فريق طوفان الأقصى

METABOLISM

Modifide N. 7

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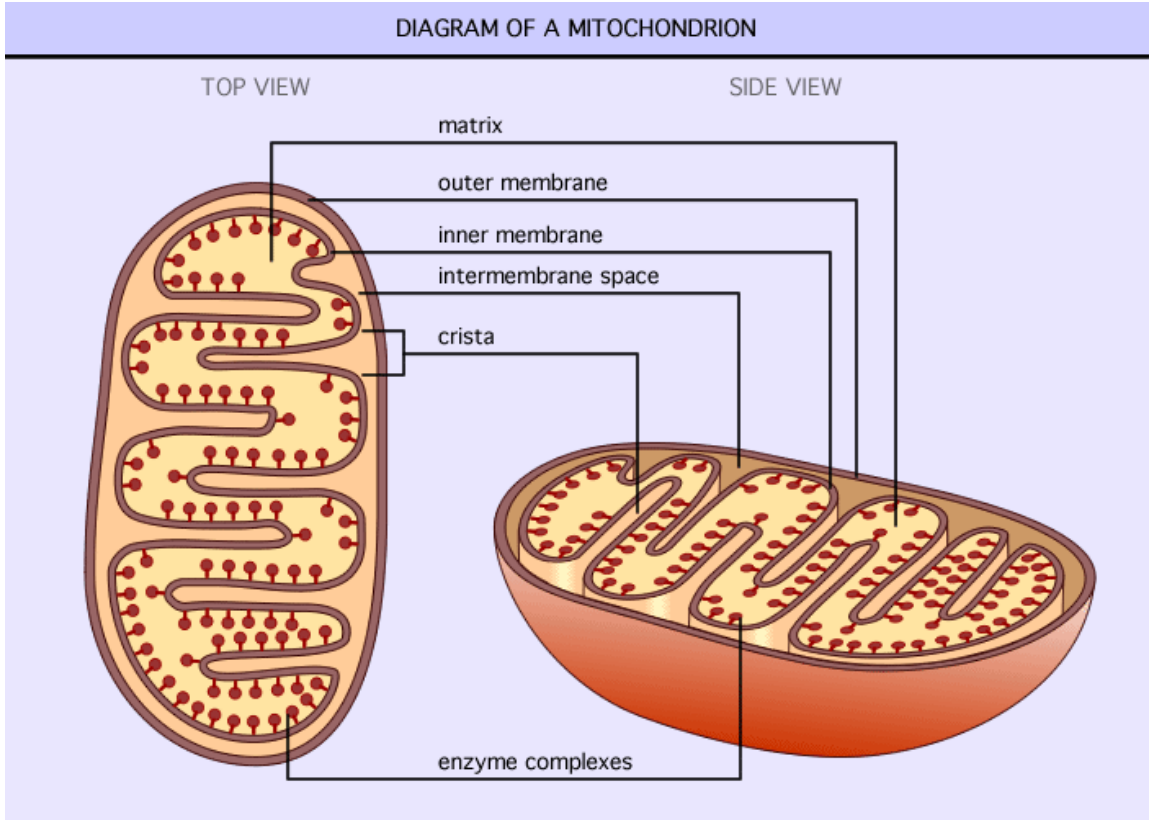
ELECTRON TRANSPORT, SHUTTLES, AND OXIDATIVE PHOSPHORYLATION

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 complement in this slide:** In ETC there are redox reactions where NADH & $FADH_2$ donate their electrons to the components of ETC , once these electrons are donated , they pass from complex to another till they reach the Oxygen which is the ultimate acceptors , while they are passing , there will be type of energy release , that will be captured by the cell and used in a mechanism to activate ATP synthase enzyme to phosphorylate ADP and produce ATP.
- The major purpose of metabolism is to extract energy from food , where it is converted to electrons carried by NADH & $FADH_2$ “reducing powers or electron rich molecules ” from glycolysis or another metabolic way such as TCA cycle, fatty acid oxidation, oxidation (utilization) of ketone bodies or amino acids or oxidative phosphorylation, then they're oxidized to extract energy that is used to synthesize ATP.



OXPHOS takes place in the mitochondria of the cell

Mitochondria consist of 2 membranes- the [1]outer and the[2] inner membranes.

The outer is freely permeable to molecules MW<10K

The [3]intermembrane space contains the enzymes that catalyze the interconversions of adenine nucleotides

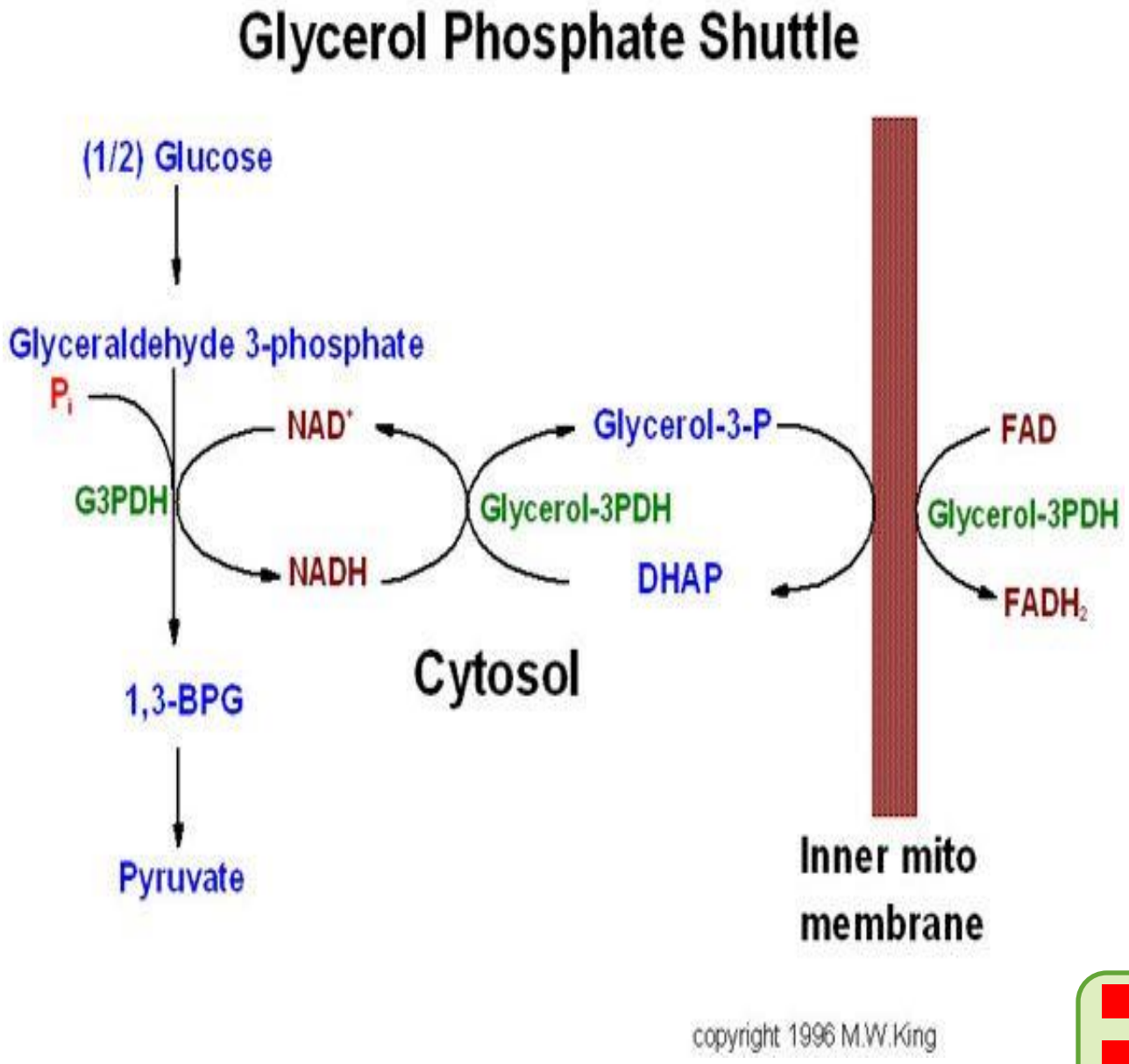
The inner membrane space has many folds directed towards the [4]mitochondrial matrix.

