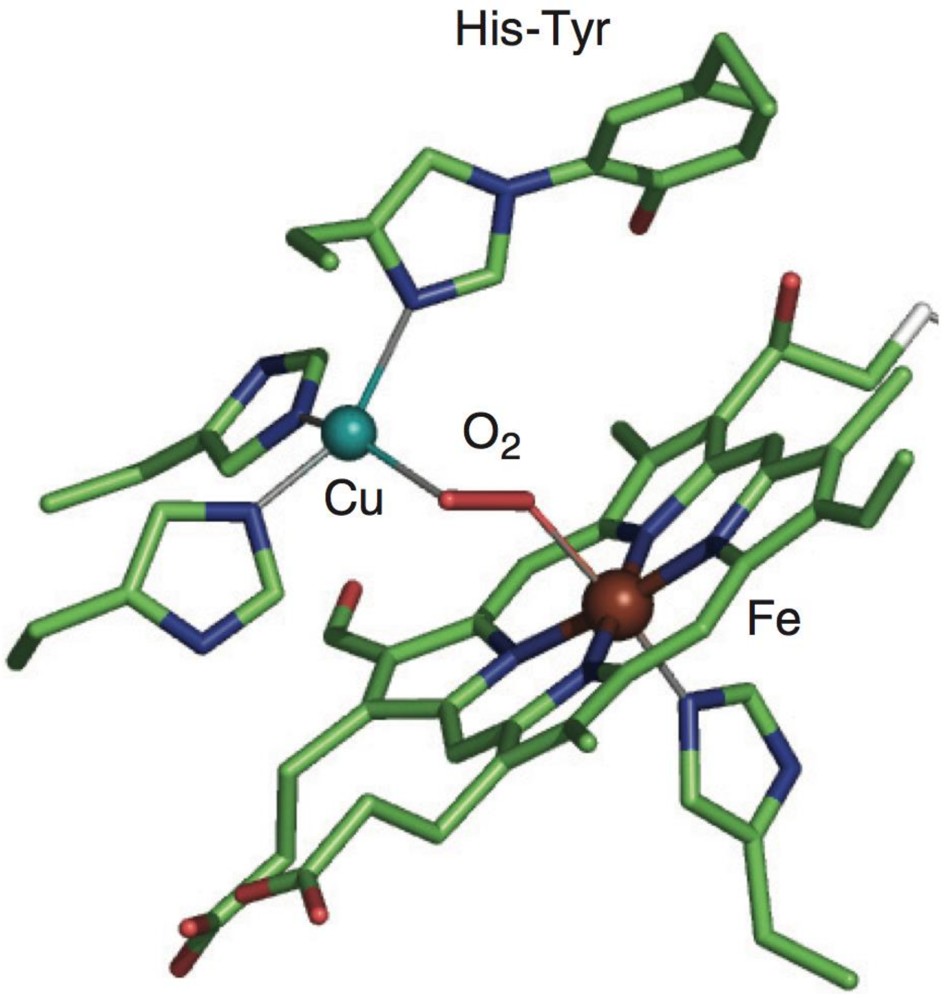
Iron-sulfur centers

Copper Containing Proteins

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

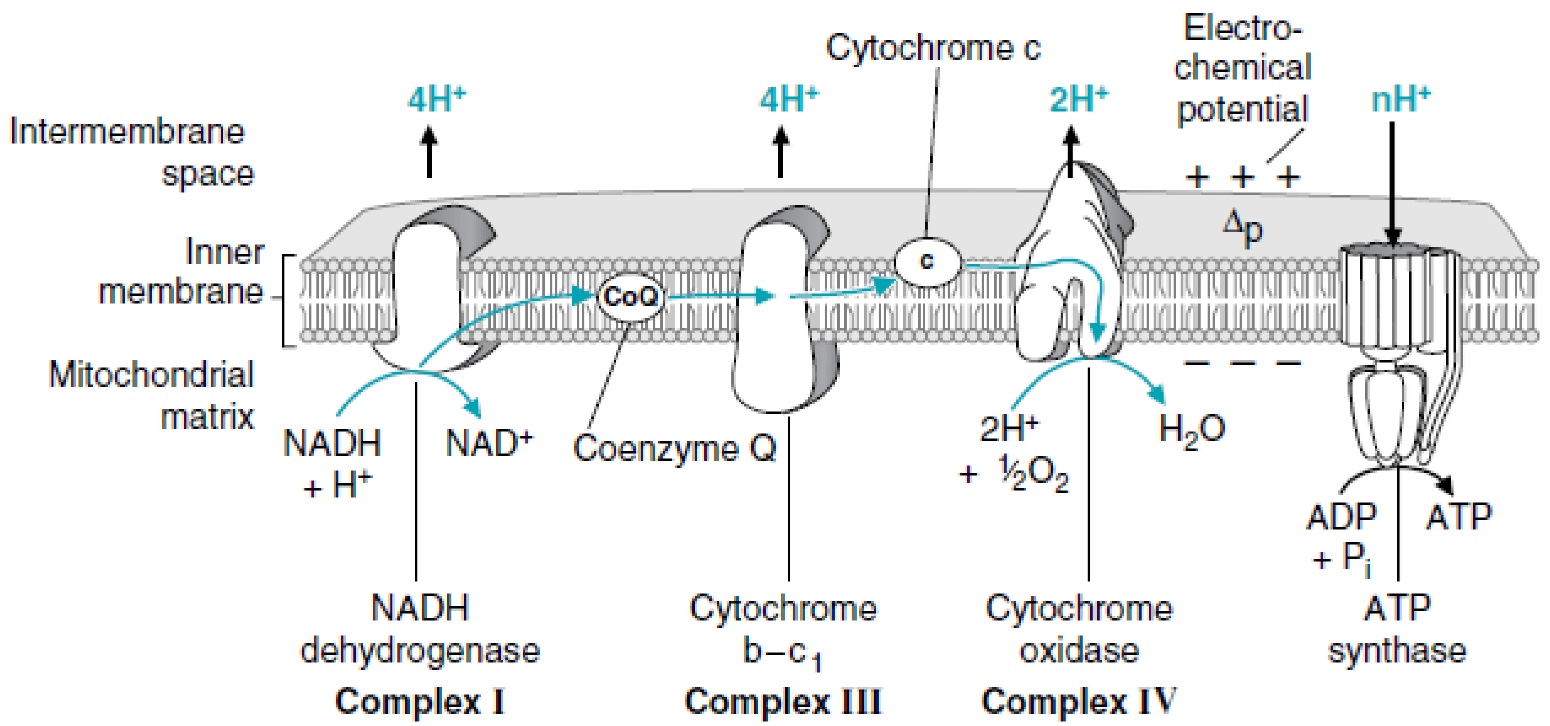
1 e-

Cu^{2+}------------------→Cu^{1+}

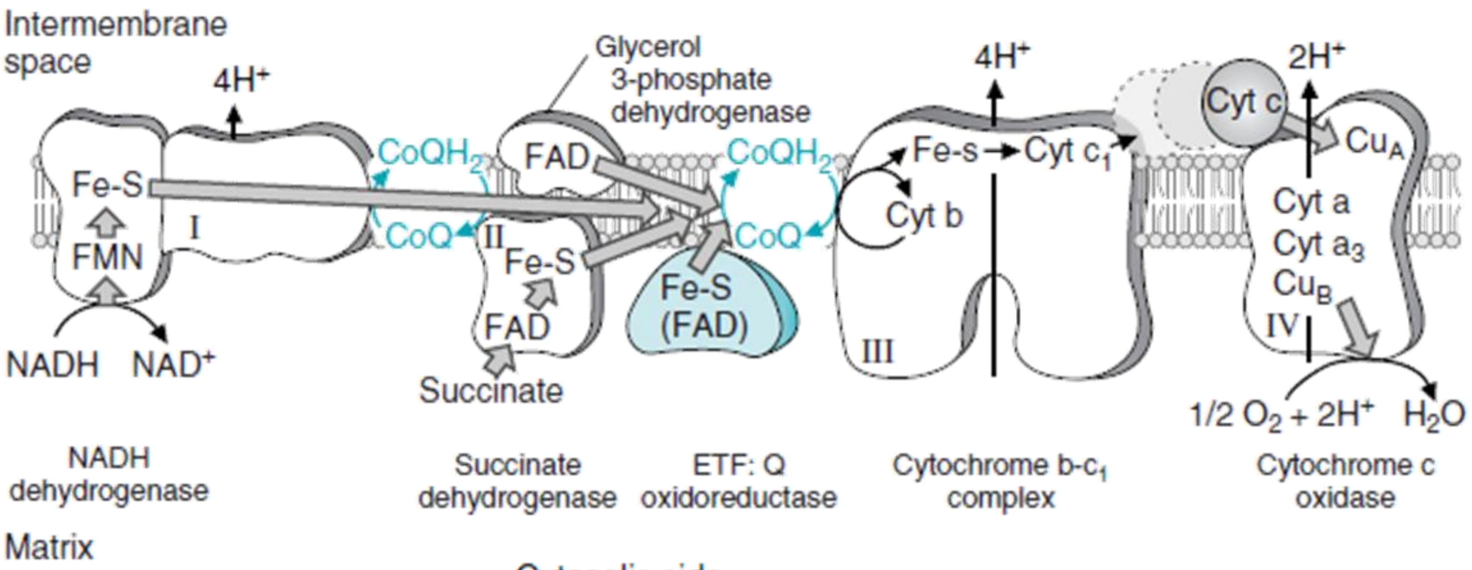


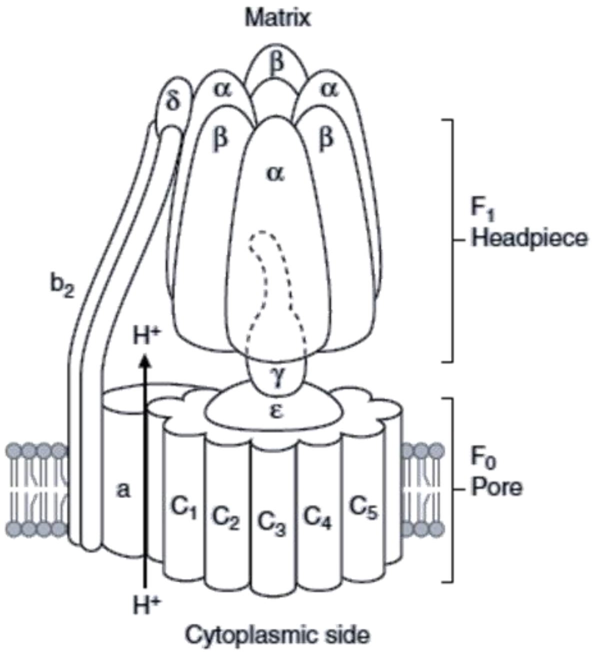
Requirements of OxPhos

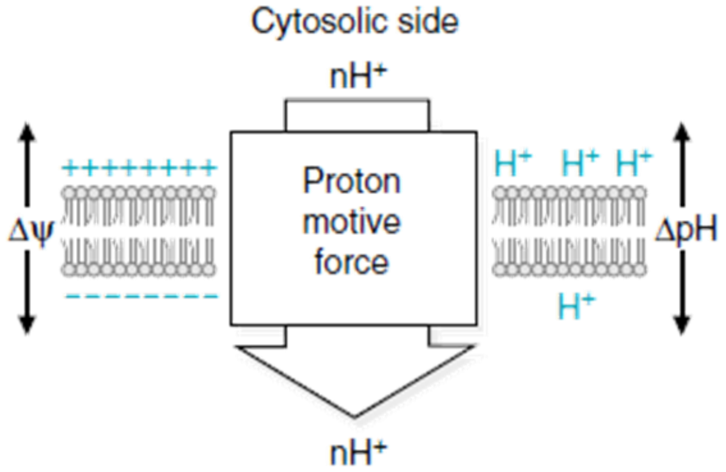
- **Redox reaction: electron donor (NADH or FADH2) & electron acceptor (O2)**
- **An intact IMM**
- **ETC of proteins: 4 complexes+1 soluble protein +CoQ**
- **ATP synthase**



