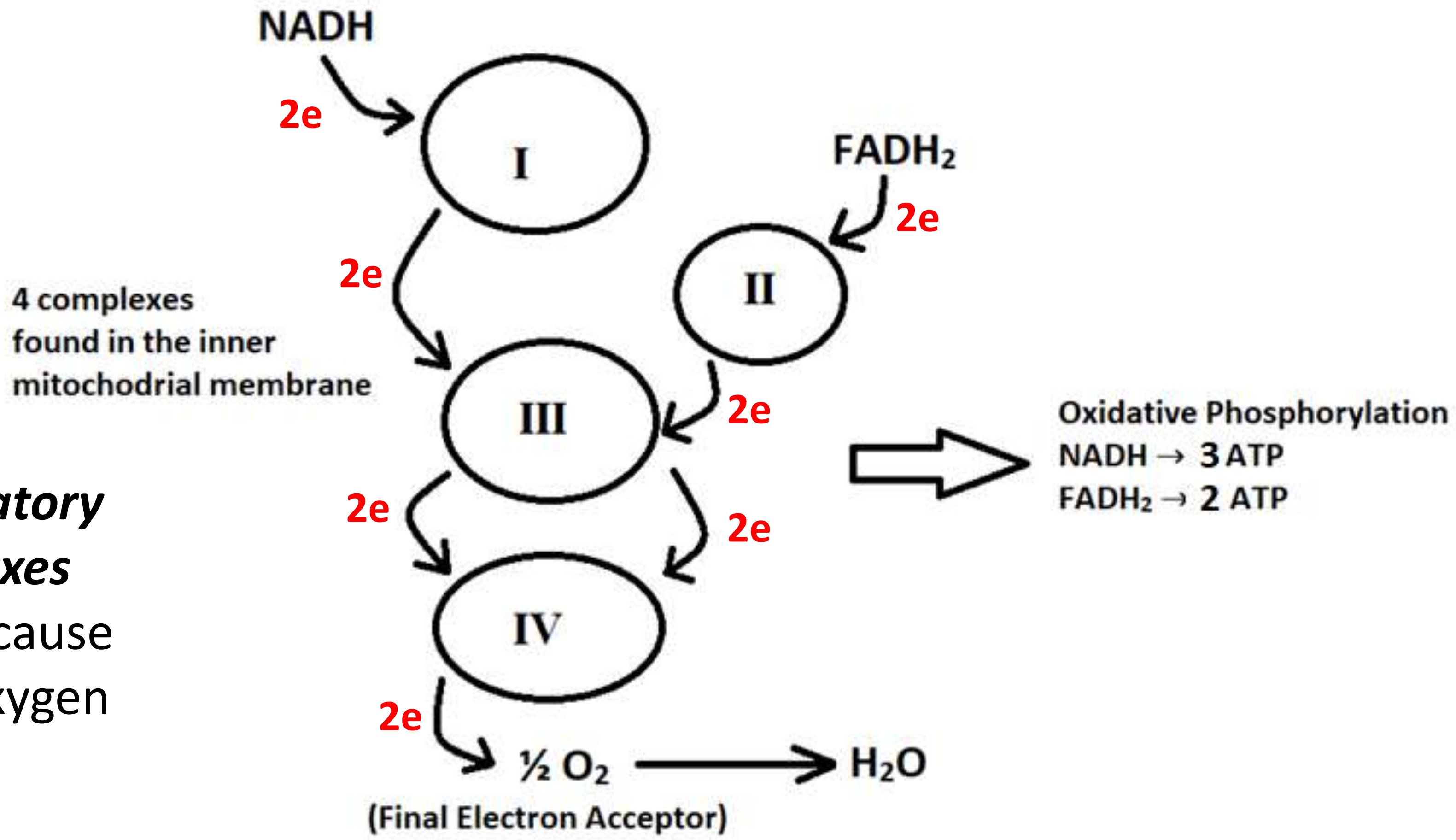
سلسلة نقل الالكترونات

Electron Transport chain and Oxidative phosphorylation (OXPHOS)

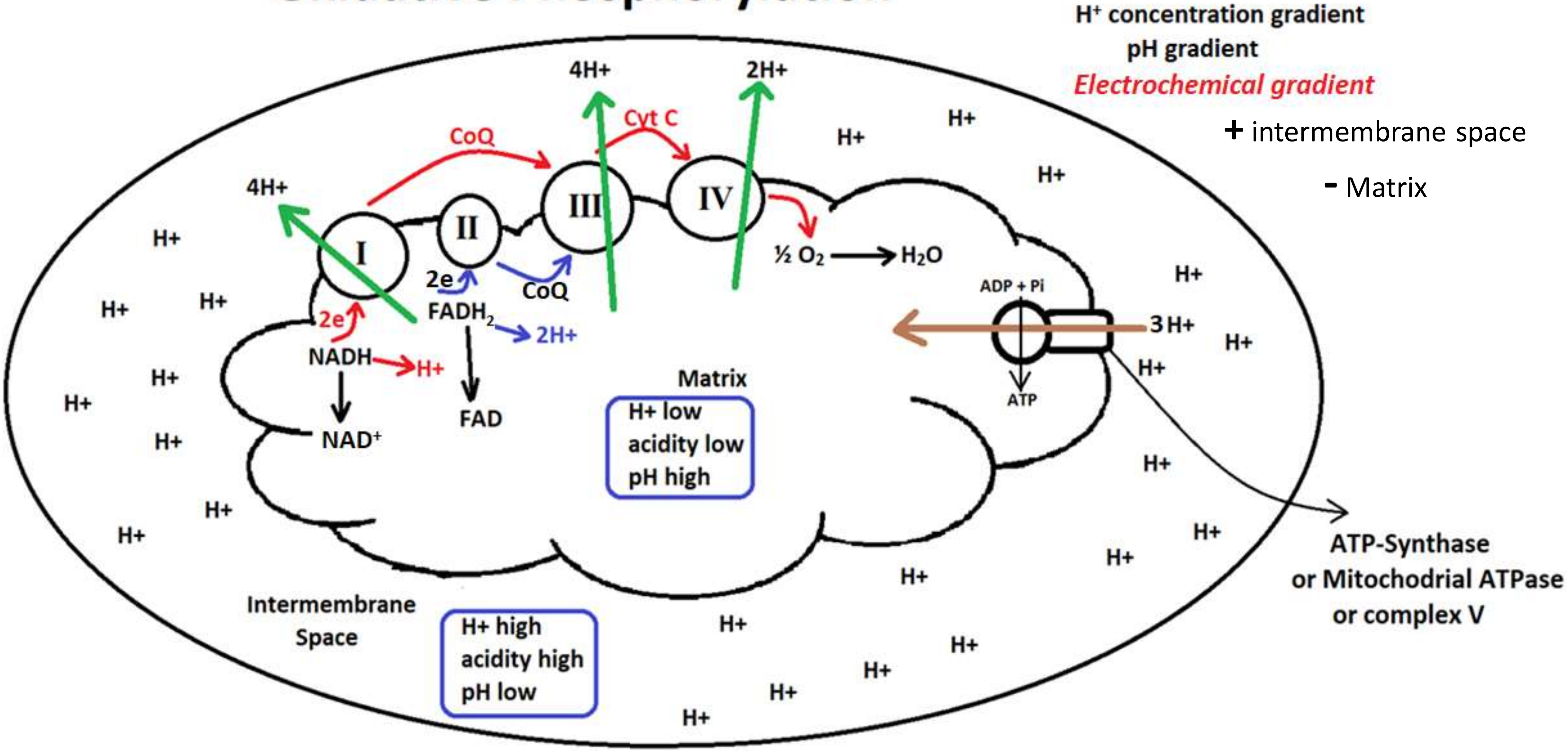
Oxidative phosphorylation: Production of ATP from NADH and $FADH_2$ using electron transport chain and Oxygen

- All NADH and $FADH_2$ produced in cellular respiration (carbohydrate, Fat, and protein oxidation) give their electrons to Oxygen through Electron Transport chain (ETC)



These complexes called ***Respiratory complexes or Enzyme complexes***
ETC called ***respiratory chain*** because the final electron acceptor is Oxygen that we breath

Oxidative Phosphorylation



In Words

- The process of ETC (Electron Transport Chain) occurs in the inner Mitochondrial membrane and the final electron acceptor is oxygen which will be reduced to H_2O
- We have 3 coupled reactions 3 عمليات مرتبطة مع بعض

نقل

1. Electron transfer from one complex to another is Exergonic process (produce energy)
2. The energy produced from electron transfer used to actively pump H^+ from the matrix to the intermembrane space creating (يخلق) pH (H^+) gradient/electrochemical gradient ميل

الميل يمثل طاقة مخزنه تدفع يعود

3. This gradient represent potential energy (Proton motive force) drive H^+ to return back to the matrix through ATP-synthase which synthesize ATP (using H^+ gradient for ATP-synthesis = ***Chemiosmosis***)