NOTE: **We will talk about proteins that are located in the inner membrane and the actions that will take place in the intermembrane space and how they are related to the synthesis of ATP by oxidative phosphorylation**

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
			PYRUVATE CARBOXYLASE
		Carbamoylphosphate synthetase I	FATTY ACYL-COQ DH
			ENOYL HYDRASE
			BETA-HYDROXYACYL-COA DH
			BETA-KETOACYL-COA THIOLASE

- **NOTE:** this table is NOT for memorization ,however, it tells you that each one of mitochondrial structures has its specific enzymes to do specific function (specialization), in our case we will talk about inner membrane so we will see most of the enzymes and proteins that are needed for ETC and oxidative phosphorylation



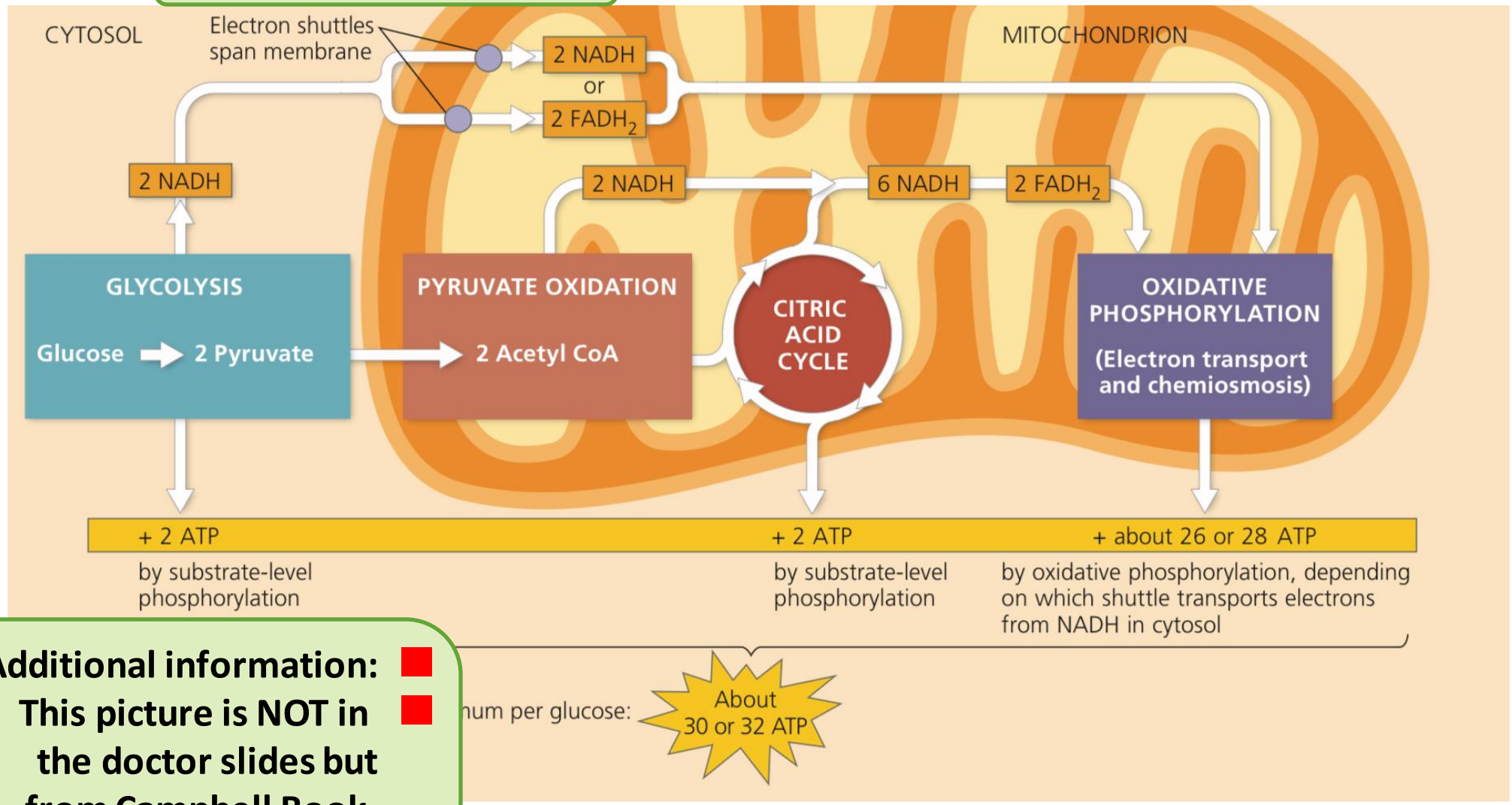
α-Glycerol Phosphate-Dihydroxyacetone Phosphate shuttle

NOTE: Shuttle is a transporter / vehicle that transports something from one place to another.

- 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

NOTE:
(Cyt)→ in the cytosol
(Mit) → in the mitochondrial matrix

Here!



Additional information:

This picture is NOT in the doctor slides but from Campbell Book, just to clarify to you what the shuttle is

- **The complement in this slide:** shuttles in the cells are not bus like structures , however, they are pathways that help to transfer some molecules from one place to another, as it is mentioned before that NADH is a principle molecule in ETC , some NADH are formed in cytosol and others in the mitochondria, the problem is the **NADH** molecules which are in the **cytosol**; must be transferred to mitochondrial membrane so they can participate and donate their electrons to ETC , but **NADH can't** simply **fused into** the mitochondria, so there must be a shuttle mechanism.

The complement in this slide:

- NADH in the cytosol is produced in one of the glycolytic reactions in which

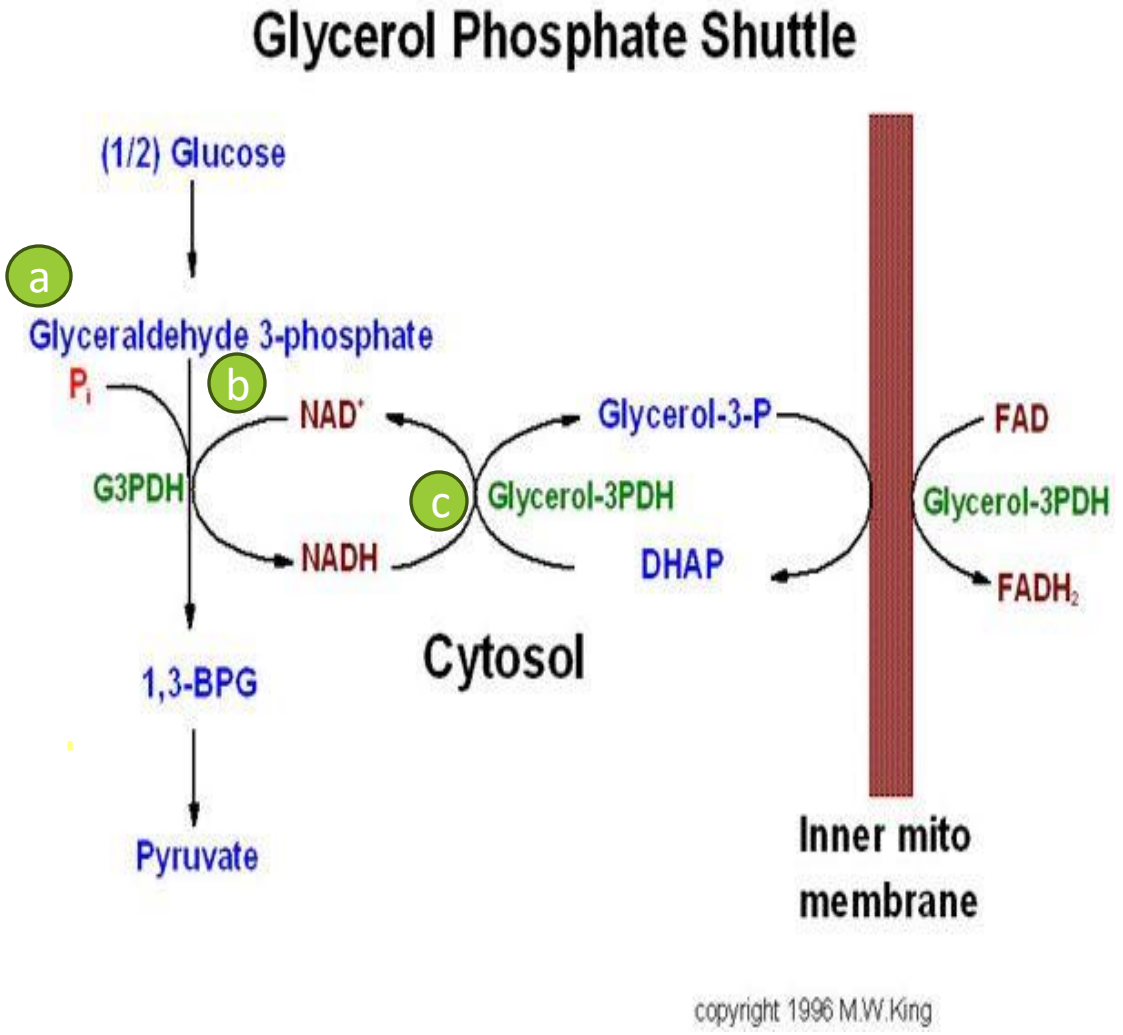
a. the glyceraldehyde 3-phosphate (G3PH) dehydrogenase oxidize (G3PDH) → 1,3_bisphosphoglycerate (1,3-BPG)

b. it requires NAD+ and inorganic phosphate (Pi)→ produces NADH that must transferred to ETC for oxidation

- In order to transfer NADH, there is an **enzyme** call glycerol 3 Phosphate dehydrogenase (**Glycerol_3PDH**) in the **cytosol** ,

c. it reduces dihydroxyacetone phosphate (DHAP), which is an intermediate in the glycolysis, in the presence of NADH → converting it into Glycerol-3-P

*it is **not** a **flavoenzyme, instead it requires NAD**

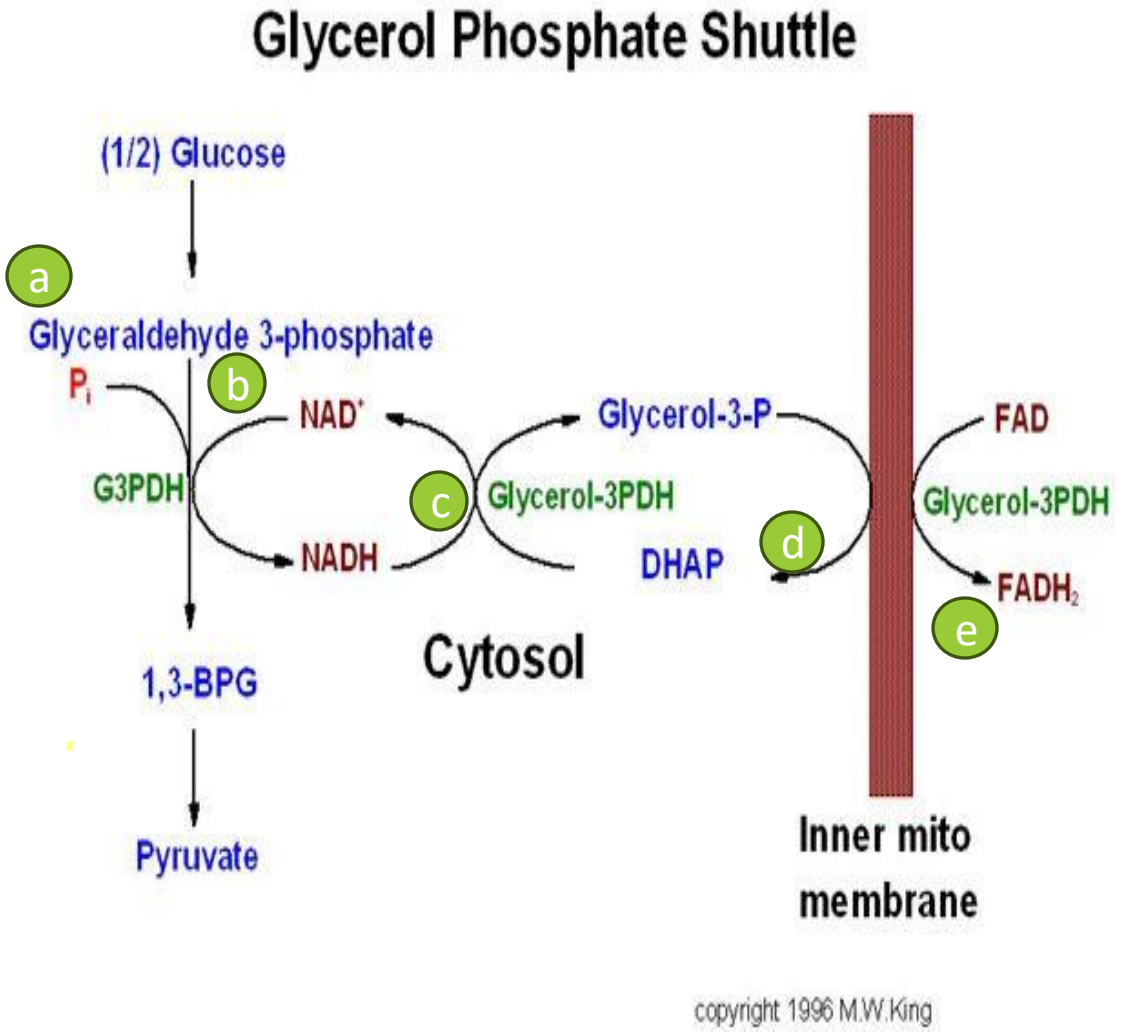


The complement in this slide:

- In the **mitochondria inner membrane** there is an enzyme like the previous one, glycerol 3 Phosphate dehydrogenase (**Glycerol_3PDH**)

d. that will regenerate (DHAP) from Glycerol-3-P,

e. it is a **flavoenzyme** which requires FAD →that will covert to FADH2 besides the (DHAP)that will be used to pick another NADH



Additional information:A flavoenzyme is an enzyme that contains a flavin group, such as flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN), as a prosthetic group

- **The complement in this slide:**

The 2 purposes of this process:

1. Transfer this NADH pair of e- from cytoplasm to the mitochondria customs transferring the electrons in a shuttle in the form of FADH2.