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







ANATOMY OF THE RESPIRATORY CHAIN

1. Complex I: NADH Reductase.

NADH + e-> CoQ

2. Complex II: Succinate-CoQ Reductase,

Succinate+ e →CoQ.

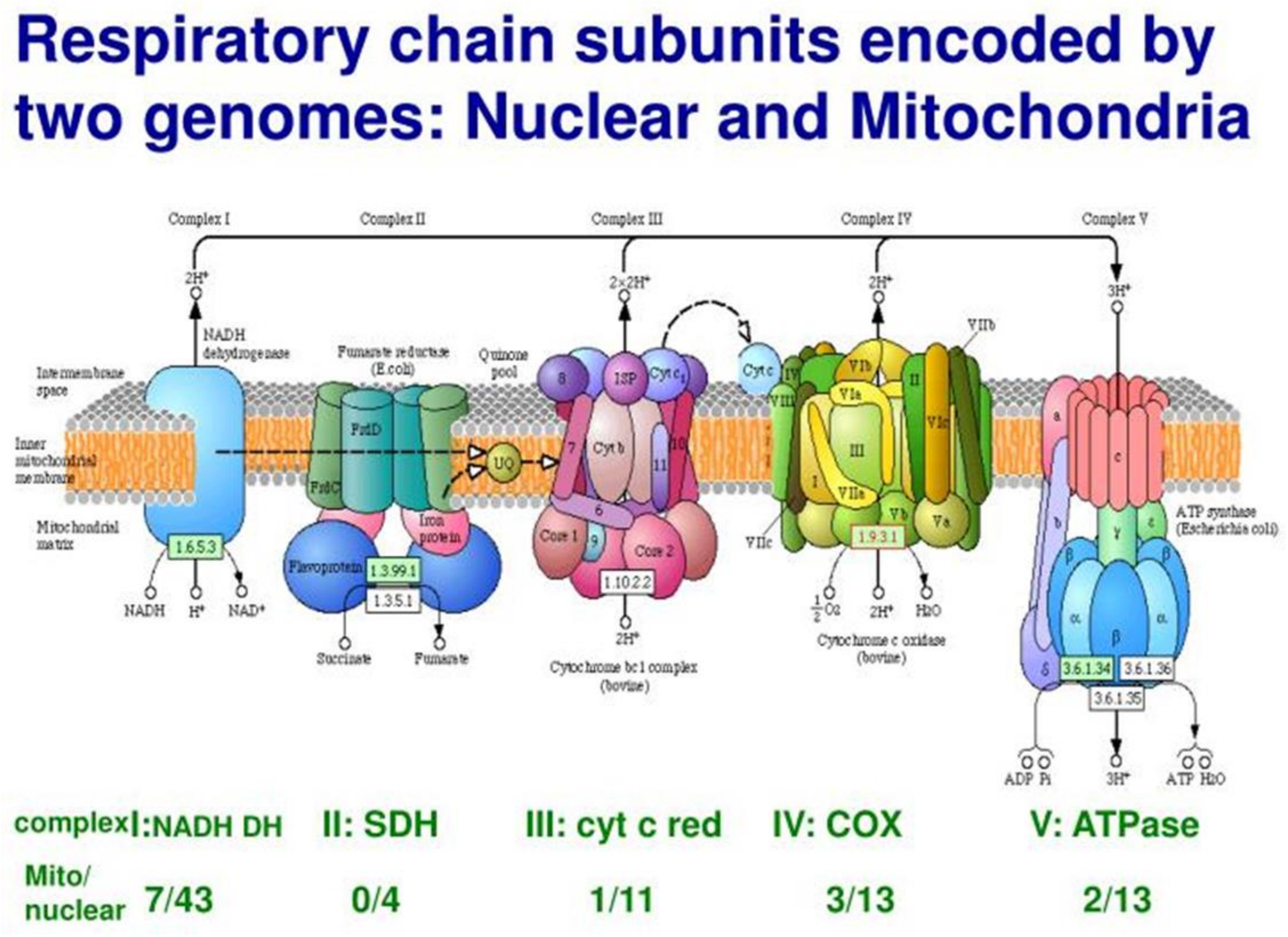
3. Complex III, Cytochrome C Reductase,

CoQ +e →Cyt c

4. Complex IV, Cyt Oxidase,

Cyt c+e → Oxygen

5. Complex V: ATPase



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

NADH-Q oxidoreductase

More than 25 polypeptide chain

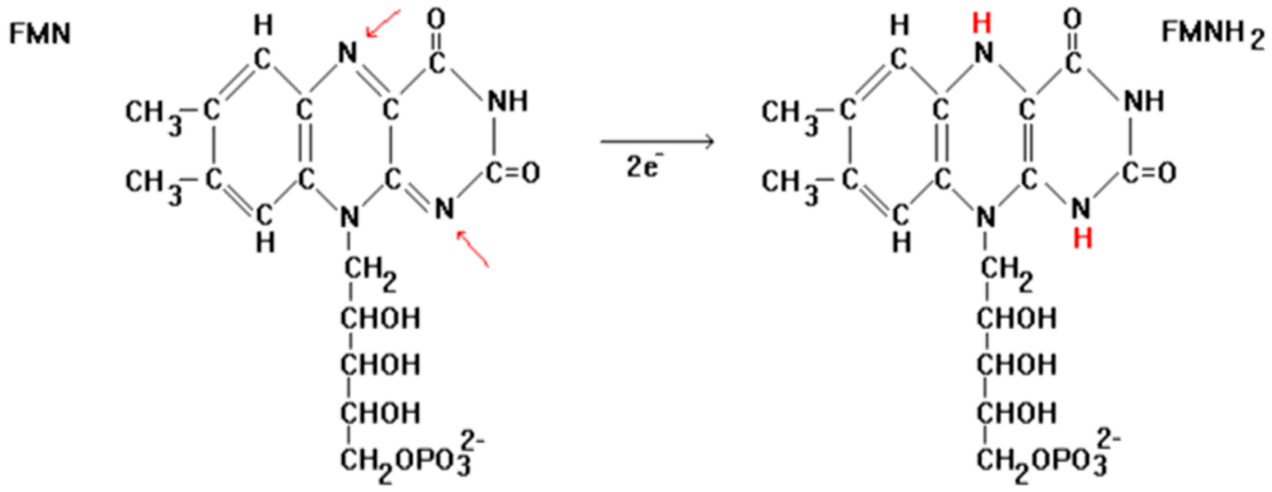
A huge flavoprotein membrane-spanning complex