This process called **Oxidative** **Phosphorylation**

Started with Oxidation of NADH and $FADH_2$ by ETC

End with phosphorylation of ADP to ATP by ATP-synthase

Tightly coupled

اذا عملية منهم توقفت العملية الثانية تتوقف مباشرة

Proposed by ***Peter Mitchell 1961***

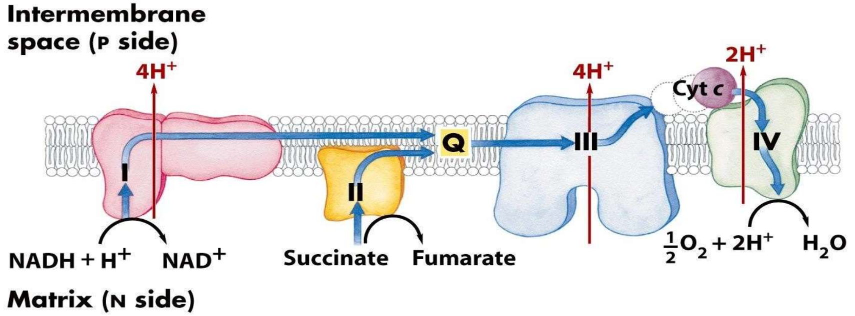
Get Nobel prize

The product of Oxidative phosphorylation is:

Energy + H_2O

Notes:

- Outer Mitochondrial membrane is **permeable** (منفذ) almost to every thing due to presence of **Porins**
- Inner Mitochondrial membrane (Convolution = Cristae) is **impermeable** (غير منفذ) almost to everything even H^+
- ETC itself do **NOT** produce ATP
- Complex I pumps $4H^+$
  Complex III pumps $4H^+$
  Complex IV pumps $2H^+$



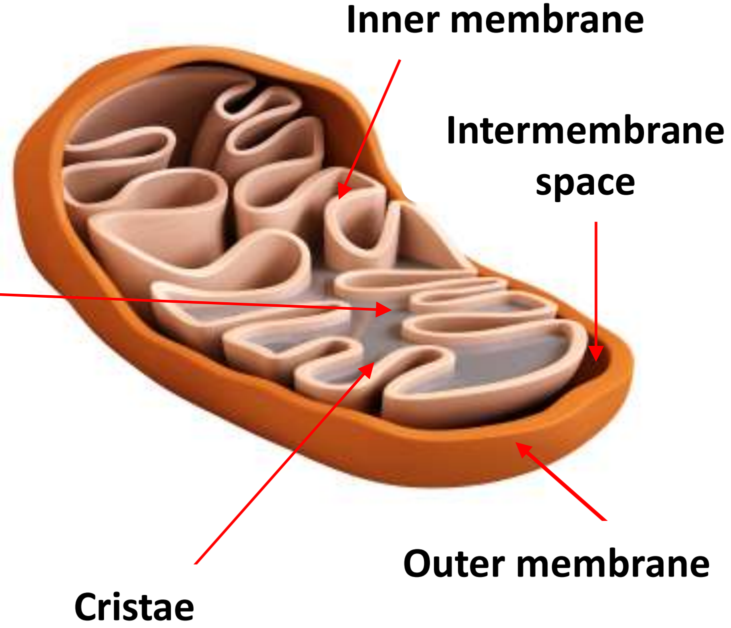
- Complex II can NOT pump H^+ because **it does NOT span** (غير مخترق بشكل كامل) the inner Mitochondrial membrane
- Each **3 H^+** return to the Matrix through ATP-synthase → produce **1 ATP**
- NADH use complexes I, III, IV → result in pumping 10 H^+ ($^{10}/_{3} \approx 3$ ATP)
- $FADH_2$ use complexes II,III,IV → result in pumping 6 H^+ ($^{6}/_{3} = 2$ ATP)

Matrix

contain circular mtDNA + RNA + Ribosomes + enzymes for

- oxidation of pyruvate
- Degradation of amino acids
- fatty acids " β-Oxidation"
- tricarboxylic acid cycle (Krebs cycle)

It also contains NAD^+, FAD, ADP and Pi



Standard Reduction Potential (E°)

قوة / رغبة كسب

Standard Reduction potential (E°): the tendency of a compound to accept electrons (to be reduced)

e

A + B ⟶ A- + B+

- Reduction potential (E°) of A is higher than B

E° > zero (Positive) → high tendency to accept electrons كلما كان موجب اكثر كان اقوى في الكسب

E° < Zero (negative) → low tendency to accept electrons higher tendency to lose electrons

كلما كان سالب اكثر تكون رغبة المادة في خسارة الالكترونات اكبر

➢ **The compound with higher E → Reduction; the other one will be oxidized**

E.g.

$NAD^+ + 2e + 2H^+ \longrightarrow NADH + H^+$ E= -0.32

$\frac{1}{2} O_2 + 2e + 2H^+ \longrightarrow H_2O$ E= +0.816

Determine which will be oxidized and which will be reduced then combine the reaction

$NADH + H^+ \longrightarrow NAD^+ + 2e + 2H^+$ E= +0.32

$\frac{1}{2} O_2 + 2e + 2H^+ \longrightarrow H_2O$ E= +0.816

$NADH + H^+ + \frac{1}{2} O_2 \longrightarrow NAD^+ + H_2O$ Enet (ΔE) =1.136 **Total Voltage**

المادة التي لها اقل E يحدث لها Oxidation
لذلك نعكس تفاعلها ونعكس اشارة E

- **E unit is Volt**

Relation between ΔE and ΔG

$$\Delta G° = - n F \Delta E°$$

n: عدد الالكترونات المنقولة
F: Faraday's constant 23.06 Kcal/volt
ΔE: Total voltage
اشارة ΔG تكون عكس اشارة ΔE

So ΔE can be used to determine the feasibility / spontaneity of the reaction

امكانية حدوث
إذا كان تلقائي او لأ

For the pervious reaction
ΔG = - (2) x (23.06) x 1.136 = **- 52.6Kcal/mol**

يعني عند انتقال الالكترونات من NADH الى Oxygen في سلسلة نقل الالكترونات الطاقة الناتجة تكون 52.6 Kcal

E.g.

$FAD + 2e + 2H^+ \longrightarrow FADH2$ E= -0.2

$\frac{1}{2} O_2 + 2e + 2H^+ \longrightarrow H_2O$ E= +0.816

calculate ΔE and ΔG ??

$FADH2 \longrightarrow FAD + \cancel{2e} + \cancel{2H^+}$ E= +0.2

$\frac{1}{2} O_2 + \cancel{2e} + \cancel{2H^+} \longrightarrow H_2O$ E= +0.816

$FADH2 + \frac{1}{2} O_2 \longrightarrow FAD + H_2O$ Enet (ΔE) = +1.016

ΔG = - (2) x (23.06) x (1.016) = - 41Kcal/mol

يعني عند انتقال الالكترونات من $FADH_2$ الى Oxygen في سلسلة نقل الالكترونات الطاقة الناتجة تكون 41 Kcal

- ΔG for e-transfer from NADH to oxygen = - 52.6Kcal/mol ; and from $FADH_2$ to oxygen = -41Kcal/mole

Efficiency of coupling for NADH

$$\text{Efficiency} = \frac{\text{الطاقة المستغلة}}{\text{الطاقة الناتجة}}\ x\ 100\% = \frac{3\ x\ 7.3}{52.6}\ x\ 100\% = \mathbf{41\%}$$

Efficiency of coupling for FADH2

$$\text{Efficiency} = \frac{\text{الطاقة المستغلة}}{\text{الطاقة الناتجة}}\ x\ 100\% = \frac{2\ x\ 7.3}{41}\ x\ 100\% = \mathbf{35\%}$$

The rest of the energy

-Heat

-Transport of Ca^{+2}, Pi, ADP, ATP

- Proteins of ETC use chemicals (Prosthetic groups) to carry and transfer electrons since amino acids can NOT carry electrons
- Electrons move from low Reduction potential (E) to high Reduction potential (E)