2. Regenerate NAD+ in order to continue the glycolytic reaction

- **NOTE:**

If NADH & FADH2 are oxidized in ETC ,NADH will produce **more** ATP than FADH2

So there will be a **loss** in energy in cells that used this type of shuttles (α-**Glycerol Phosphate**-Dihydroxyacetone Phosphate **shuttle**)

- **NOTE:**

There is no shuttle from the cytosol to the intermembrane space, because The outer is freely permeable to molecules MW<10K

NOTE: SUMMARY

NADH from glycolysis

Cytoplasmic enzyme Glycerol-3PDH

Reduction of DHAP, in the presence of NADH, producing Glycerol-3-P

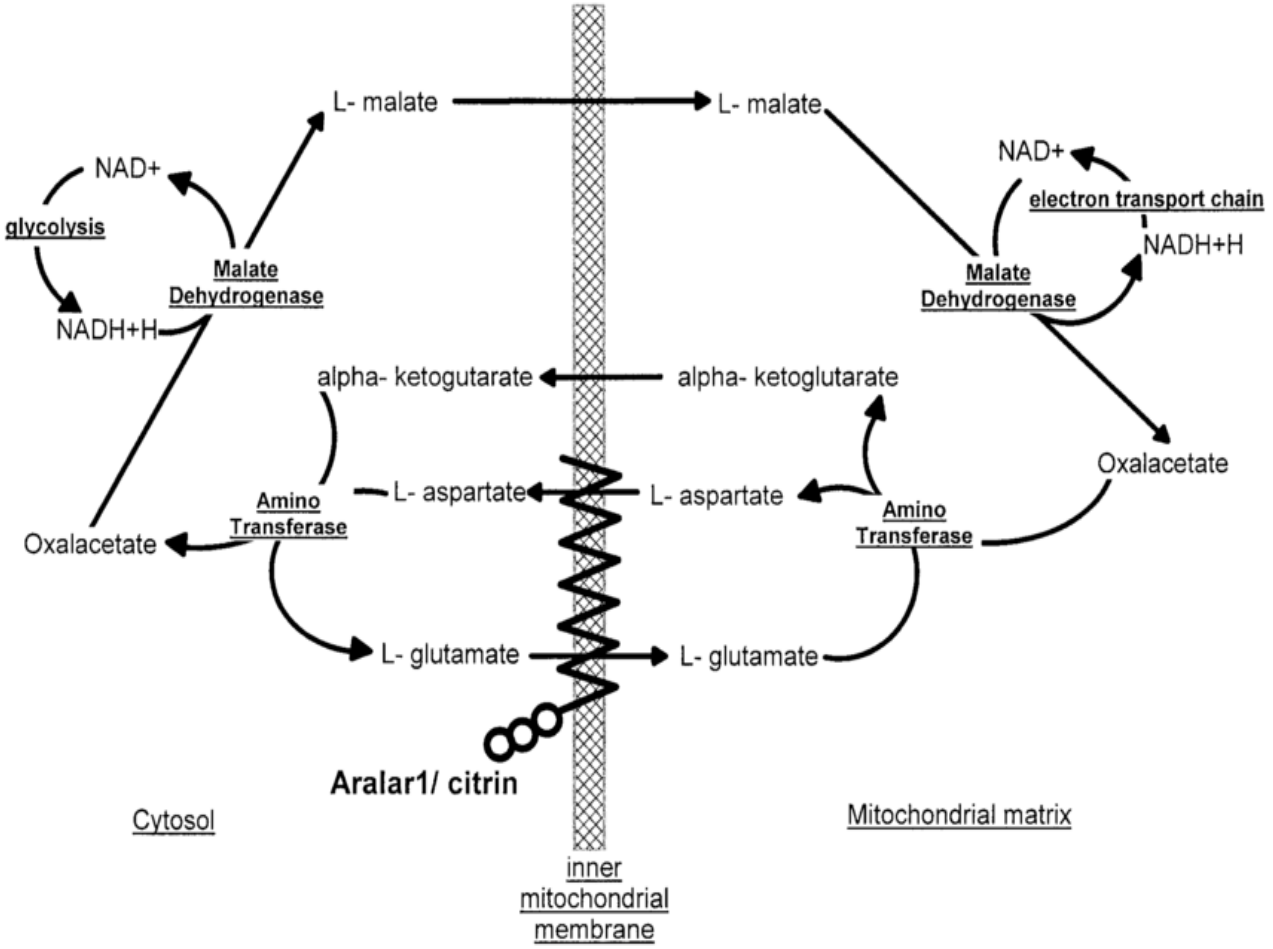
Mitochondrial enzyme Glycerol-3PDH (**flavoenzyme)**

Oxidation of Glycerol-3-P into DHAP, producing **FADH2** on the mitochondrial side

- **NOTE:** The previous shuttle included the transfer of electrons from NADH in the cytosol to $FADH_2$ in the mitochondrial matrix, but in the next shuttle we are going to study electrons that will be transferred from NADH in the cytosol to another NADH in the mitochondrial matrix.

- **NOTE:**
- (Cyt)➔ in the cytosol
- (Mit) ➔ in the mitochondrial matrix

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

- **The complement in this slide:**
- Firstly, the **cytoplasmic enzyme** malate dehydrogenase reduces oxaloacetate in the presence of NADH (a product of glycolysis), which will result in the conversion of oxaloacetate to malate. The purpose of this reaction is: to **regenerate** NAD+ (so that glycolysis wouldn't stop) and to **transfer** the electrons from the cytoplasmic NADH to the mitochondrial NADH.
- Secondly, malate will be transported from the cytosol to the mitochondrial matrix via specific transport protein (portal).
- Thirdly, the **mitochondrial** malate dehydrogenase will convert malate to oxaloacetate and regenerate NADH (both NADH and oxaloacetate are produced in the mitochondrial matrix).
- The result: Transfer of the electrons from the cytoplasmic NADH to another -equivalent- NADH in the mitochondrial matrix.

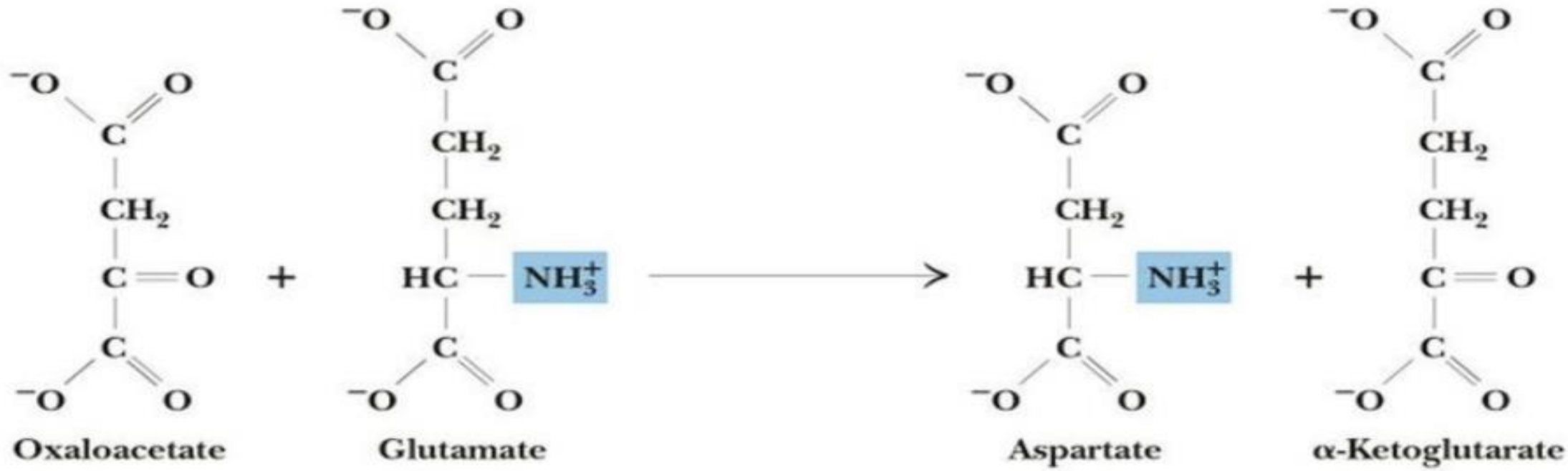
- **The complement in this slide:**
- Now, **mitochondrial** oxaloacetate must in a way or another come back to the cytosol, and there is no specific membrane protein to transport oxaloacetate across the inner mitochondrial membrane.
- Oxaloacetate can return back to the cytosol through **transamination reactions** between keto acids and amino acids to **reproduce** and **regenerate** oxaloacetate in the **cytosol**.
- These transamination reactions occur in both the **cytosol** and the **mitochondrial matrix**.
- Then, the regenerated oxaloacetate will be reduced again by cytoplasmic malate dehydrogenase and the cytoplasmic NADH will be oxidized into NAD+ and the cycle repeats itself again and again.