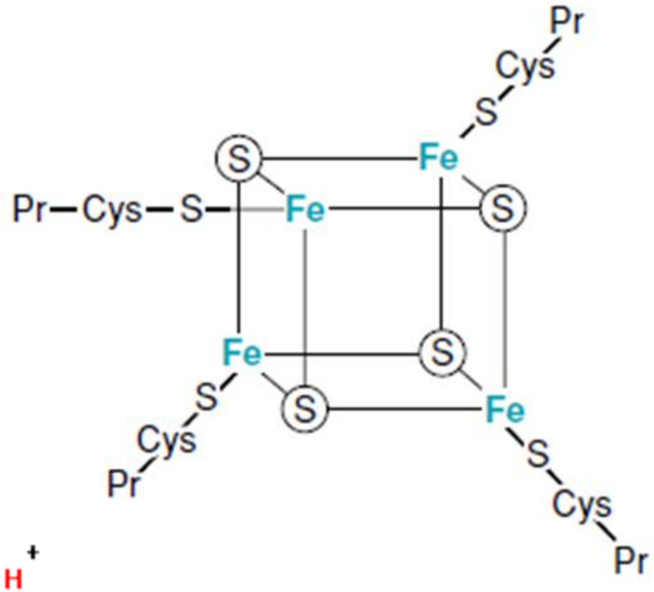
The FMN is tightly bound

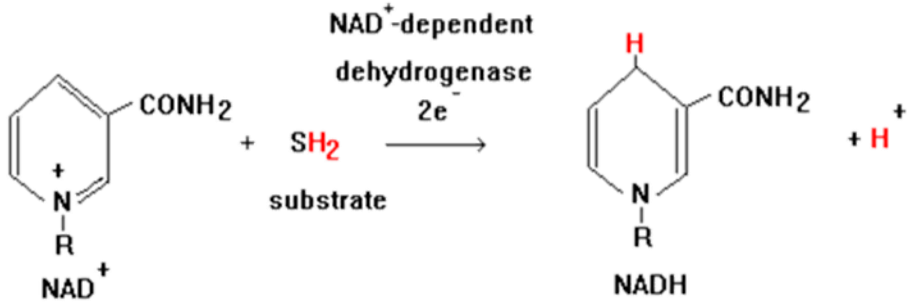
Seven Fe-S centers of at least two different types

Binds NADH & CoQ

4 H+



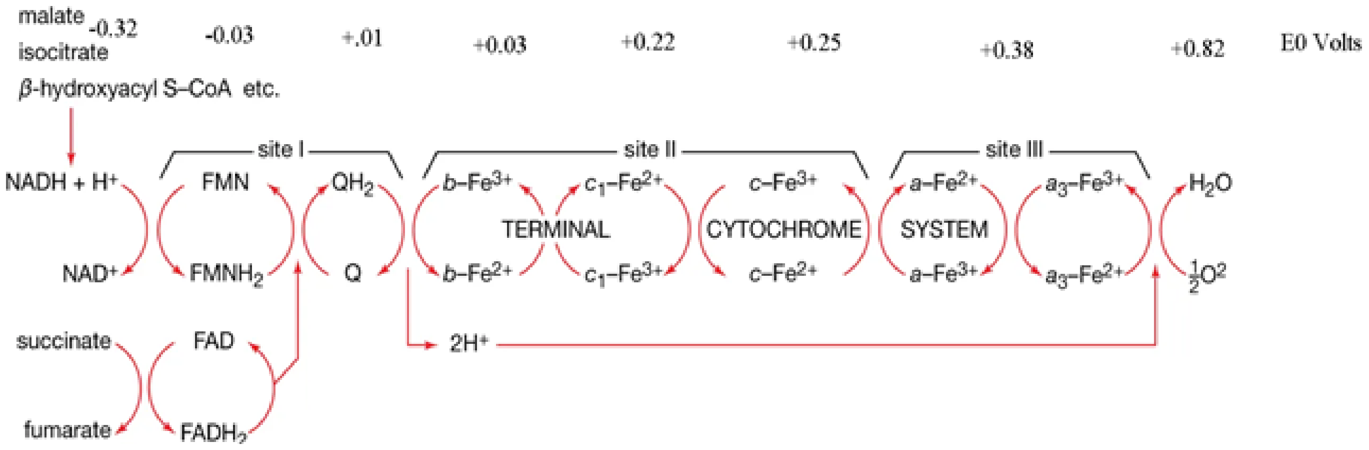


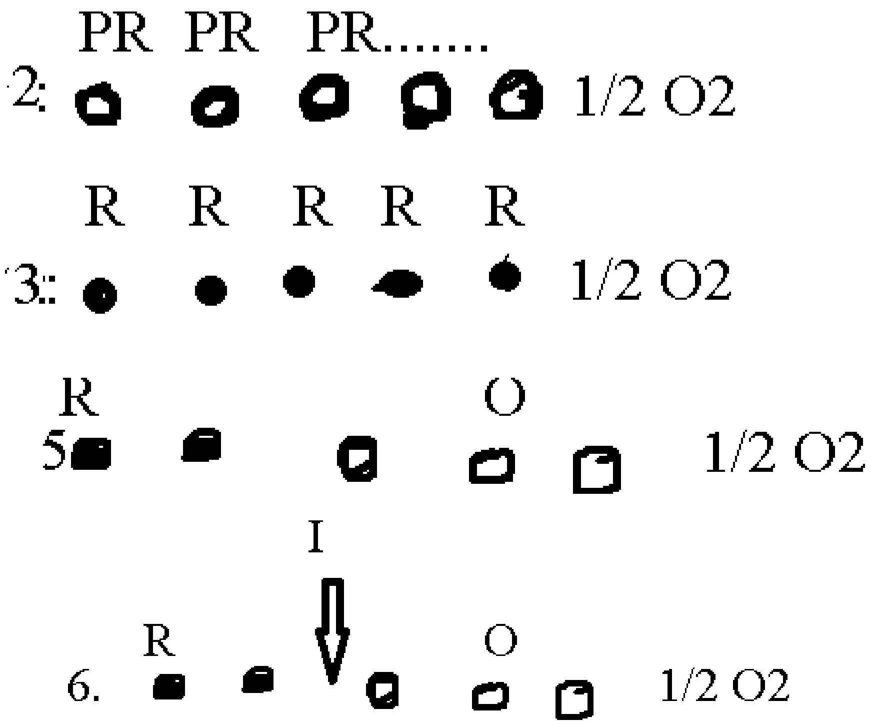


Sequence of carriers in ETC

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.

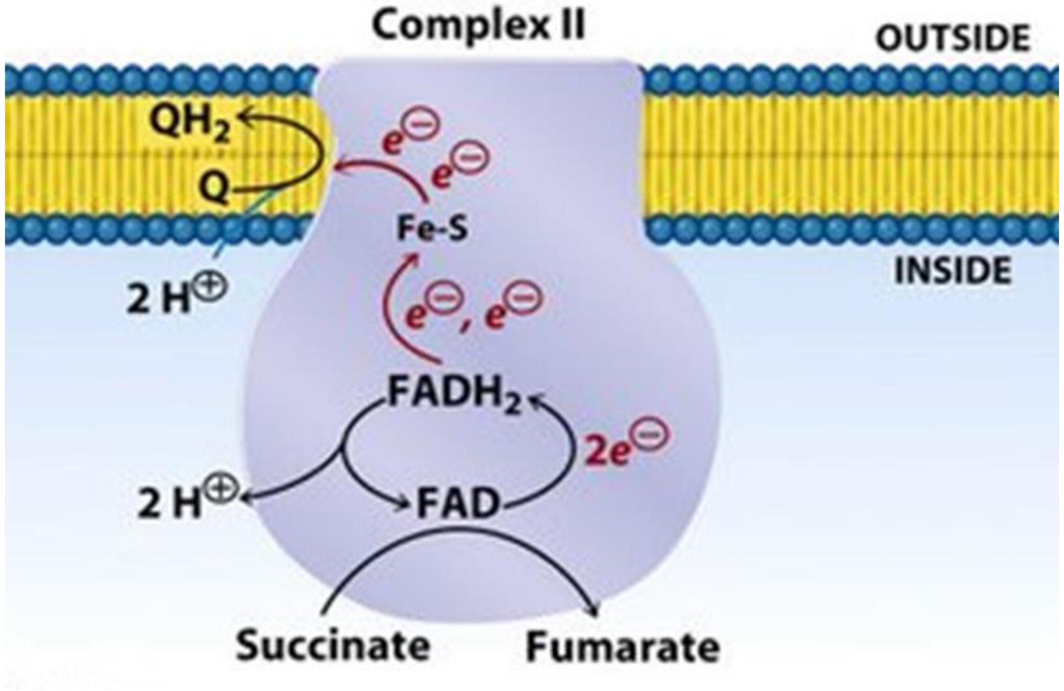
1.