Q: which in ETC has the highest Reduction Potential ?? **Oxygen**

- كل مادة في السلسلة سيكون لها شكلان

- Oxidized form قبل الكسب
- Reduced form بعد الكسب

Q: the reduced form of Oxygen is ……………. **H_2O**

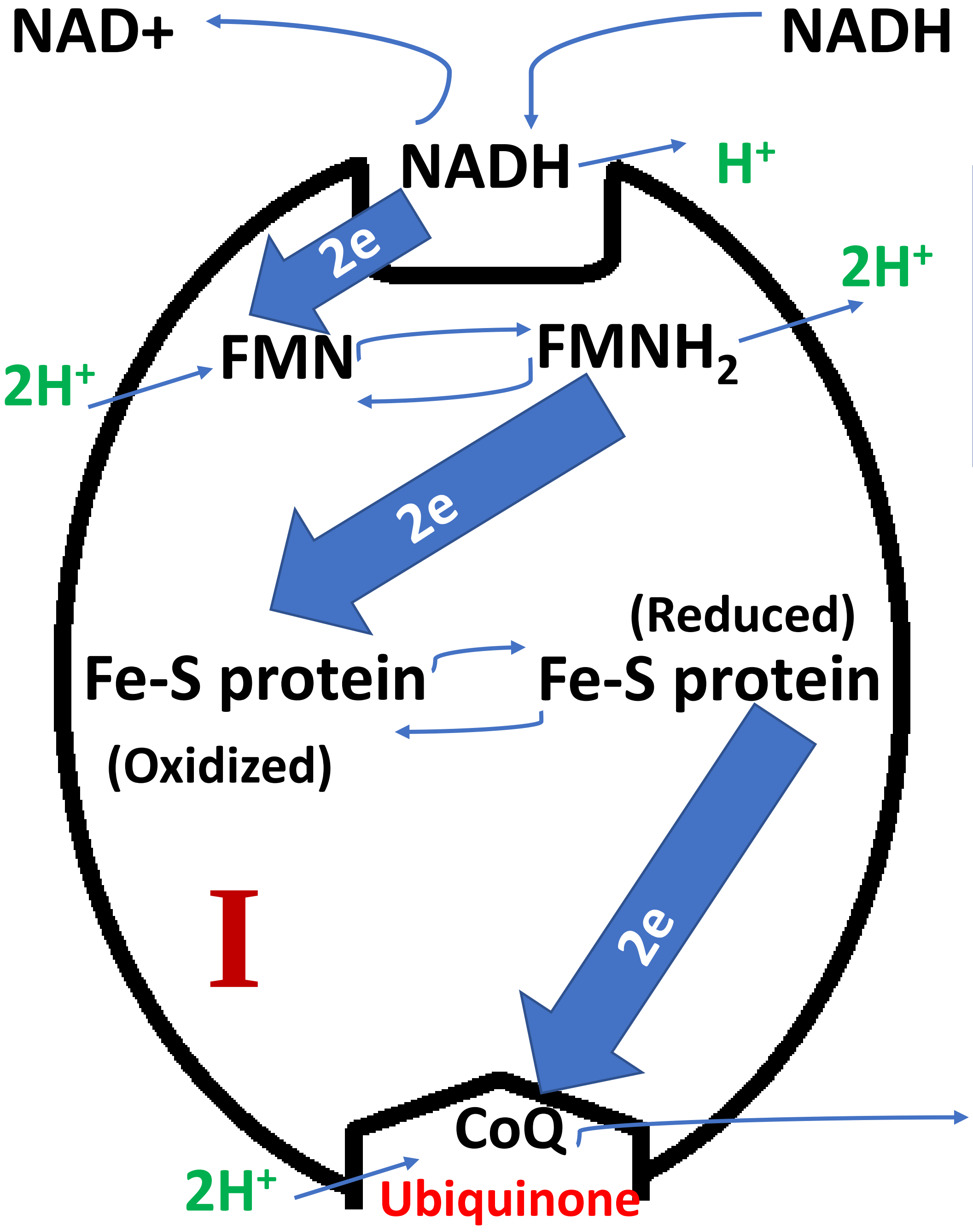
Complex I: Take electrons from NADH and donate CoQ

NADH-Dehydrogenase
NADH-CoQ Oxidoreductase

CoQ is lipid; So it’ solubIe in the inner mitochondrial membrane, it can move (Mobile) to transfer electrons to complex III

FMN: Flavin Mono-Nucleotide related to FAD and accept 2H

CoQ is the ONLY non-protein electron carrier in the ETC the rest are Proteins



$CoQH_2$ ➡ **Move to complex III**
Ubiquinol

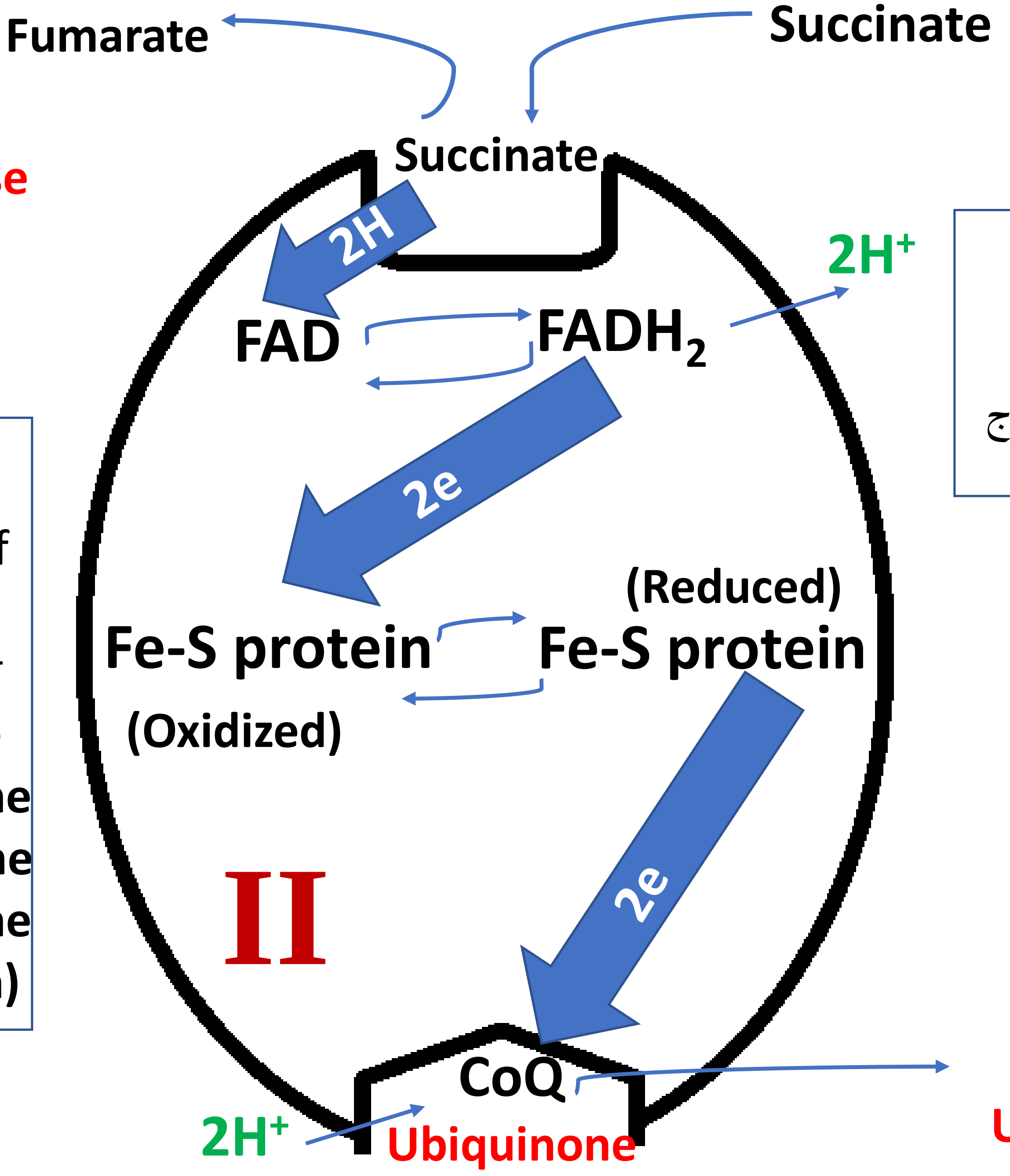
الملخص بالعربي

NADH بتعطي FMN
FMN بتعطي Fe-S Center
Fe-S center بعطي CoQ
CoQ بتحول ل $CoQH_2$ وبروح
يعطي Complex III

Complex 1 pump $4H^+$ For each 2 electron transferred through complex I

Complex II: Take electrons from Succinate and donate CoQ

Succinate-Dehydrogenase
Succinate-CoQ Oxidoreductase



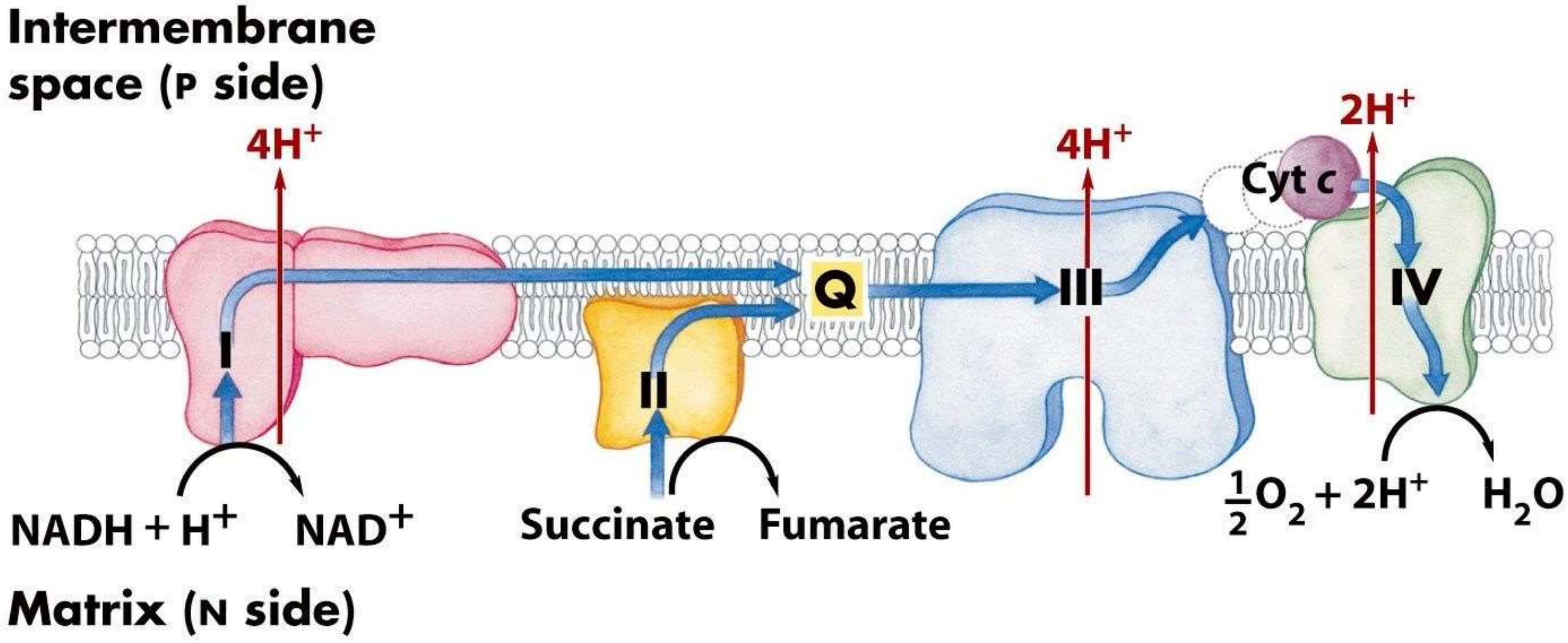
$CoQH_2$ ➡ **Move to complex III**
Ubiquinol