- **NOTE:** If the cells require pyruvate for gluconeogenesis, pyruvate could be produced by transamination between oxaloacetate and alanine.

- **NOTE:** Generally speaking, oxaloacetate is found in the mitochondrial matrix in catalytic amounts to be used in the TCA cycle.
- But if the cell requires oxaloacetate in the cytosol, transamination reactions will occur to transport oxaloacetate to the cytosol for other purposes.

- **The complement in this slide:** Recall the transamination reaction of oxaloacetate and glutamate

- **Attachment:**



- **Note:**
- Transamination reaction involves the engagement of oxaloacetate & L-glutamate -> to produce L-aspartate & alpha-ketoglutarate on the mitochondrial side, which can cross the membrane by specific transporting protein to the cytosol.
- And VISE VERSA on the cytosolic side, except that OAA doesn't cross the membrane.
- ADDITIONAL EQUATION :-
- OAA + L-glutamate <—> L-aspartate + alpha-ketoglutarate; where from left to right occurs in the mitochondrial matrix, from right to left occurs in the cytosol

- **NOTE:**
- Now, all the electron carriers (NADH and $FADH_2$) produced from all the stages of catabolism are located within the mitochondrial matrix.
- So the cell is ready to oxidize them and make use of the energy they carry to produce ATP.
- The cell makes use of the energy that electron carriers carry to produce ATP through **the electron transport chain,** which is going to be our next topic.

- **The complement in this slide:**
- Like any other metabolic pathways, the electron transport chain consists of **redox reactions**.
- Some carriers will accept electrons from the donor (NADH or $FADH_2$), and once they accept them; they will donate these electrons to the next protein complex carrier, and after accepting the electrons the carrier will donate them to the next carrier and so on…
- The carriers in the electron transport chain are **arranged** in the **inner mitochondrial membrane** according to their **redox potential**.
- Electron transport chain consists of 4 protein complex carriers, each carrier will accept electrons from the previous carrier (or reducing agent), and will donate these electrons to the next carrier (or oxidizing agent), as the electrons will flow through them.
- In the electron transport chain, the electrons will flow from the carrier with the **lower** (more negative) redox potential to the carrier with the **higher** (more positive) redox potential.
- Notice that NADH has the **lowest** redox potential, while **oxygen** has the **highest** redox potential, which means that oxygen has **high affinity** for electrons and is going to be **reduced**.

- **The complement in this slide:**
- Now, how do those carrier protein complexes function and do their work?
- They do their work of accepting and donating electrons by having some extra-prosthetic groups within their structure.
- Each complex of the 4 complexes in the electron transport chain has a specific prosthetic group (or groups) which will assist that complex to accept and donate electrons.

- **NOTE:**
- The carriers in the electron transport chain are: 4 protein complexes, 1 simple protein carrier (not complex) and 1 non-protein carrier (lipid in structure).
- In the next slide we are going to study the non-protein electron carrier.

- **NOTE:** Ubiquinone (or coenzyme Q), a non-protein electron carrier, which is lipid in nature.

- **NOTE:** Coenzyme Q, also known as coenzyme Q10

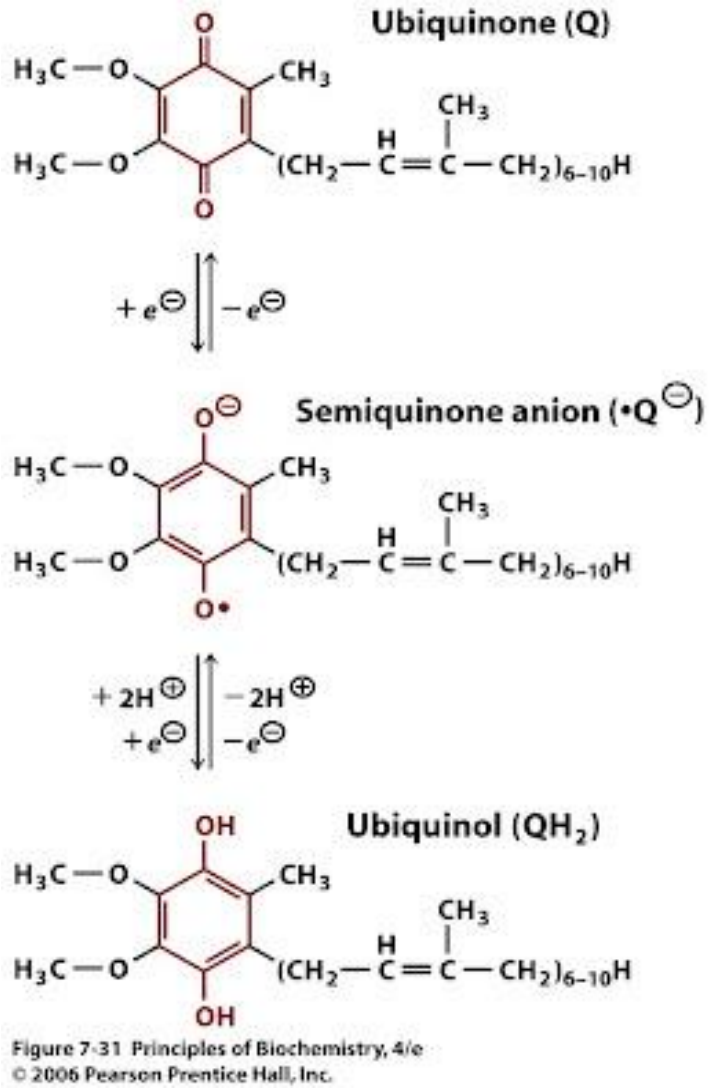


Figure 7-31 Principles of Biochemistry, 4/e
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Carriers of Electron Transport Chain
The chain of carriers is called : Electron Transport Chain Or Respiratory Chain.