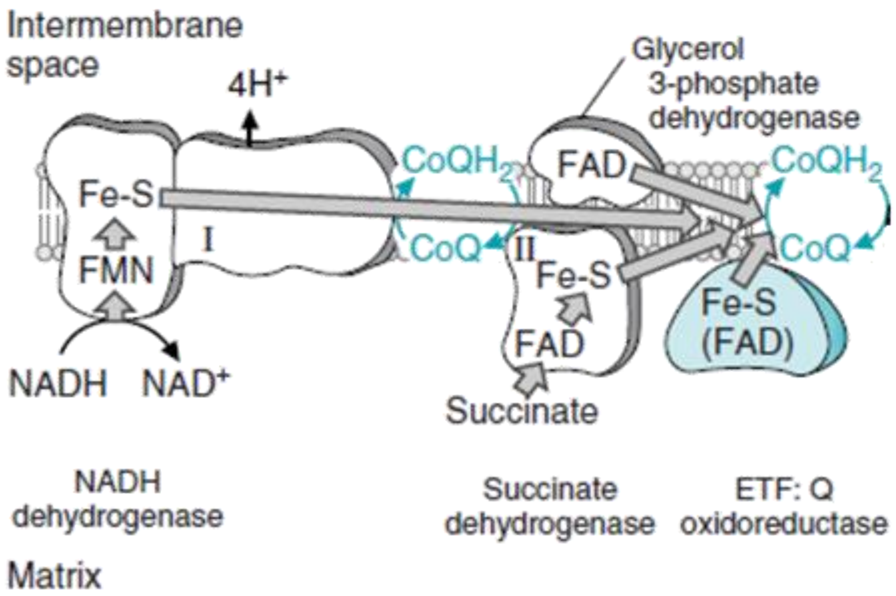




Oxi–Red Components of the ETC "Succinate Dehydrogenase" – Complex II

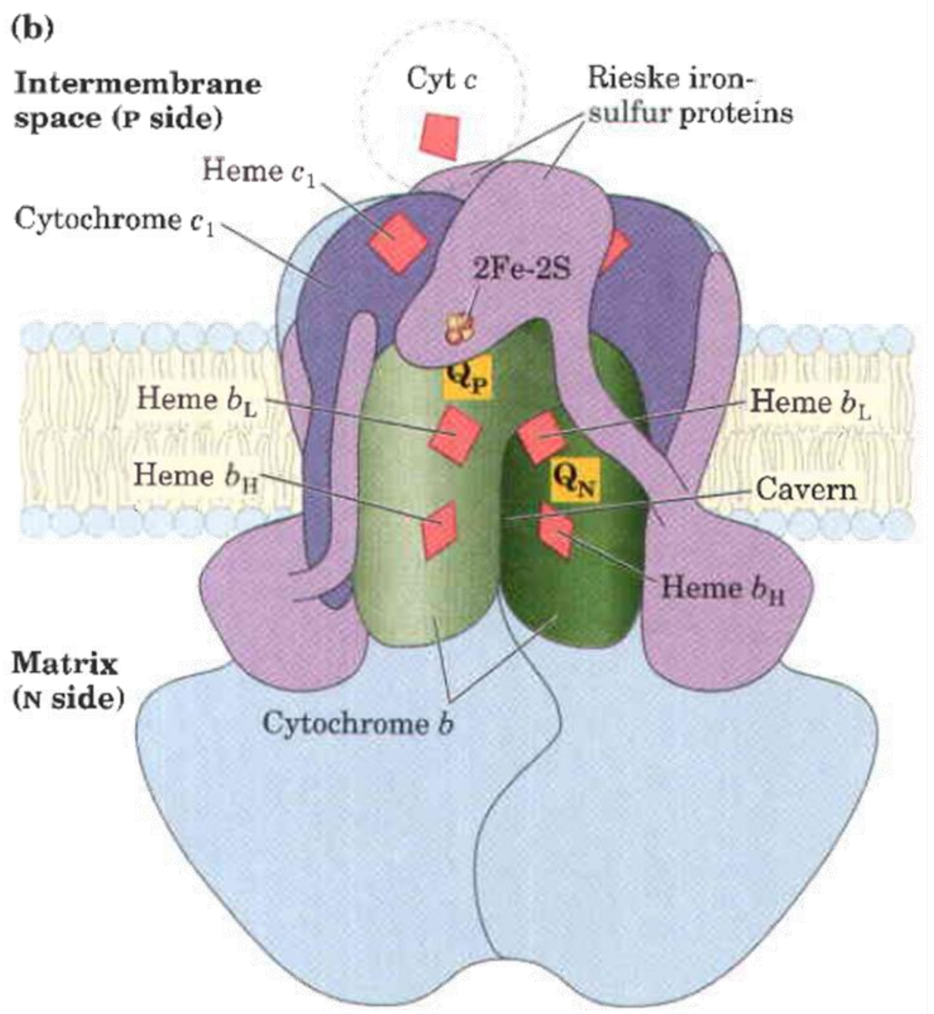
- Succinate Dehydrogenase & other flavoproteins
- TCA cycle
  - ETF-CoQ oxidoreductase (ex. fatty acid oxidation)
  - ≈Substrates oxidized by FAD-linked enzymes bypass complex-I
  - Three major enzyme systems:
  - Succinate dehydrogenase
  - Fatty acyl CoA dehydrogenase
  - Mitochondrial glycerol phosphate dehydrogenase
  - 0 kcal, H+?





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**
- **Contain two CoQ binding sites**
- **4H+**

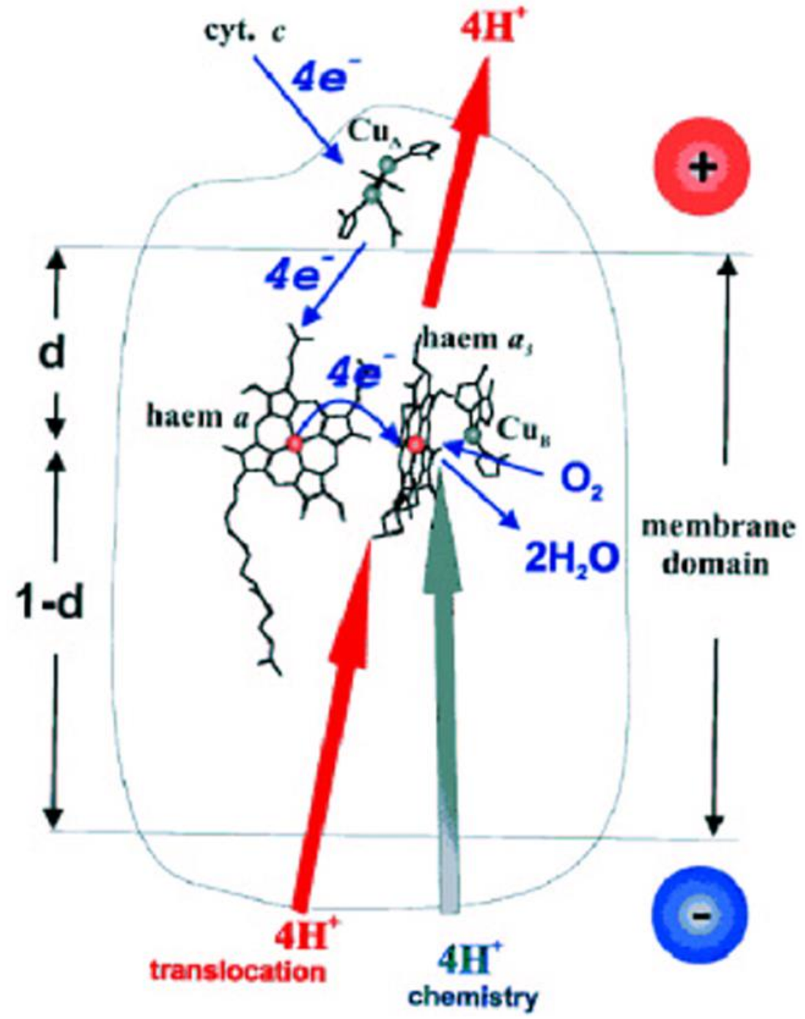


Oxi–Red Components of the ETC "Cytochrome c oxidase" – Complex IV

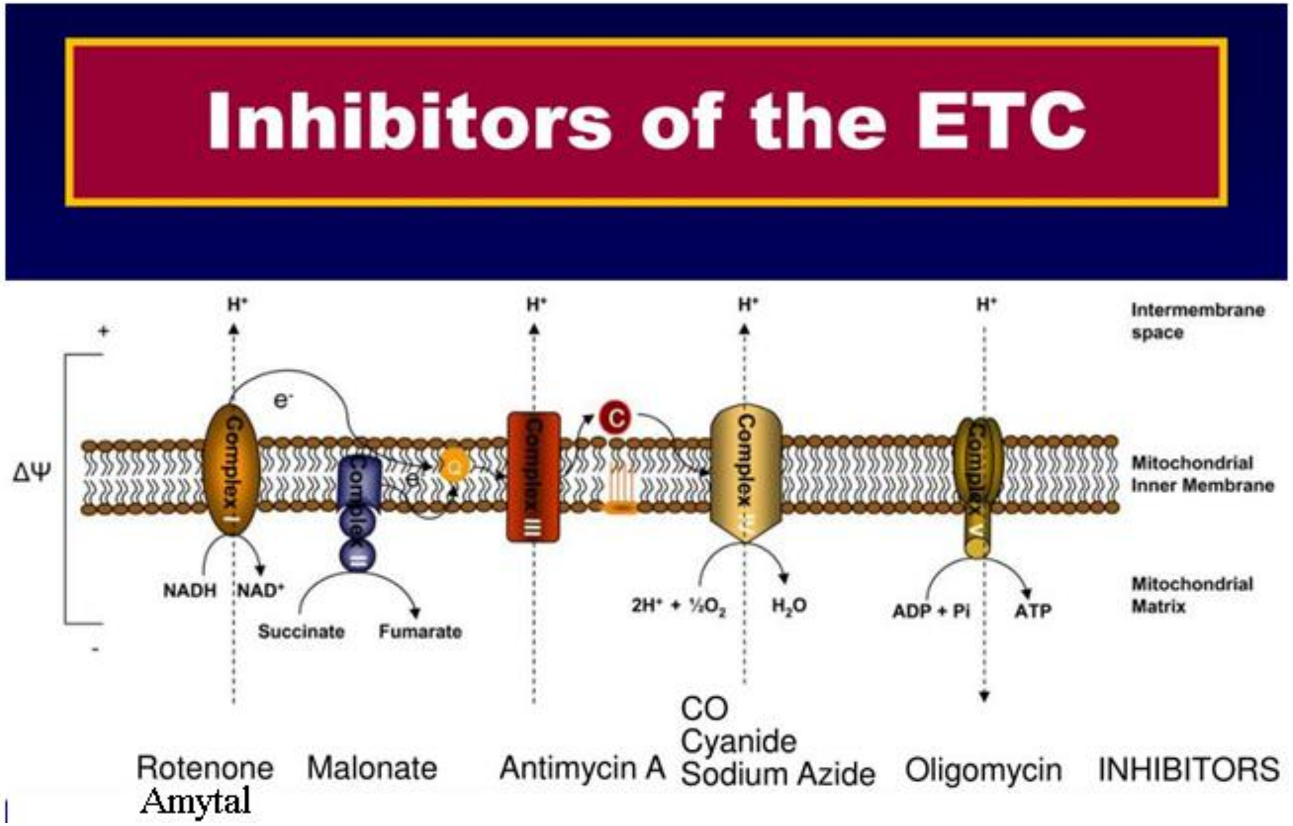
- **Passes electrons from Cytocrome c to O2**
- **Contains cytochrome a and a3**
- **Contains two copper sites**
- **Contains oxygen binding sites**
- **O2 must accept 4 electrons to be**