الملخص بالعربي

Succinate بتعطي FAD
FAD بتعطي Fe-S Center
Fe-S center بعطي CoQ
CoQ بتحول ل $CoQH_2$ وبروح
يعطي Complex III

Complex II is actually the enzyme that catalyze Step6 of TCA cycle

Complex II can NOT pump H^+ because it does not span the inner mitochondrial membrane
It's embedded but not span the inner mitochondrial membrane (NOT transmembrane protein)

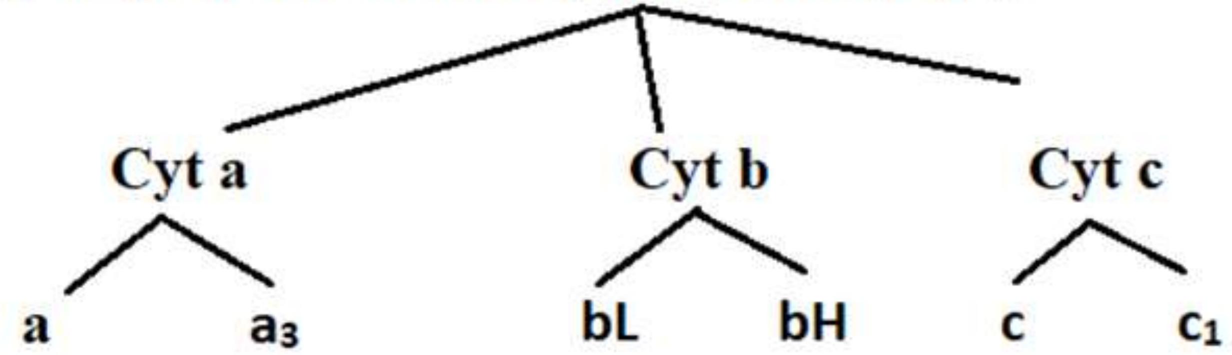


طرفي الخارجية الطبقة

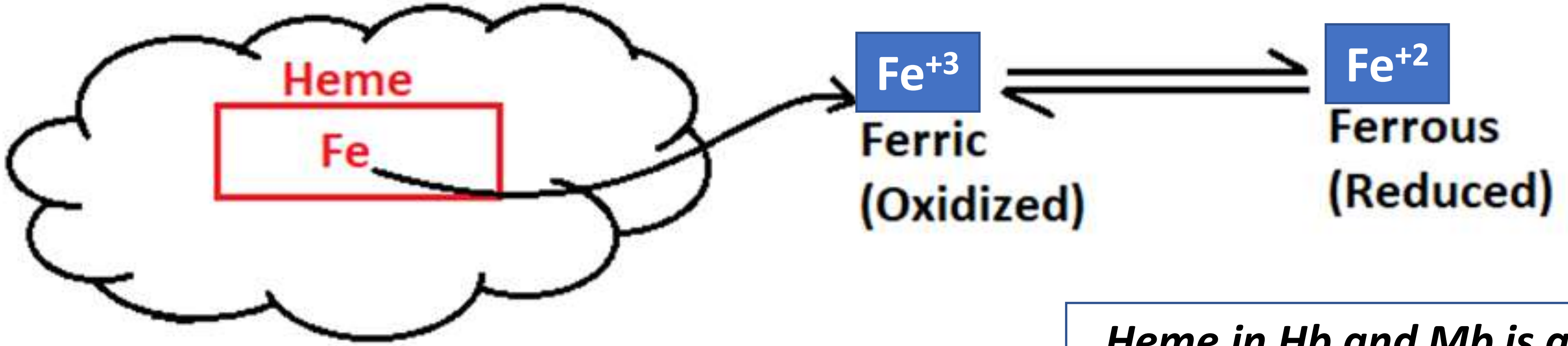
Note that ***Cyt c*** is a peripheral protein that is located on the outer leaflet of the inner Mitochondrial membrane

مش مغموس

(Not embedded within the inner mitochondrial membrane) complex III and IV contain a family of proteins called Cytochromes (Cyt)



* these proteins consists of single polypeptide chain + Heme



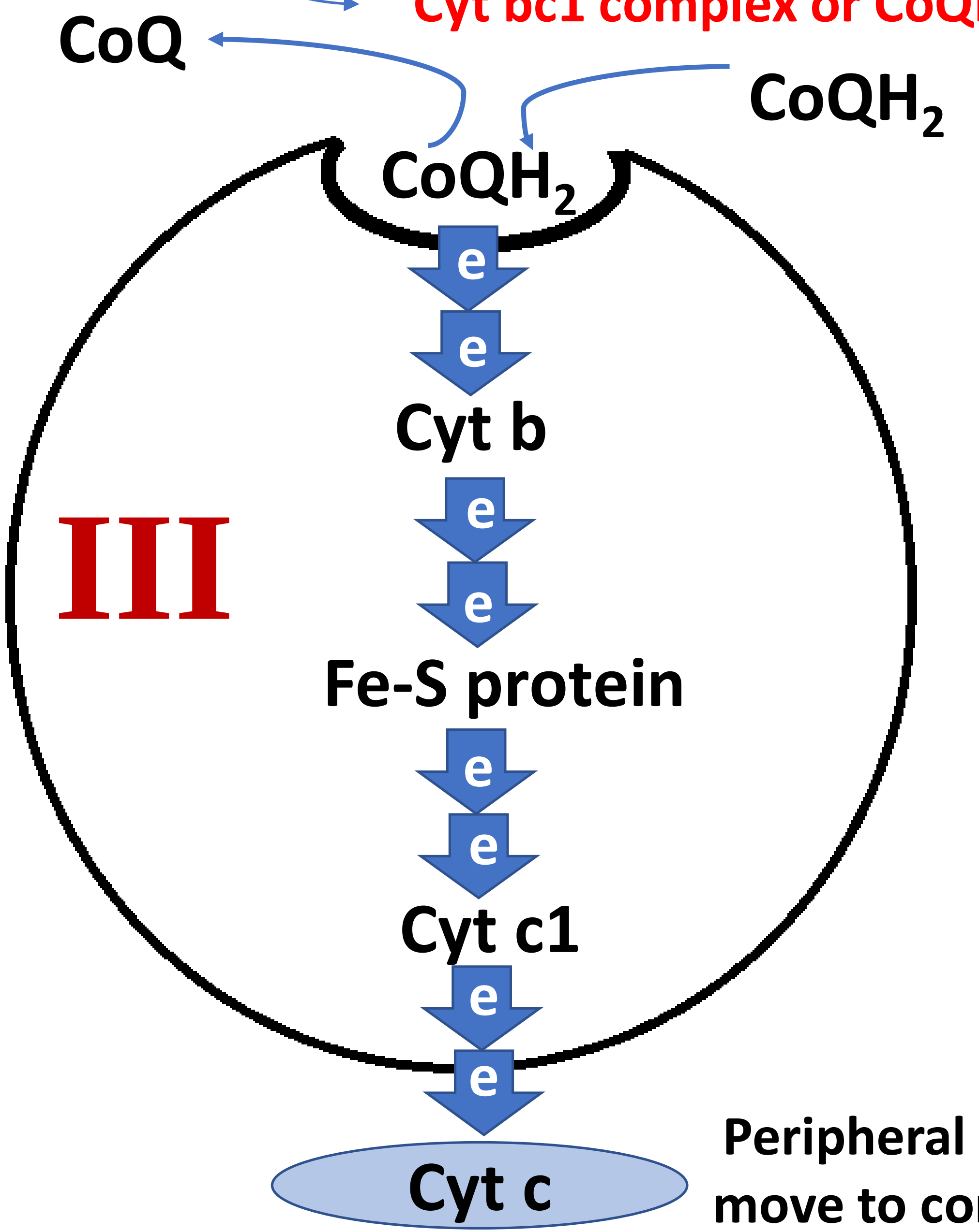
Heme in Hb and Mb is always Fe^{+2} and function in O_2 transport

☆So, this family can carry only 1 electron on Iron

☆Cytochromes named according to the type of Heme they contain

Complex III: take electron from CoQH2 and Donate Cytochrome c

Cyt bc1 complex or $CoQH_2$- Cytc Oxidoreductase



Peripheral protein move to complex 4

From now on electrons move one by one

الملخص بالعربي

Cyt b بتعطي CoQH2
Fe-S Center بتعطي Cyt b
Cyt c1 بعطي Fe-S center
Cyt c بعطي Cyt c1
Cyt c هو بروتين طرفي يتحرك
وينقل الالكترون ل Complex IV