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

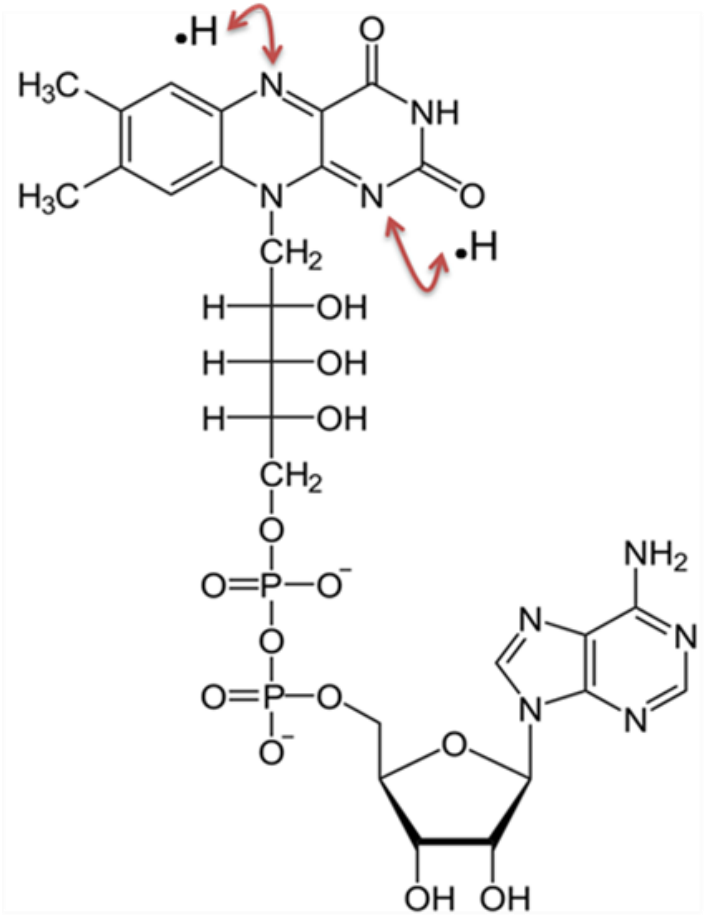
- **NOTE:**
- Because ubiquinone is **lipid** in nature; it can easily **diffuse** in the membrane and change its **location** from one site to another depending on **the bindings sites** which will determine to which carrier ubiquinone will bind.
- Notice the aromatic ring and the long hydrocarbon chain (isoprenoid tail).

- **The complement in this slide:**
- When ubiquinone accepts one electron, it is converted to semiquinone.
- When the semiquinone also accepts another electron, it is converted to ubiquinol.
- Ubiquinone accepts electrons in the electron transport chain from protein complex 1 and protein complex 2.
- Once ubiquinone is loaded with electrons from complex 1&2, it will donate these electrons to protein complex 3.
- So, ubiquinone moves to the site where it accepts electrons, and moves to the site where it donates these electrons.
- It's lipid nature (isoprenoid tails) gives it the ability to move freely in the lipid bilayer.
- Ubiquinone doesn't move or diffuse randomly; it moves to specific sites where there are binding sites for it so it could accept or donate electrons.

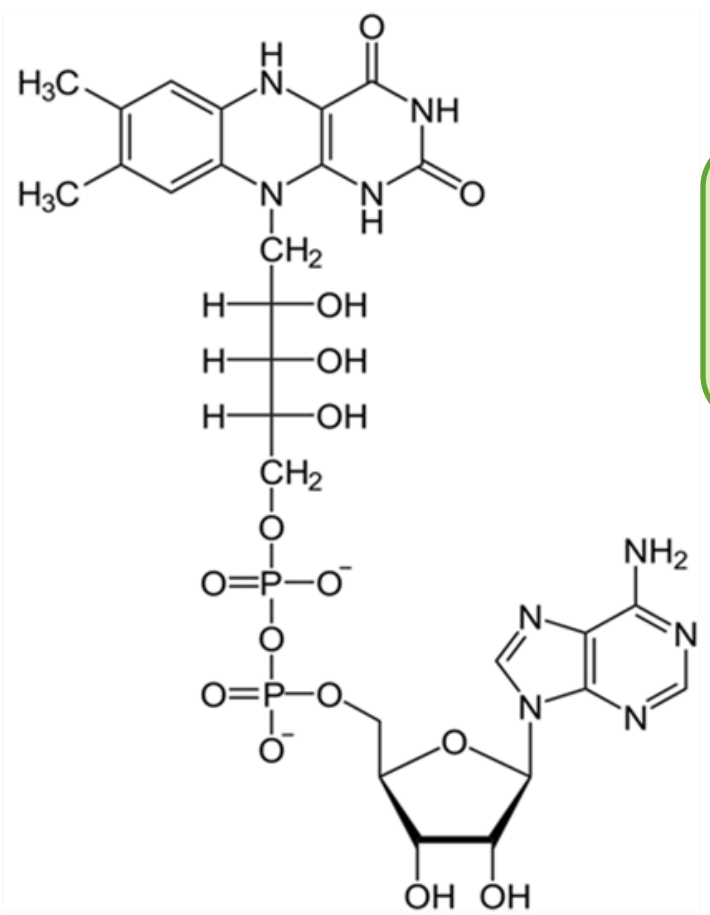
- **NOTE:** Nowadays, a lot of people take coenzyme Q10 as a supplement to increase the energy in their bodies.

NOTE: Another coenzyme (a prosthetic group actually), Flavin Adenosine Dinucleotide, also known as FAD