  reduced to 2 H2O (2H+/2e-)
- **Cytochrome c is one electron carrier**

  Cyt c_{red} + $4H^+$ + O2 → Cyt c_{ox} + 2H2O

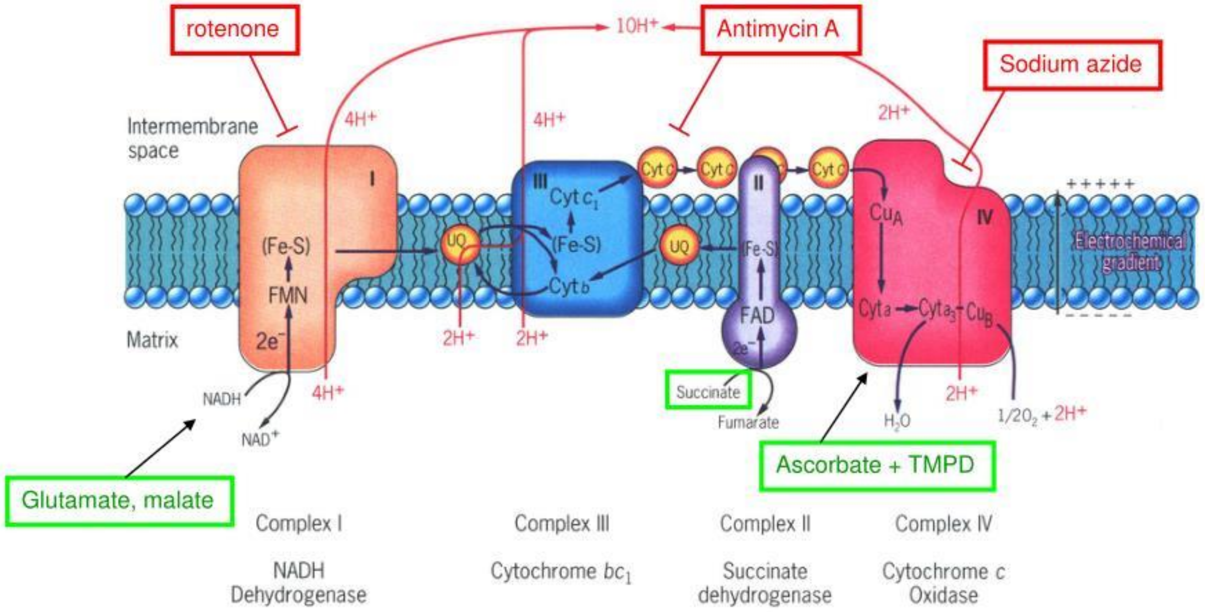


1. Amytal.(sedative)-inhibits NADH-Q Oxireductase
2. Rotenone.(insecticide)-inhibits NADH-Q Oxireductase
3. Antimycin A: inhibits electron flow between cyt b and c1, which prevents continued ATP synthesis at sites I and II as the carriers. InhibitsQ-cyttochromr c oxireductase,once reduced can not be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



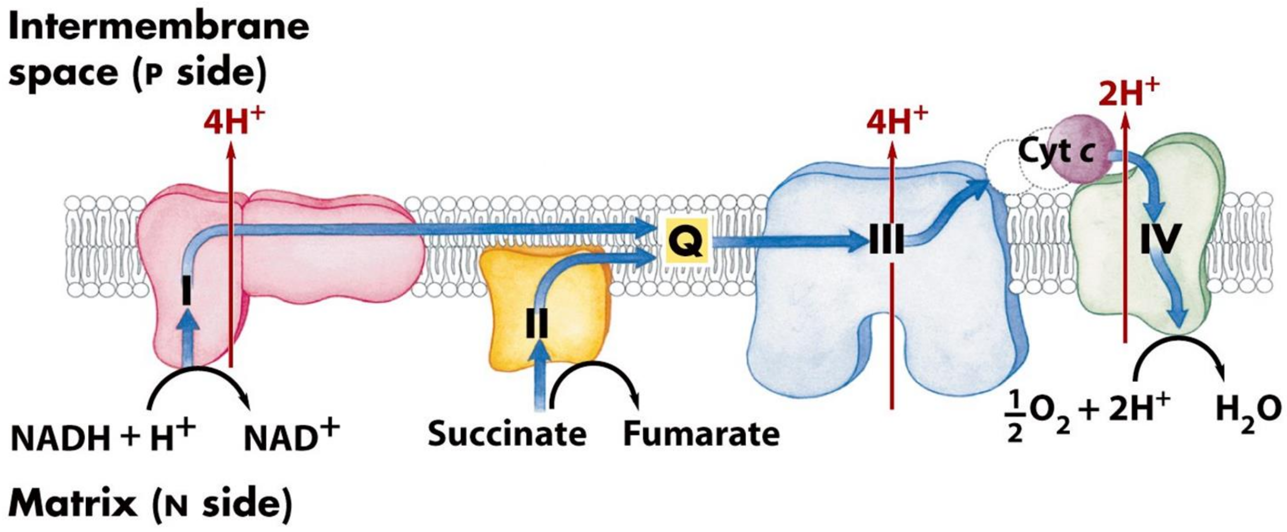
Electron transport chain inhibitors and substrates

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



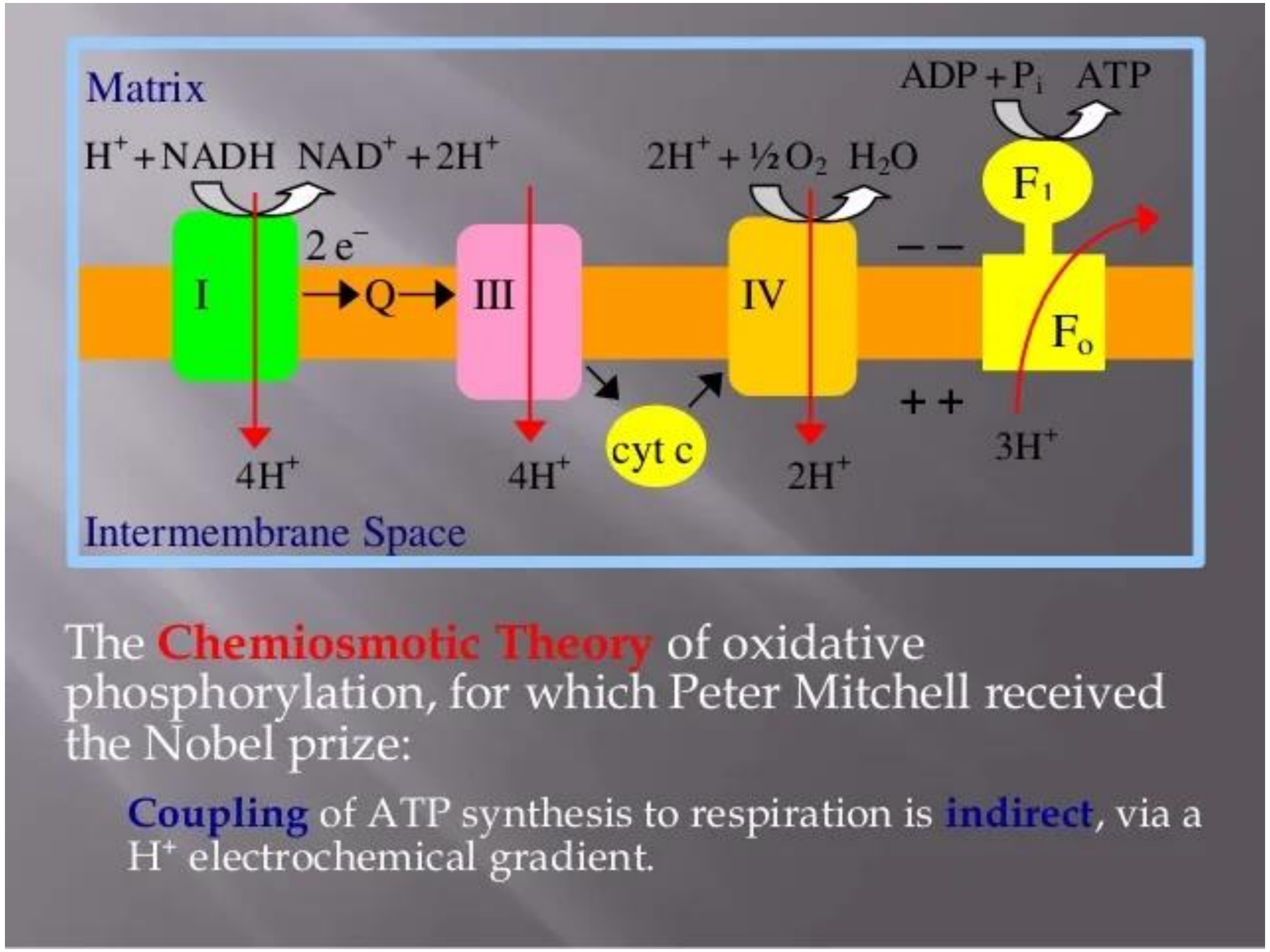
Pumping of Protons

- 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 and the energy released is used to drive the synthesis of ATP.