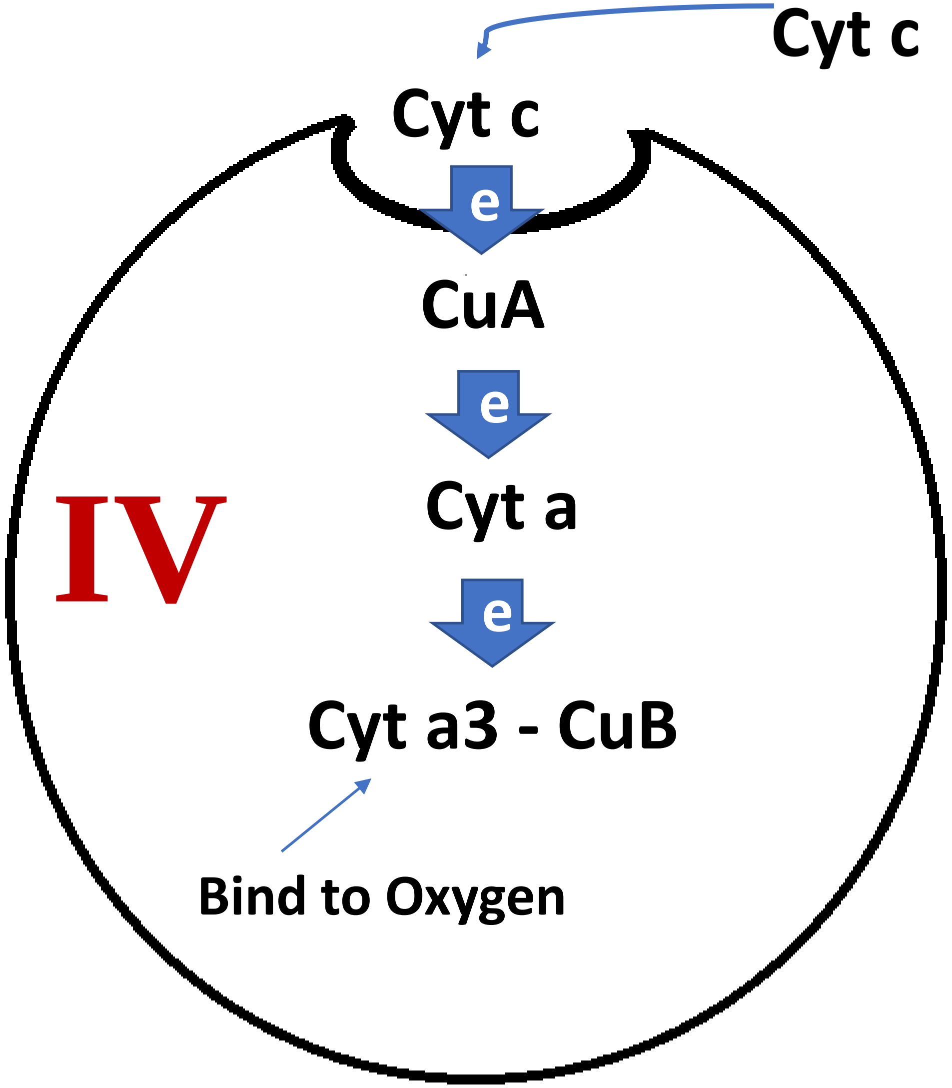
Complex 3 pump $4H^+$ For each 2 electron transferred through complex III

الطبقة الخارجية

- Found at the outer leaflet of the inner Mitochondrial membrane
- It's Mobile متحرك

Complex IV: take electron from Cyt c and Donate Oxygen

Cyt c Oxidase or Cytaa3 complex



$Cu^{+2} \rightleftharpoons Cu^{+1}$

Coppric (Oxidized) — *Copprous* (Reduced)

الملخص بالعربي

Copper A بتعطي Cyt c
Cyt a بتعطي Copper A
Cyta3-Copper B بعطي Cyt a
binuclear center
Oxygen is bound to Cyta3

Complex 4 pump $2H^+$ For each 2 electron transferred through complex IV

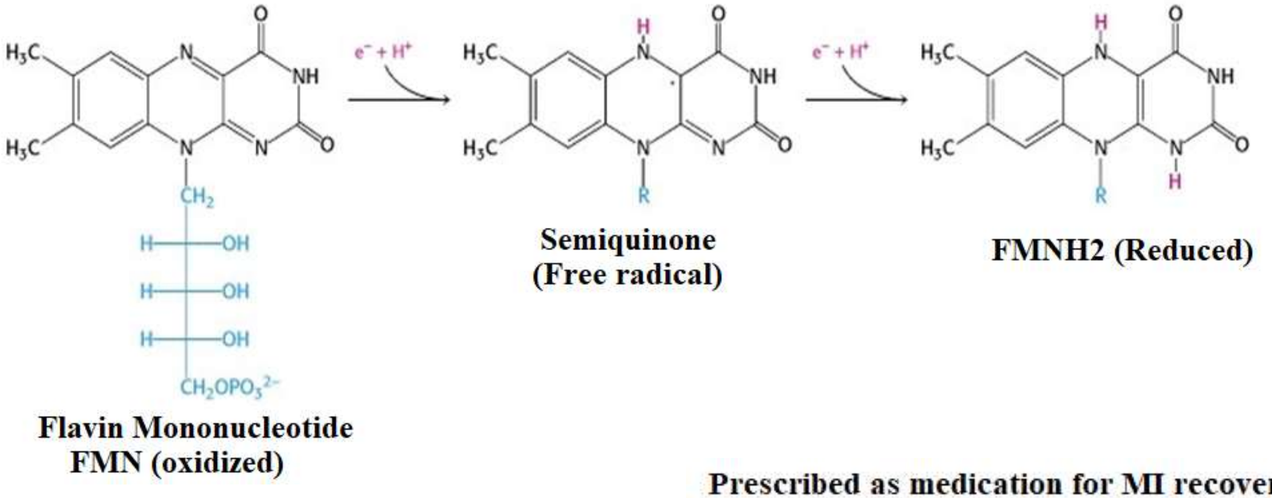
4 electrons required to reduce 1 molecule of oxygen (O_2) to 2 H_2O molecules

More information about ETC components

FMN

Flavin Mono-nucleotide

- Accept one or 2 electrons as Hydrogen
- Tightly bound to the protein



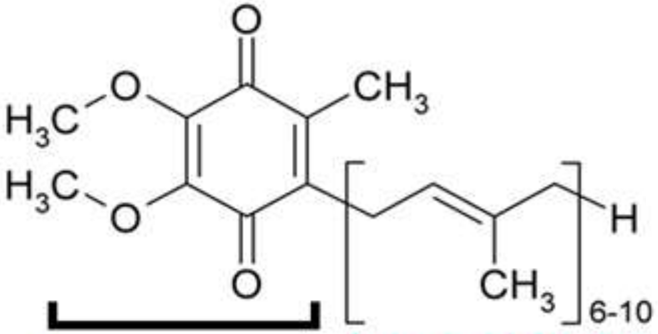
Prescribed as medication for MI recovery
increase energy production in muscles

CoQ

- Accept one or 2 electrons as Hydrogen
- Made of intermediates of cholesterol synthesis

موجود بكثرة

- Ubiquitous in biological systems



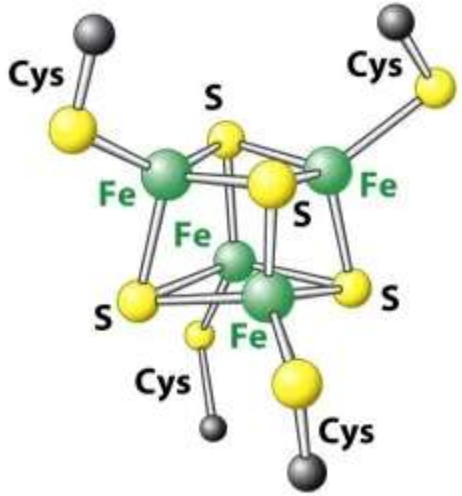
Benzoquinone
site of electron binding

Isoprenoid Unit

Hydrophobic and make it
lipid soluble

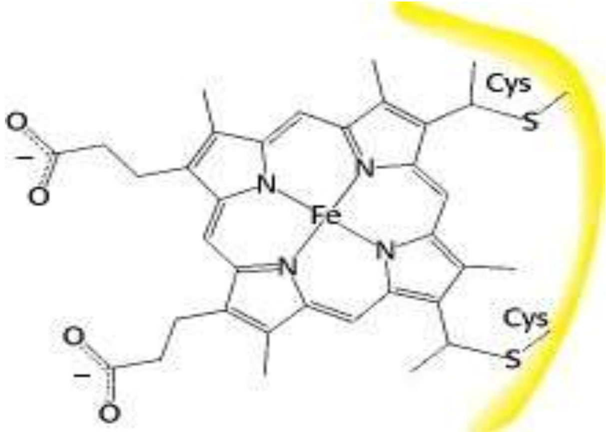
Fe-S proteins

- Contain Iron and Inorganic sulfur bound to Cysteine amino acid
- Carry one electron as electron



Cytochromes

- Contain heme group
- There are many types of heme a, b, c
- Carry one electron as electron



The only Lipid (Hydrophobic)…………**CoQ**……………………..
Mobile in the ETC……**CoQ**………………… and ……**Cyt c**…………….
Not embedded in the inner Mitochondrial membrane ………**Cyt c**……..