NOTE: Oxidized form



FAD



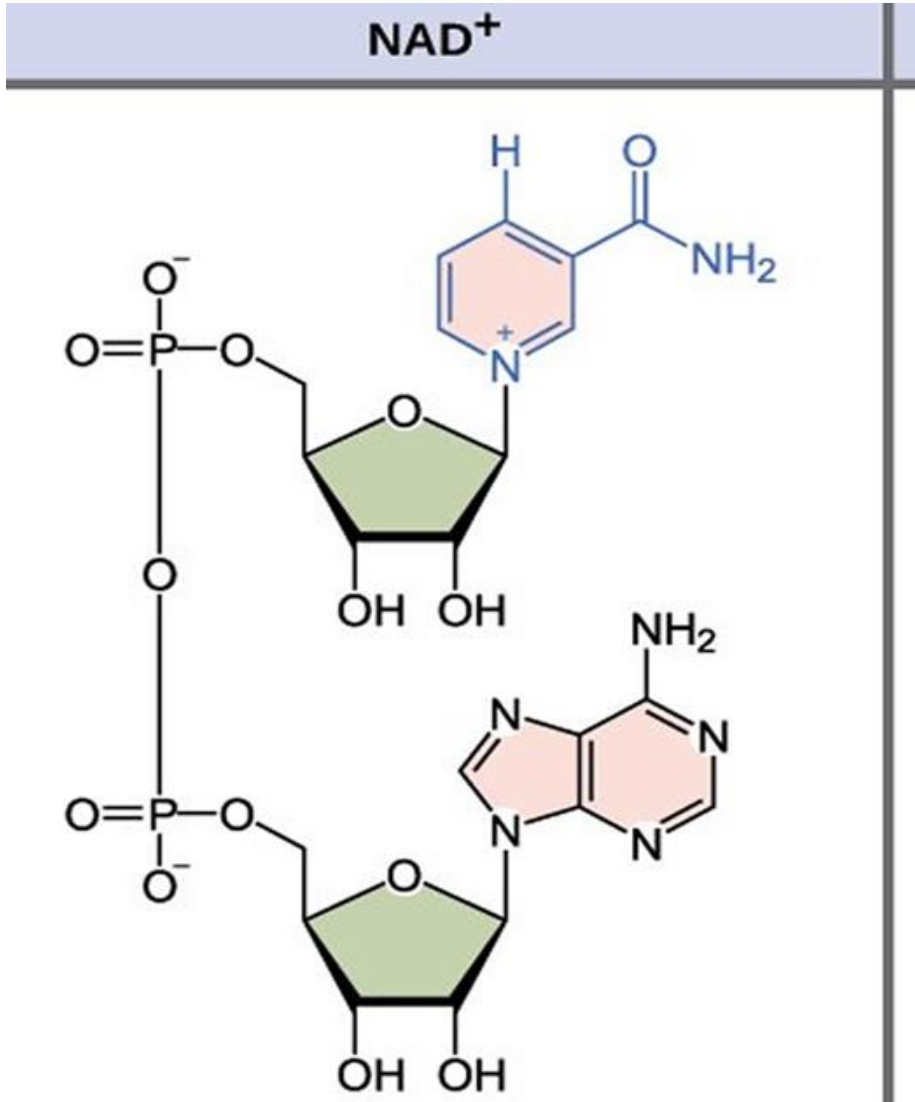
$FADH_2$

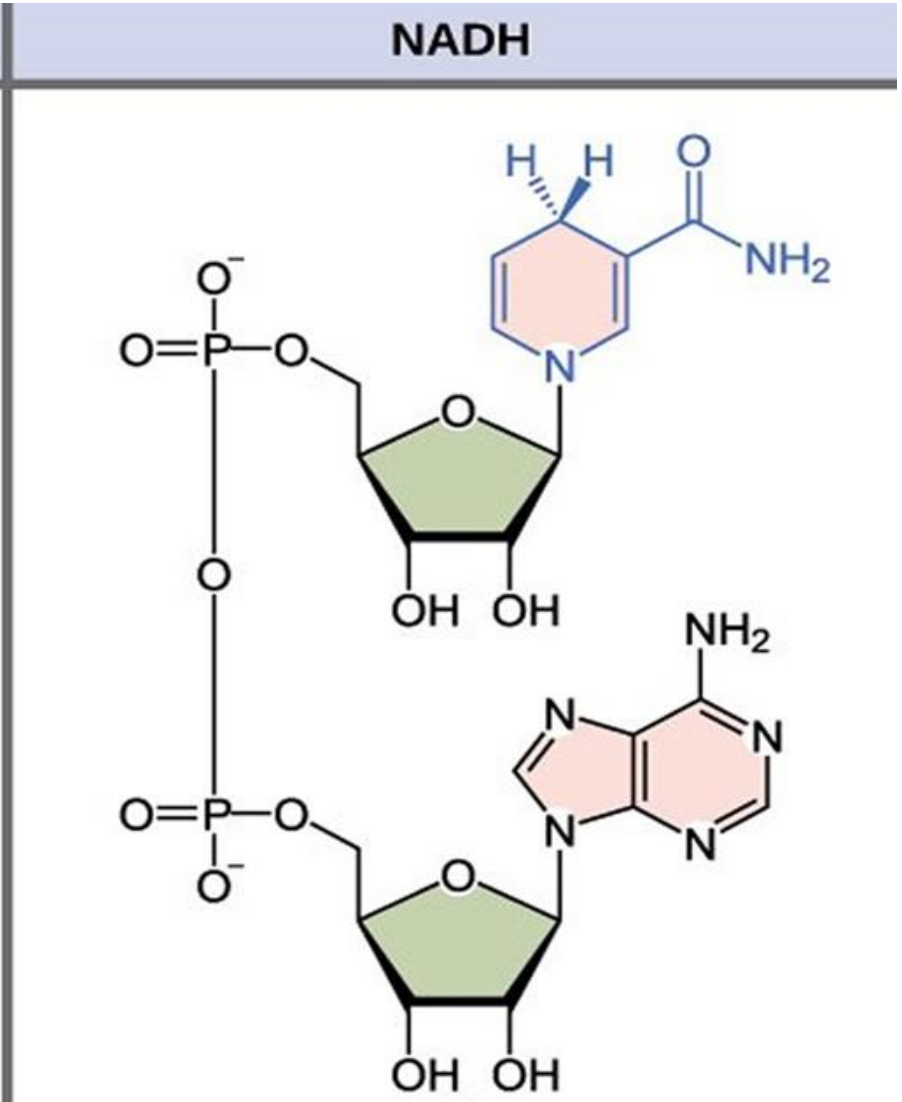
NOTE: Reduced form

- **The complement in this slide:**
- FAD is **covalently** bound to flavoproteins (that's why it is considered a prosthetic group).
- When FAD is reduced to $FADH_2$, it will carry 2 electrons and 2 protons.
- FAD is attached to protein **complex 2** which contains **succinate dehydrogenase** enzyme which plays an important role in the TCA cycle.
- Recall: succinate dehydrogenase was the only exceptional enzyme in the TCA cycle that is located in the inner mitochondrial membrane while the other enzymes are located in the matrix.
- Succinate dehydrogenase is part of the protein complex 2 in the electron transport chain, which is also called succinate reductase.
- Succinate dehydrogenase is a flavoprotein through which it's $FADH_2$ donates its electrons to the prosthetic groups of protein complex 2.

NOTE: Another coenzyme, Nicotinamide Adenosine Dinucleotide, also known as NAD+

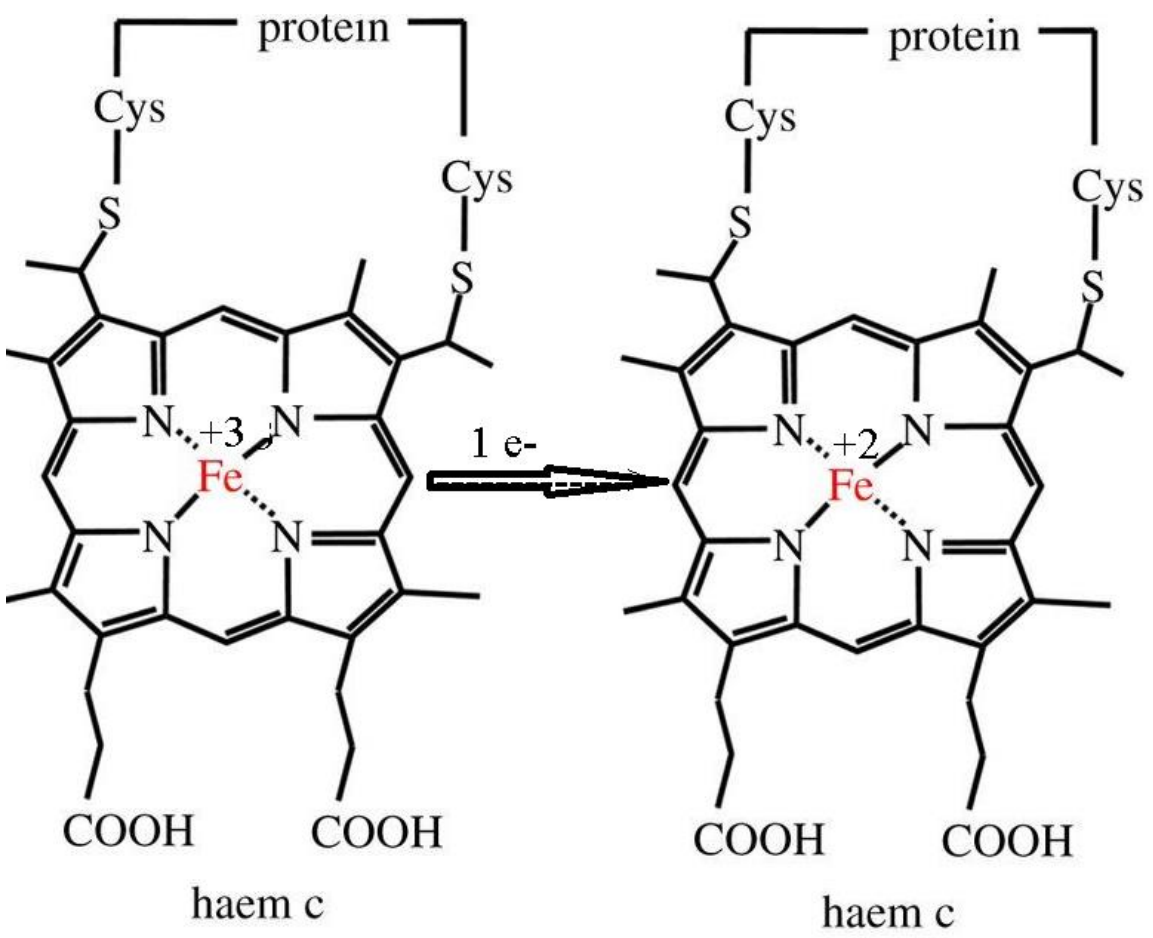
NOTE: Oxidized form





NOTE: Reduced form

- **The complement in this slide:**
- NAD+ binds 1 proton and 2 electrons to produce $NADH+H^+$.
- NADH is an electron donor to the protein complex 1 in the electron transport chain.
- Protein complex 1 will accept electrons from NADH via the prosthetic group of the complex.
- Then the electrons are going to be donated from protein complex 1 to coenzyme Q.