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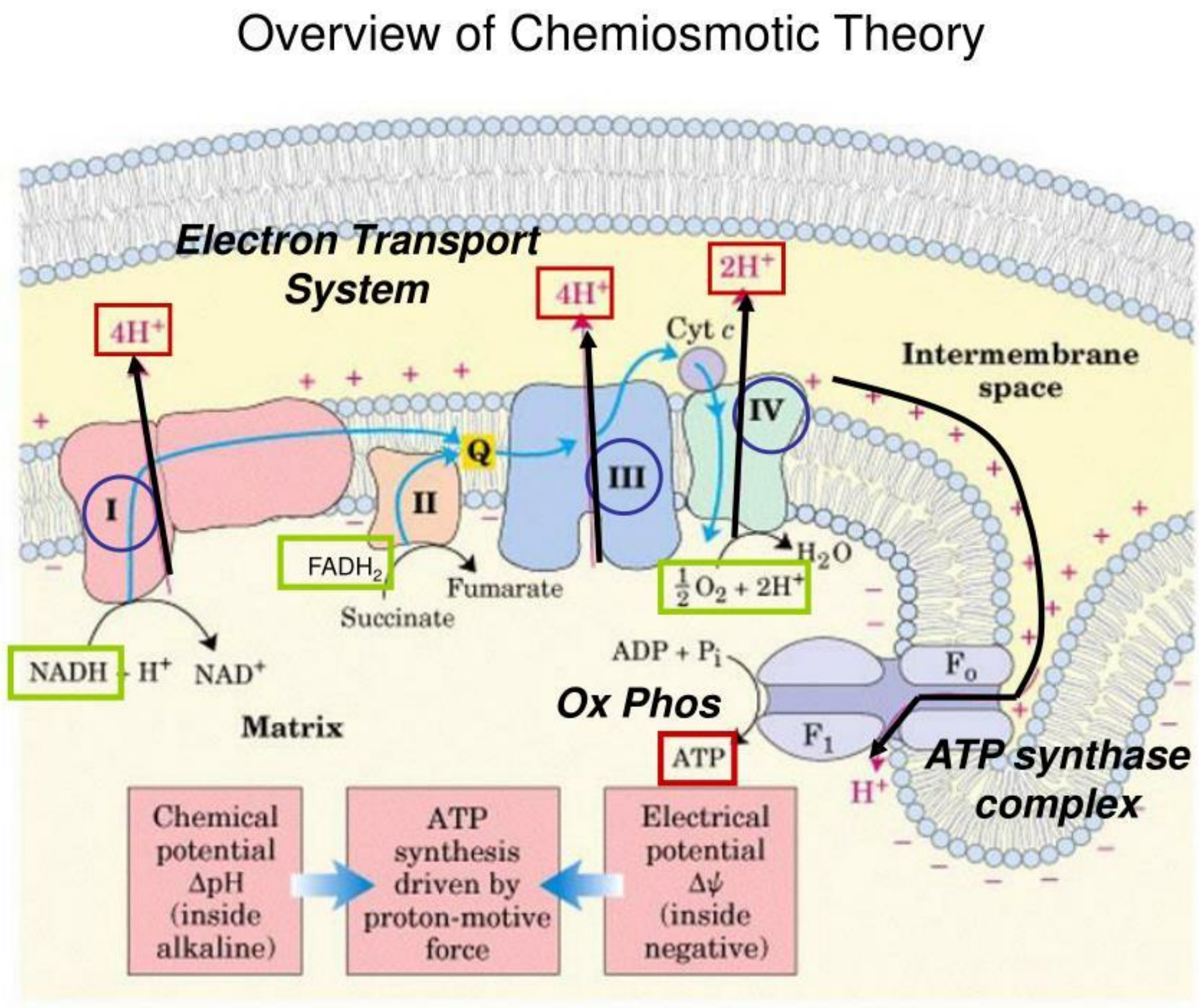
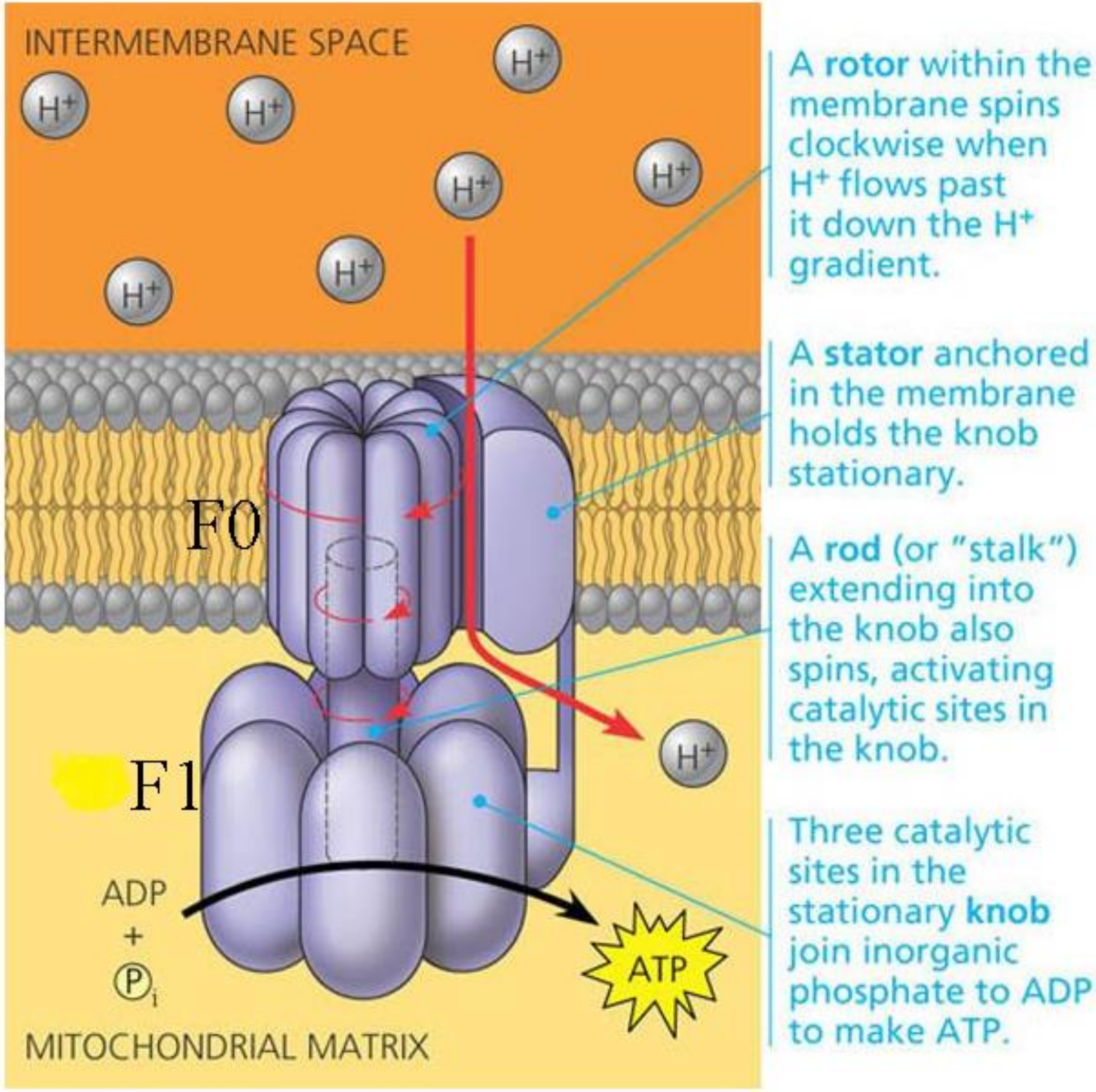


STRUCTURE AND MECHANISM OF ATP SYNTHASE-COMPLEX V

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:

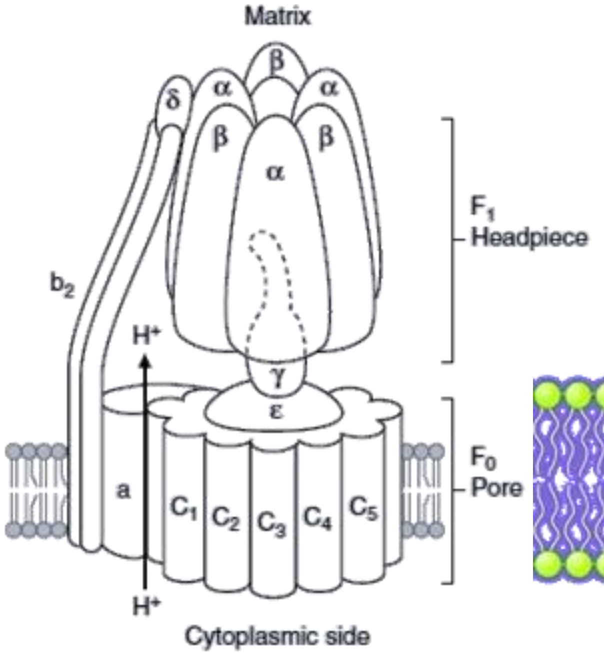
ADP +Pi---→ ATP +H2O

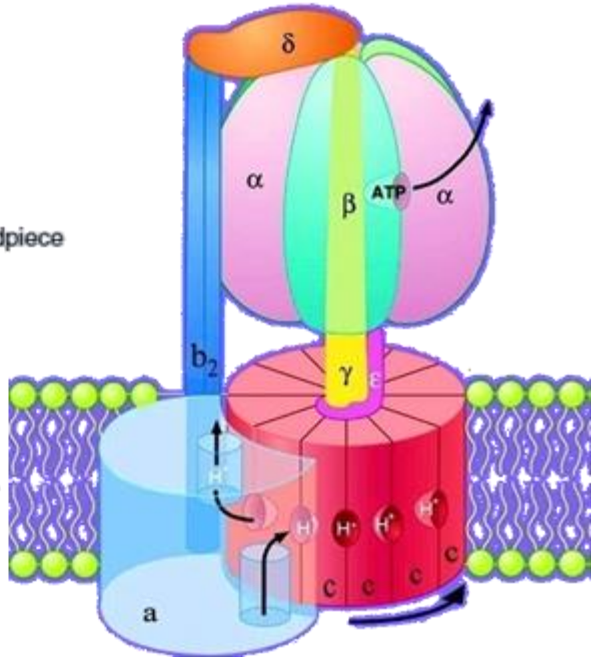
https://youtu.be/U26Jz3K1w2k

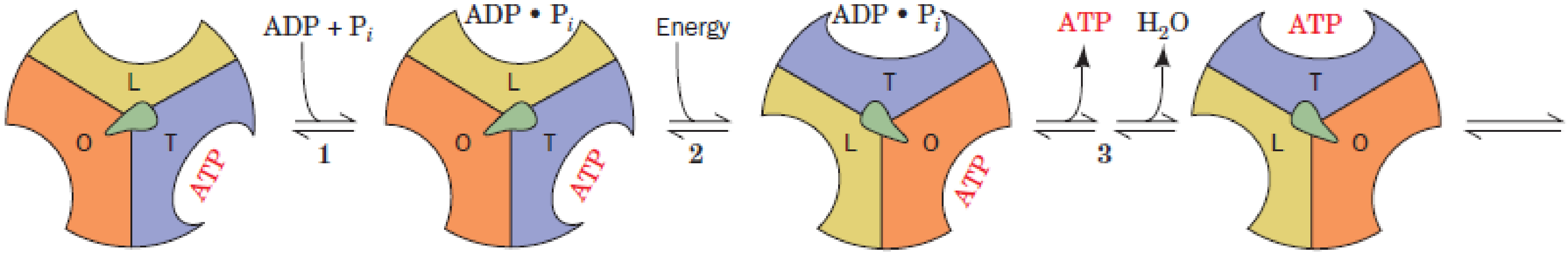


ATP Synthase

- 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

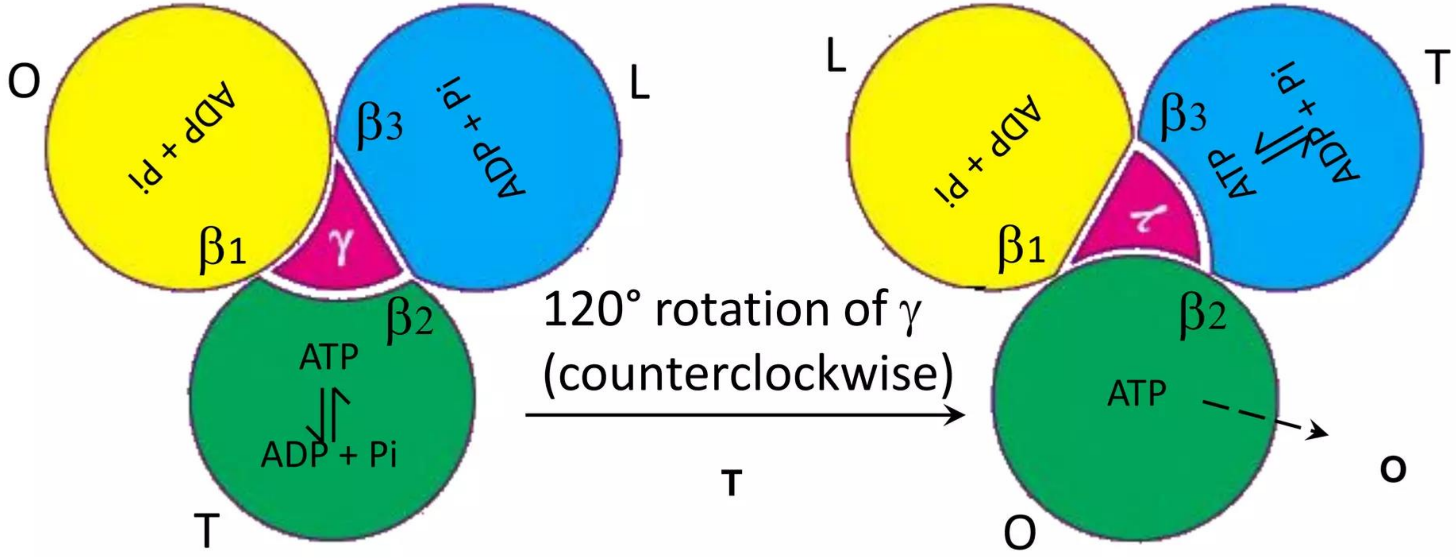






Binding-change mechanism of ATP synthesis

- 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 + $P_i \rightarrow$ ATP, but ATP can't dissociate
- G rotation causes T $\rightarrow$ O, L $\rightarrow$ T, O $\rightarrow$ L
- As a result of the T$\rightarrow$O structural change, ATP can now dissociate from what is now an O site.