ATP synthase (Mitochondrial ATPase) or Complex V

- It's a complex enzyme consists of many subunits
- According to the structure it can be divided into 2 domains

F0 : embedded in the inner mitochondrial membrane

2 types of polypeptides : **c-ring** (8 subunit), and **a** function as H^+ channel/gate

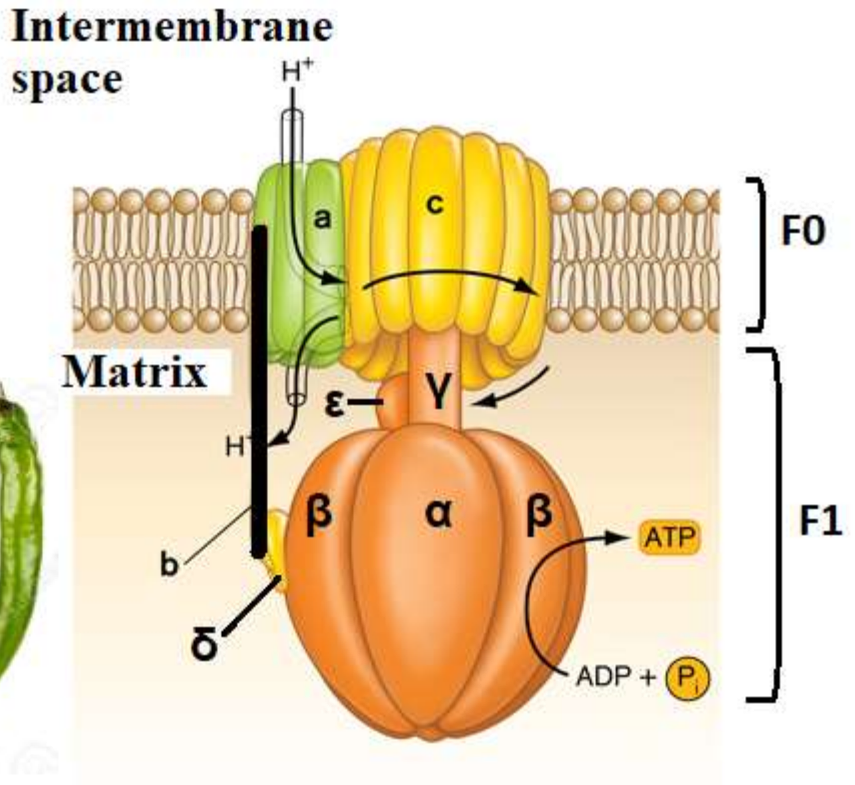
F1: project to the matrix

5 types of polypeptides **3α 3β γ δ ε**

Synthesize the ATP

β contain the active site

α Structural role

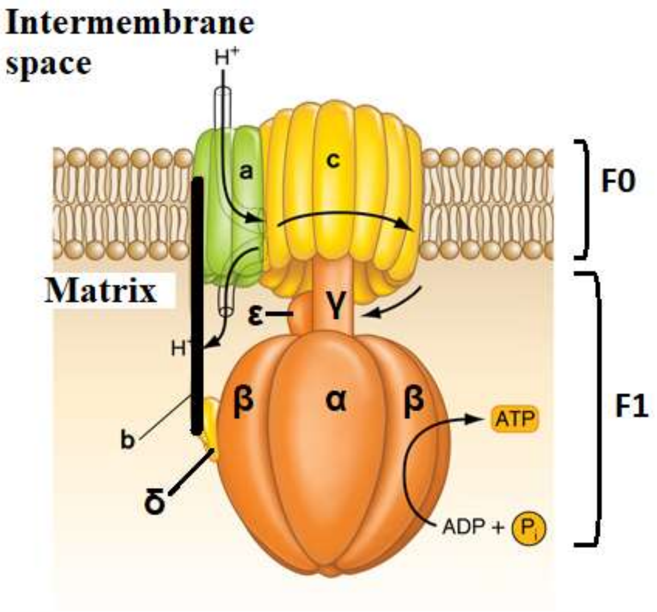


F0 and F1 connected to each other by **γ** Stalk (Angled, Curved)

This Enzyme also called ***F1/F0 complex***

How it works?

→ H^+ enter from the intermembrane space to the matrix through Fo domain
→ C ring rotate
→ **γ rotate and hit β (α and β are fixed do not rotate)**
→ conformational changes in the β-subunits that allow is to bind to ADP + Pi, phosphorylate ADP to ATP, and release ATP



- One complete rotation of the c-ring → Hit the 3 β subunits → synthesis of 3ATP

Electrochemical Energy → Mechanical Energy → Chemical Energy (ATP)

- If H^+ flow in the opposite direction from matrix to the intermembrane space

→ ATP synthase will rotate counter clockwise and it will break ATP

Substances that can lower/Inhibit ATP synthesis

Uncouplers

Inhibit ATP synthesis without affecting ETC or ATP synthase

- They simply cancel H^+ gradient → less ATP produced

E.g. 2,4 Dinitophenol; it's a small lipophilic molecule that swim inside the inner mitochondrial membrane

It takes H^+ from the intermembrane space then release it into the Matrix (ionophores)

→ No H^+ gradient

→ No ATP synthesis

ETC sill pumping H^+ so the oxidation of food will continue but less energy used for ATP synthesis because H^+ will return to the matrix through the uncoupler not through ATP synthase, most of the energy will be released as ***Heat***

الجسم يستمر بتكسير الدهون والكربوهيدرات لكن بدون انتاج ATP (انتاج قليل)

كان يستخدم قديما لتقليل الوزن Control Obesity

Side effects: Malignant Hyperthermia, bleeding eyes and Death

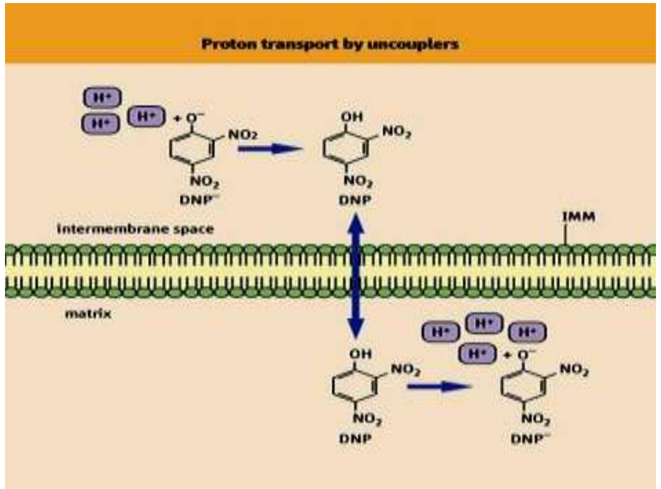
منعته منظمة الغذاء والدواء FDA banned 1938

Respiratory Inhibitors

Inhibit Electron movement through ETC or inhibit ATP-synthase

Oxidative phosphorylation will stop

Oxidation of food will stop

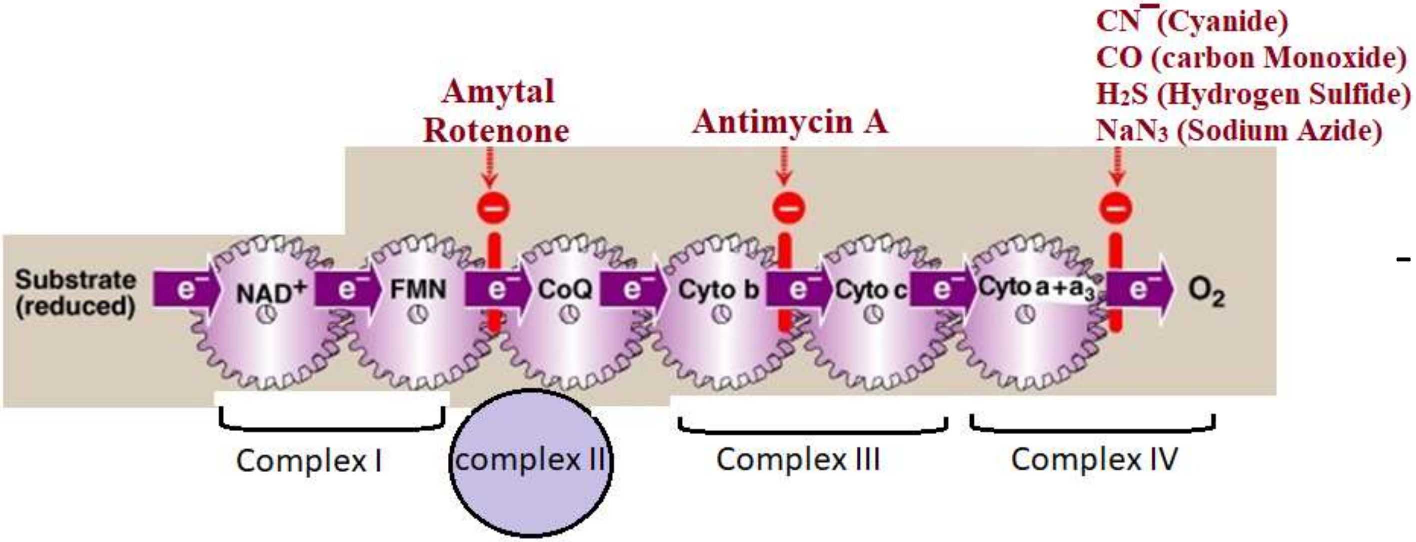


Respiratory Inhibitors:

يمنع

Substances that Inhibit electron transfer in ETC or inhibit ATP-synthase

حفظ



- **Oligomycin** (antibiotic) prevent H^+ flow through $\mathbf{F_o}$ **portion** of ATP synthase

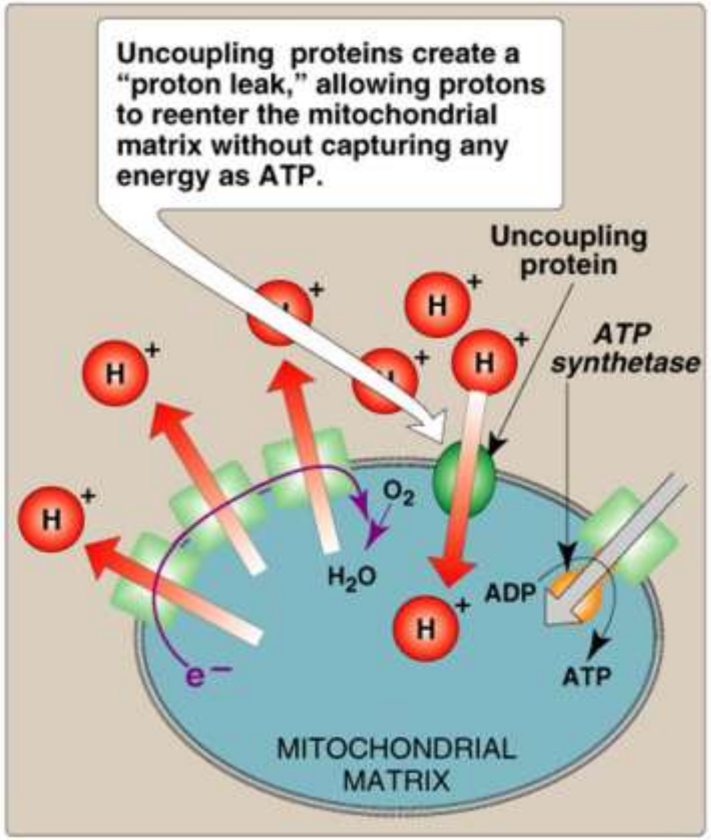
Using respiratory inhibitor will accumulate:
Reduced form before inhibition point
And the oxidized form after inhibition point

- Inhibit complex I → ATP synthesis from NADH stop, [Succinate ($FADH_2$) not affected]
- Inhibit complex II → ATP synthesis from succinate ($FADH_2$) stop, [NADH not affected]
- Inhibit complex III and IV → electron transport from NADH and succinate ($FADH_2$) stop

Q: which of the following will accumulate in the Mitochondria when exposed to Antimycin A?

a. NAD^+ **b. $CoQH_2$** c. Cyt $c_{(Fe+2)}$ d. FMN

Uncoupling Proteins (UCPs)



Physiological proteins that are normally found in the inner mitochondrial membrane. they enable تسمح H^+ to return to matrix without passing من دون المرور through ATP synthase.

All energy converted to heat not to ATP this stimulate the body to break more fat

Many types UCP1, UCP2, UCP3, UCP4….

UCP1 also called **Thermogenin** found primarily in Brown Adipose tissue, it convert fat to heat

Brown Fat found in high amount in infants الرضع and young children, then decline يقل in adults.

Some Societies المجتمعات have high concentration of UCPs, So they don't suffer from Obesity السمنه (Yamen)

The production of heat through UCP called ***non-shivering thermogenesis*** انتاج الحرارة

note:

- The main factor that affect the rate of Oxidative phosphorylation is ATP/ADP ratio
- High **ADP** increase the rate of oxidative phosphorylation
- If no ADP present then ATP-synthase cannot work then oxidative phosphorylation will stop

Note: Krebs Cycle is not the only source of NADH and $FADH_2$, there are other pathways produce NADH and $FADH_2$ that must be undergo oxidative phosphorylation in the mitochondria

E.g. Glycolysis in cytoplasm produce 2 NADH; these NADH must be transported to mitochondrial matrix to synthesize ATP

But, inner mitochondrial membrane is impermeable to NADH, what we do?

NADH has 2 electrons that should be transferred to complex I and NADH can NOT cross to matrix; so we try to transport the 2 electrons using Shuttles (2 Types)

a. Glycerol-3-P Dehydrogenase shuttle

NADH in the Cytosol give its 2 electrons to Dihydroxyacetone-P producing Glycerol-3-P by enzyme called Glycerol-3-P Dehydrogenase
→ Then Glycerol-3-P enter the mitochondria
→ In mitochondria Glycerol-3-P give the 2 electron to FAD which is found inside mitochondrial Glycerol-3-P Dehydrogenase enzyme
→ Then $FADH_2$ give to CoQ

NADH (Cytosol)→→→→ $FADH_2$ (mitochondria)
3 ATP 2 ATP

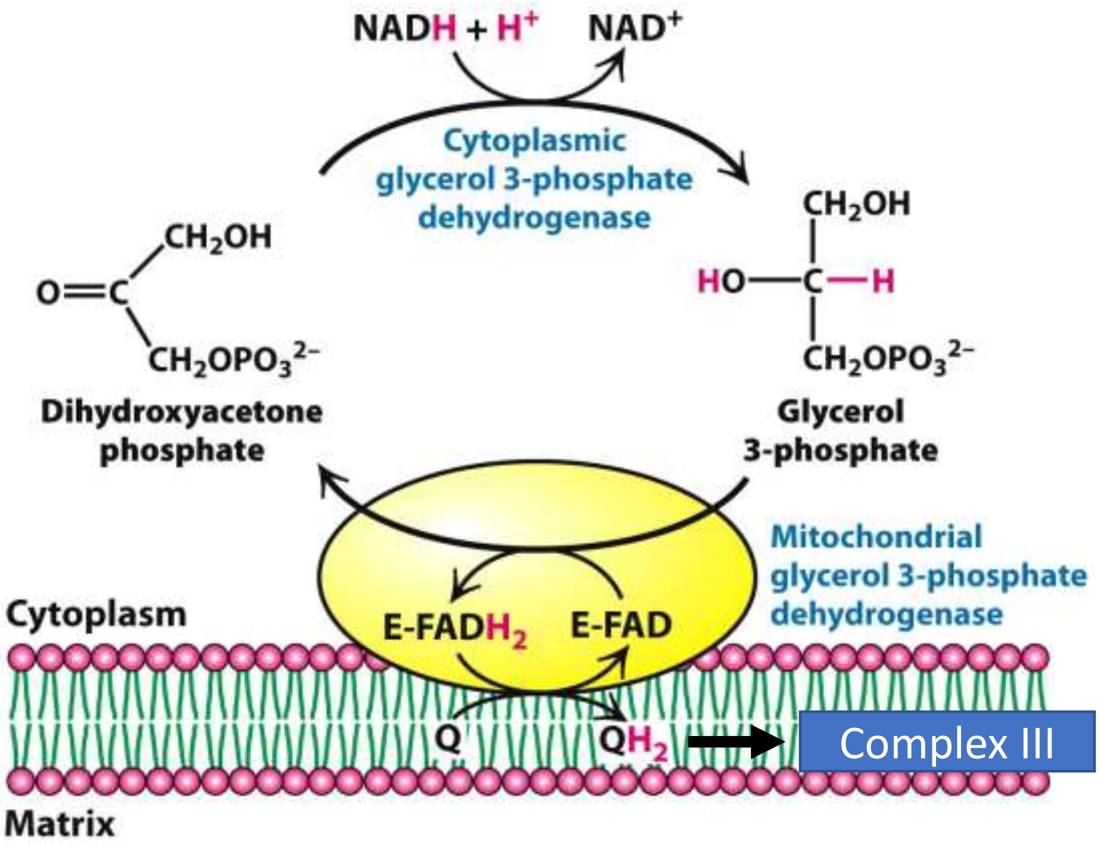
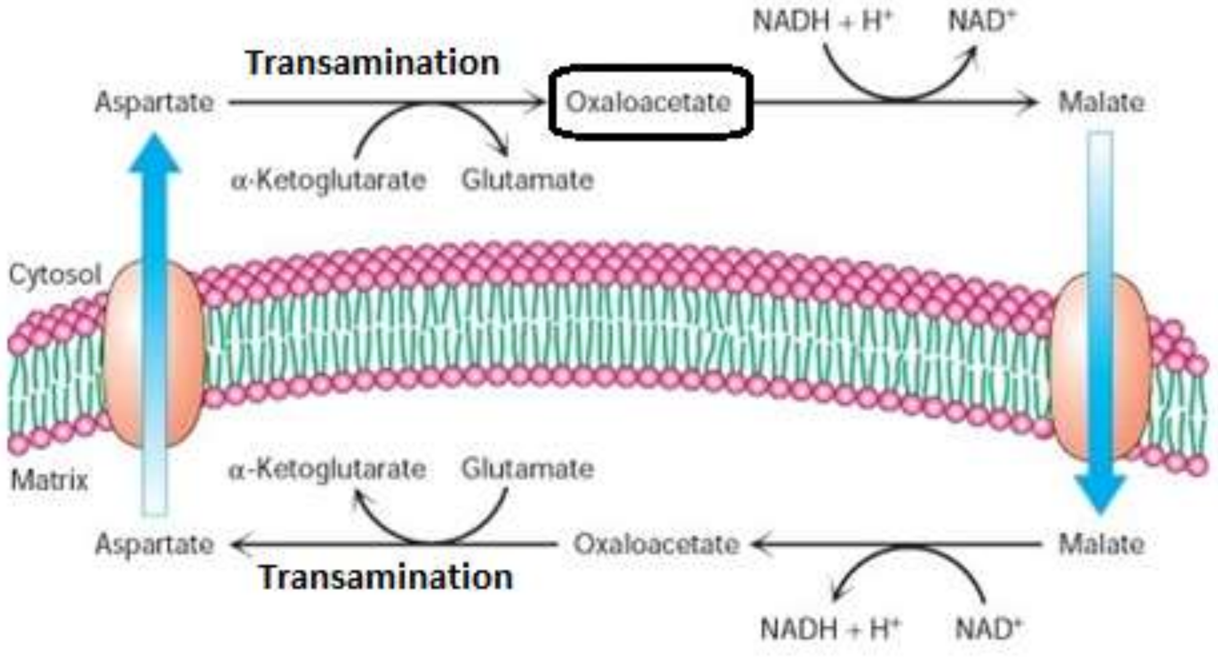