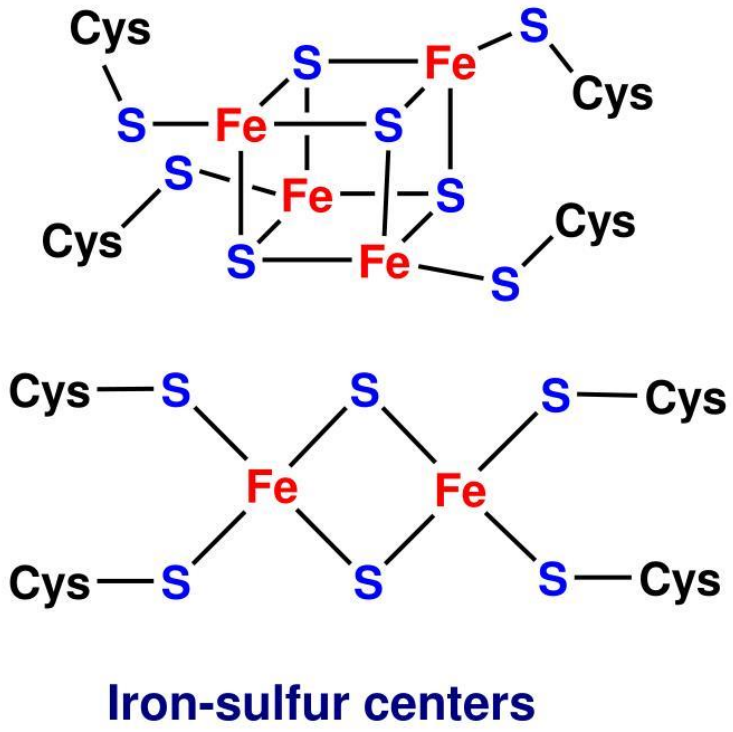


Cytochromes (heme proteins)

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 of cytochromes (a , b, & c).

- **The complement in this slide:**
- Recall the heme group, which is a prosthetic group in hemoproteins, such as proteins in some complexes of the electron transport chain.
- Hemoproteins contain heme as a prosthetic group in protein complex 3 and protein complex 4 in the electron transport chain.
- These hemoproteins which contains at least one heme group are called cytochromes.
- The structure of heme contains an iron cation coordinated with 4 nitrogens coming from pyrrole rings.
- Cytochrome is also found in some complexes (3 and 4) in the electron transport chain.
- Each one of the 3 and 4 complexes have different groups of cytochromes as prosthetic groups.
- There are different types of cytochromes (e.g: a, b, c) which differ in 2 things: the arrangement of the conjugated bonds in the pyrrole rings and the type of the side chains attached to these pyrrole rings.
- Accordingly, some different types of the cytochrome may differ in their function or in their behavior during the reaction

NOTE: Proteins which have iron and sulfur, whether they are sulfur atoms or sulfur from the sulfhydryl groups of cysteines.



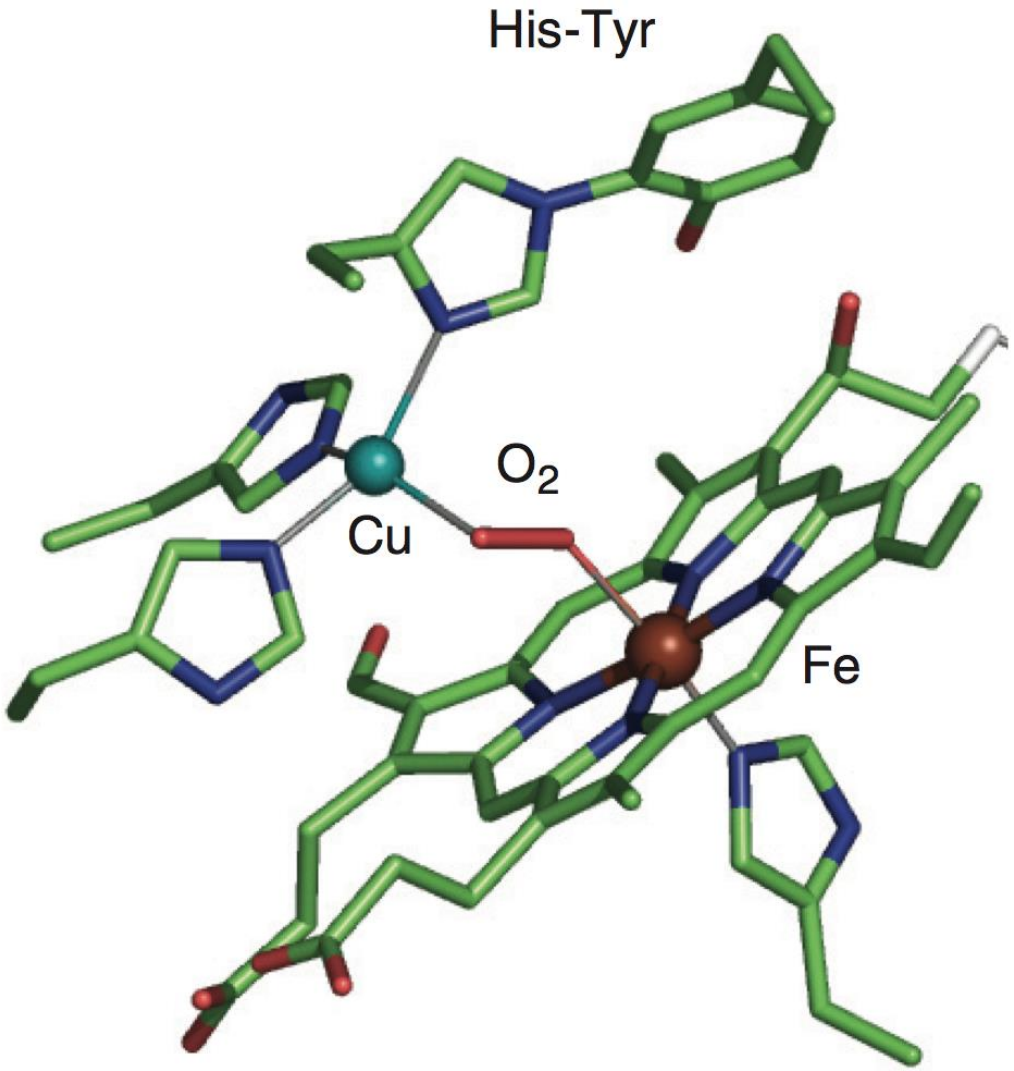
Iron-sulfur centers

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.

NOTE: They are small proteins found within the electron transport chain complex proteins, such as complex 1 and complex 2.



Copper Containing Proteins

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

1 e-

Cu2+----------→Cu1+

- **NOTE:**
- **Copper-containing proteins also assist in transferring electrons.**
- **Found in the complex 4.**

Carriers that carry 2e-

- NADH
  FADH2
  Coenzyme Q

Carriers that carry 1e-

- Cytochromes
  Iron sulfur centers
  Copper containing proteins