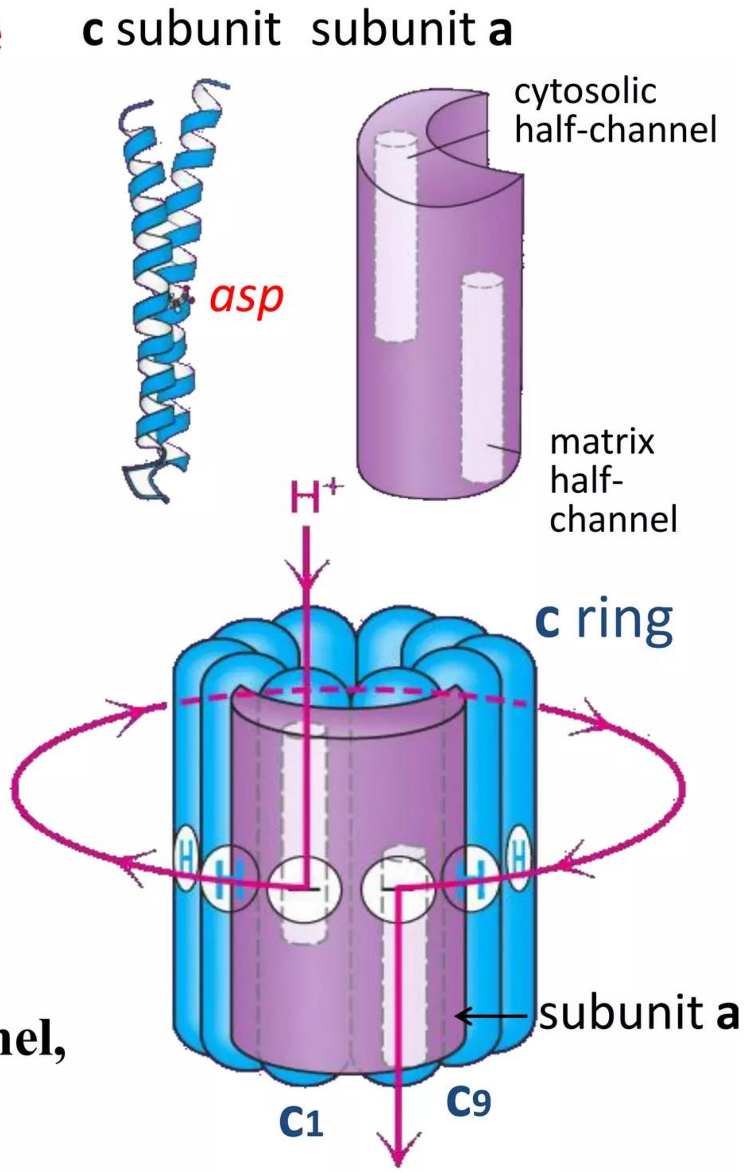
H^+ path through membrane

c ring & a subunit structure

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

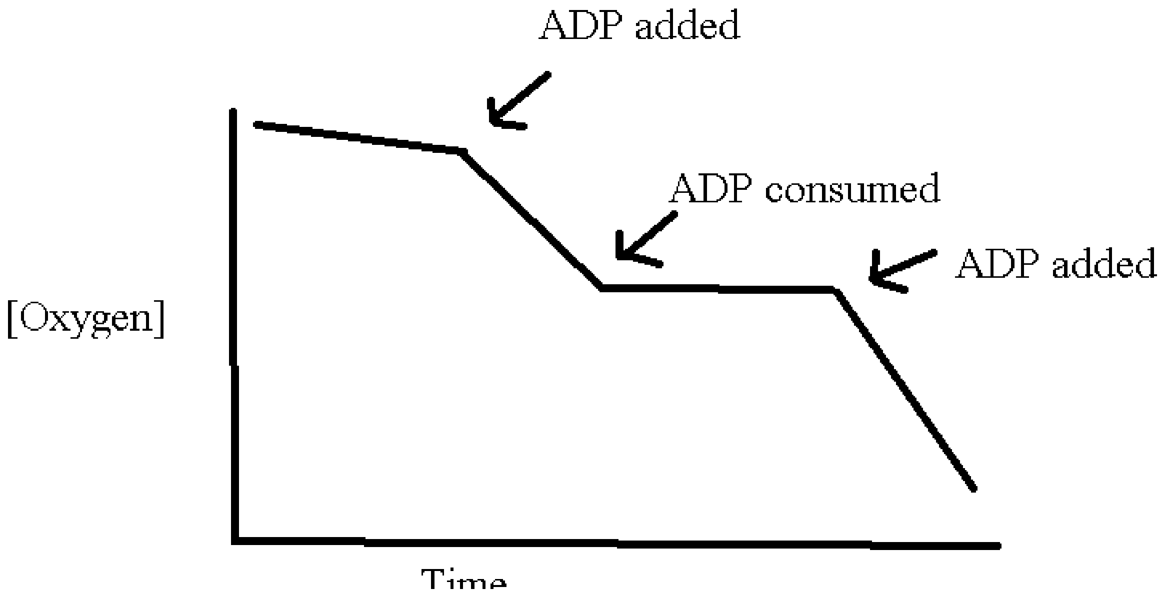
H^+ path

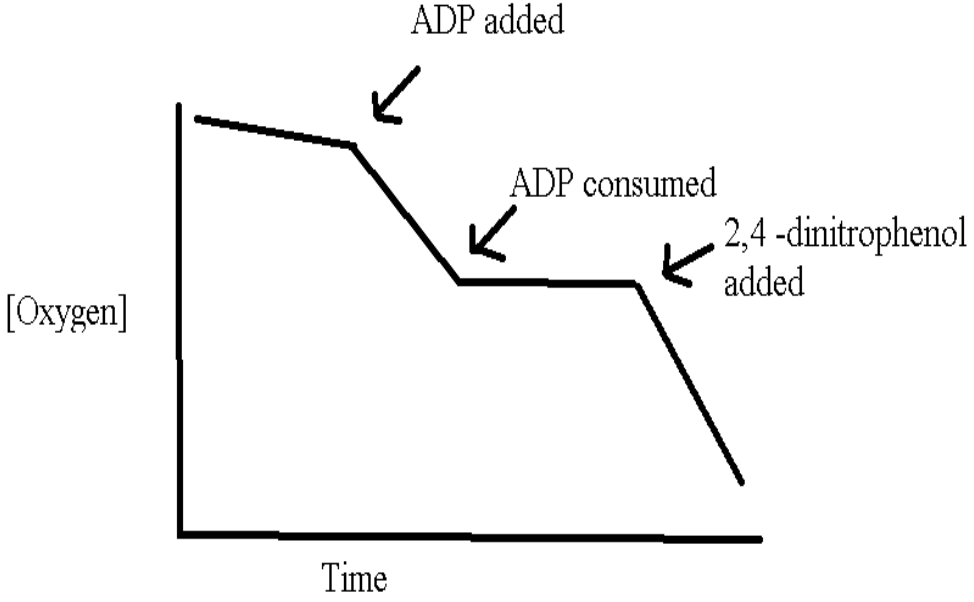
- **H^+ from cytosol diffuses via half-channel to *asp* on c ring subunit (c1)**
- **this subunit can now move to interface membrane, allowing c ring to rotate**
- **c9 now interfaces matrix half-channel, allowing H^+ to diffuse into matrix**

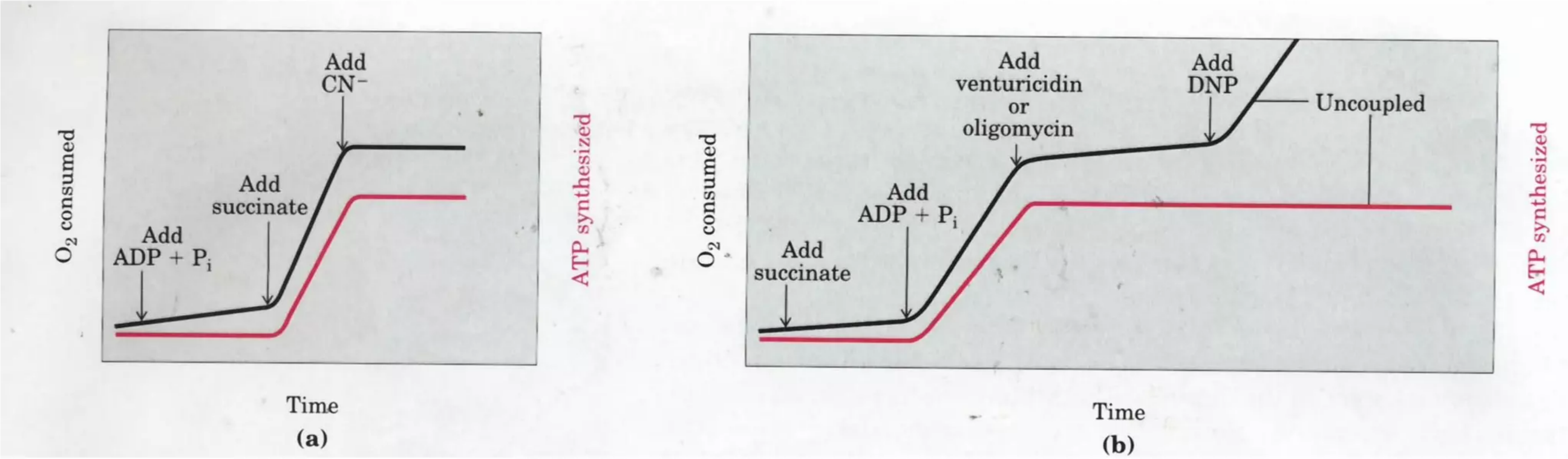


RECEPTOR OR ACCEPTOR CONTROL

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 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.





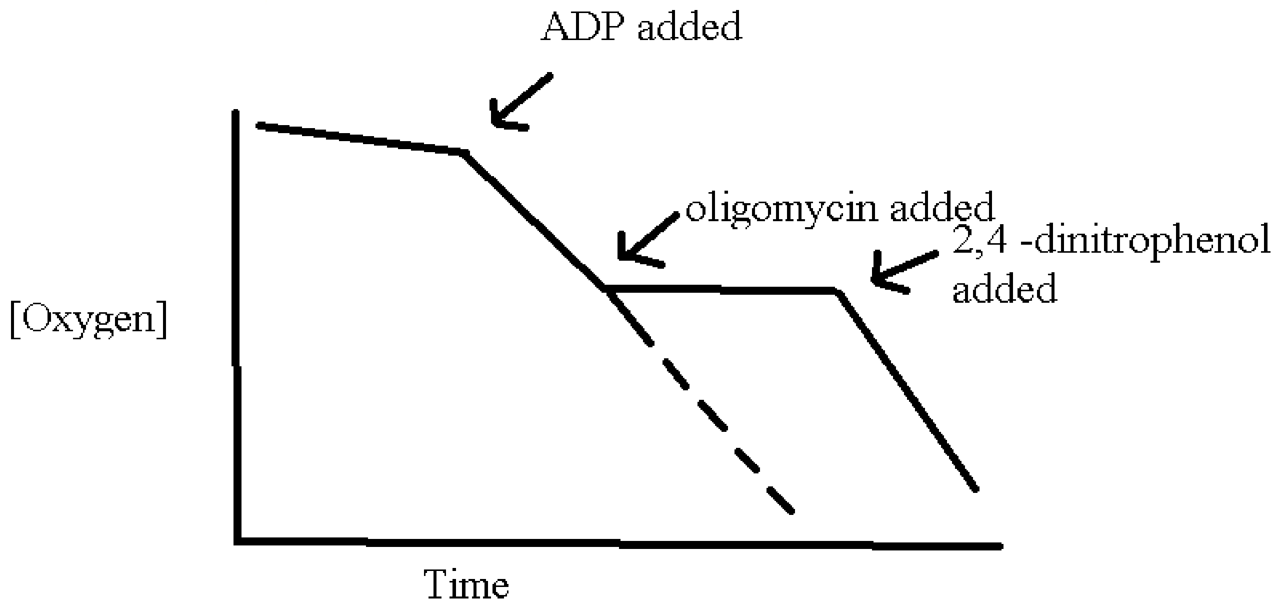


Electron transfer was found to be obligatorily coupled to ATP Synthesis in isolated mitochondria suspensions:

neither occurs without the other.

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:
- With NADH it is 3
- With succinate it is 2
- With ascorbate it is 1
- The overall equation for respiratory chain phosphorylation:

NADH+ H+ +3ADP+3Pi--→ NAD + H_2O + 3ATP

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)**