Figure 18.34
Biochemistry, Seventh Edition

- b. Malate – Aspartate Shuttle

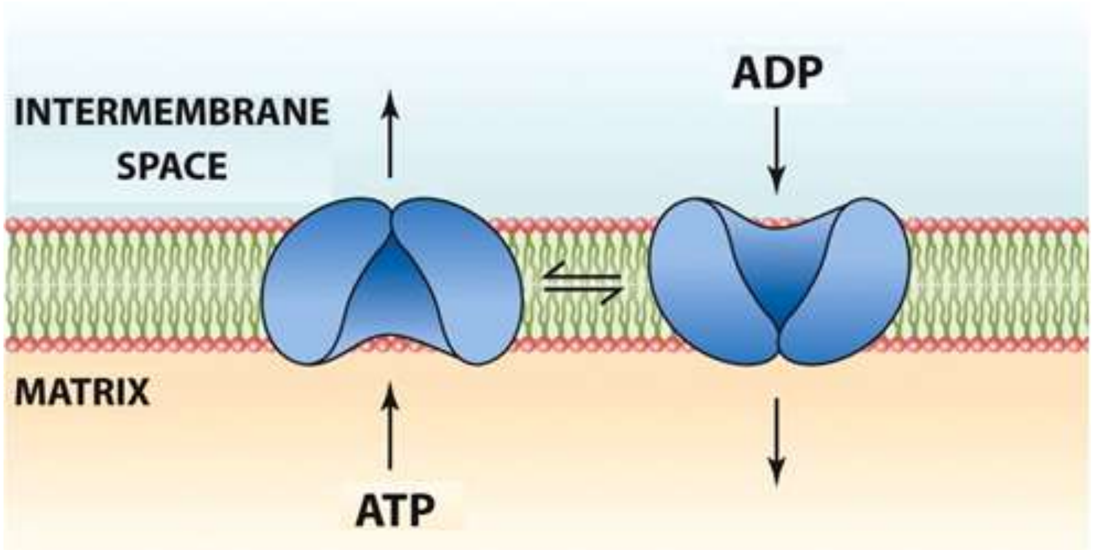
NADH of the Cytosol give its 2 electrons to Oxaloacetate producing Malate by enzyme called Malate Dehydrogenase
→ Then Malate enter the mitochondrial Matrix
→ In mitochondrial Matrix Malate give the 2 electron to NAD^+ (Step8 in Krebs cycle)
→ Then NADH give to Complex I
→ Malate converted to oxaloacetate then to Aspartate
→ Aspartate get out of mitochondria to Cytosol
→ In the Cytosol Aspartate converted back to Oxaloacetate

NADH (Cytosol)→→→→ NADH (mitochondria)
3 ATP 3 ATP



Note : most ATP synthesized in Mitochondria by Oxidative phosphorylation, but most ATP consumed outside mitochondria; So we must transport ATP from mitochondria to cytosol and ADP from Cytosol to mitochondria
How??

By Carrier called **ATP-ADP Translocase** which is found in the inner mitochondrial membrane
-Each ATP out , ADP in (1 : 1 Ratio)
-This carrier represent 14% of the inner mitochondrial membrane proteins
-It consume energy, this energy come from electron transfer in ETC



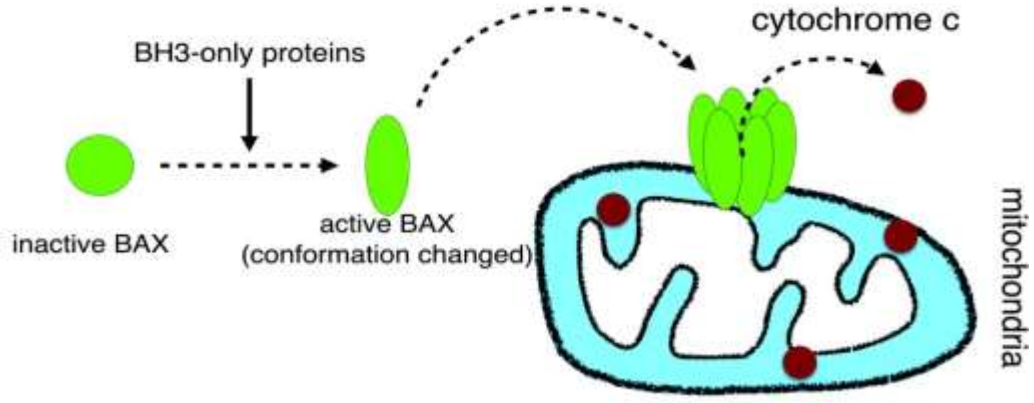
What will happen if ATP-ADP translocase is inhibited??

Inherited defects in oxidative phosphorylation

- 90 polypeptides required for Oxidative phosphorylation, 13 of them are encoded by mtDNA and the rest by nuclear DNA
- Genetic defects in OXPHOS are more likely result from mtDNA mutation
- Affect mainly CNS, muscles and Liver
- Examples: mitochondrial myopathies, Leber hereditary optic neuropathy which result in damage of optic nerve
- Maternal inheritance

Mitochondria and Apoptosis

- Apoptosis (programmed cell death) may can be initiated by ***mitochondrial mediated pathway*** where pores are formed in the outer mitochondrial membrane allowing ***Cytc*** to inter the cytosol and associate with ***proapoptotic factors***, this will activate a family of proteolytic enzymes called ***Caspases*** that cleave cell proteins and cause cell death



Notes from Dr. slides

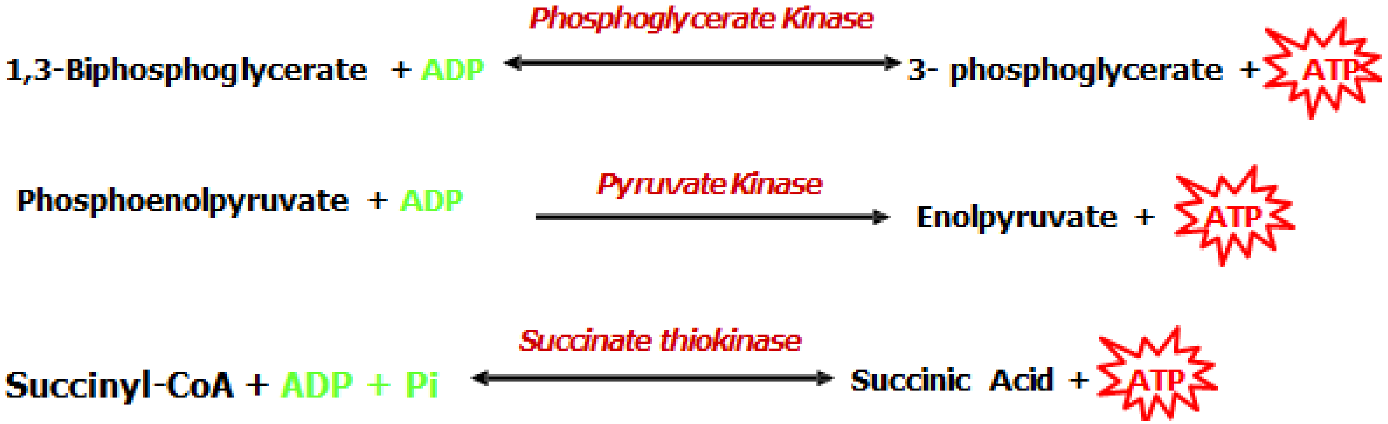
Low energy bonds: bonds that produce **less than 7Kcal** upon hydrolysis such as:

- Phosphate ester bonds (Glucose-6-P or Glucose-1-P)
- Glycosidic bonds
- Peptide bonds

High energy bonds: bonds that produce **more than 7Kcal** upon hydrolysis such as:

- Enole phosphate bond as in phosphoenol pyruvate
- Carboxyl phosphate bonds as in 1,3 bisphosphoglycerate
- Thioester bond of CoA

High energy bonds can be used in substrate level phosphorylation to regenerate ATP



P/O ratio: number of **ATP produced** for each **oxygen atom** reduced to H_2O

For NADH P/O ratio is 3:1

For $FADH_2$ P/O ratio is 2:1