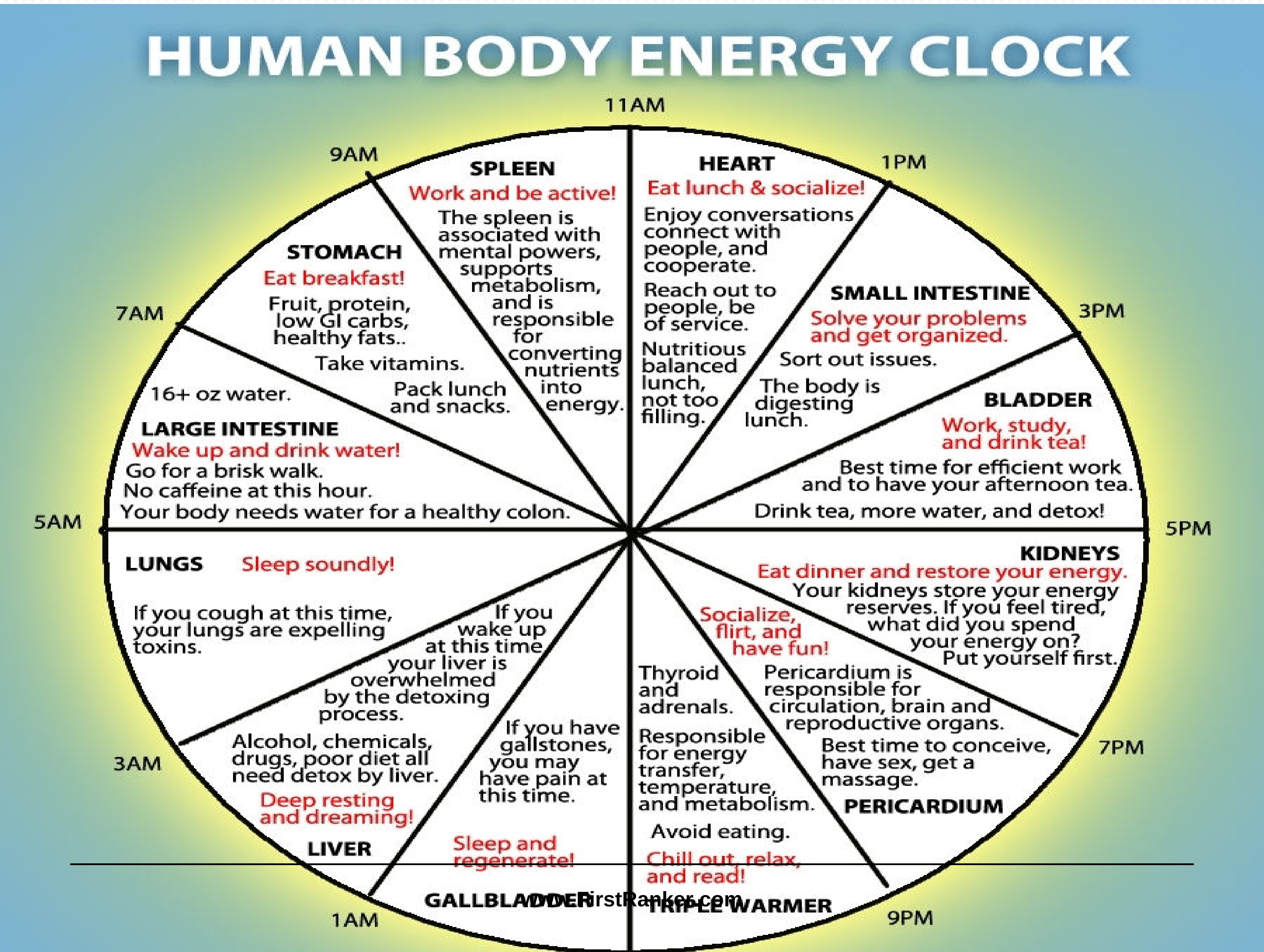
Induction To Todays Topic



Any Guesses Of Todays Topic???

Energy Metabolism

Bioenergetics

BIOLOGICAL OXIDATION

Specific Learning Objectives

Questions Which Will be Answered

- **What is system of Bioenergetics ?**

- How is chemical form of energy ATP formed (**Generation**) and utilized (**Operation**) in human body ?

- **What Factors are associated to bioenergetics system?**
  - Metabolites
  - Enzymes
  - Coenzymes
  - Cofactors
  - Hormones

- **Which disorders suffered due to defective system ?**

Synopsis

- What is Bioenergetics?
- High Energy Compounds
- Substrate Level Phosphorylation
- What is Biological Oxidation?
- Enzymes and Coenzymes of Biological Oxidation Reactions
- Electron Transport Chain (ETC)

Continued---------

- Oxidative Phosphorylation Mechanism
- Inhibitors of ETC and Oxidative Phosphorylation
- Uncouplers- Mode of Action
- Shuttle System
- Factors Involved in Oxidative Phosphorylation mechanism

Lets Get Introduced To

Human energy

- Energy is the ability to do work
- Work is one form of energy, it is known as mechanical or kinetic energy.
- Energy are found in different forms in human body Energy are of four types;-

Types of energy in human body

- 1-Chemical energy;– Storage form of energy
- 2- electrical energy for nerve impulses
- 3- Heat energy;– Product of metabolism **energy** to keep body temperature at 37degree C
- 4- Mechanical energy;– Capacity to do metabolic work (muscle to be able to move)

What Is Bioenergetics?

- Bioenergetics or biochemical thermodynamics is:
- Study of **energy changes** during biochemical reactions.

Biological Systems Conform to General Laws of Thermodynamics

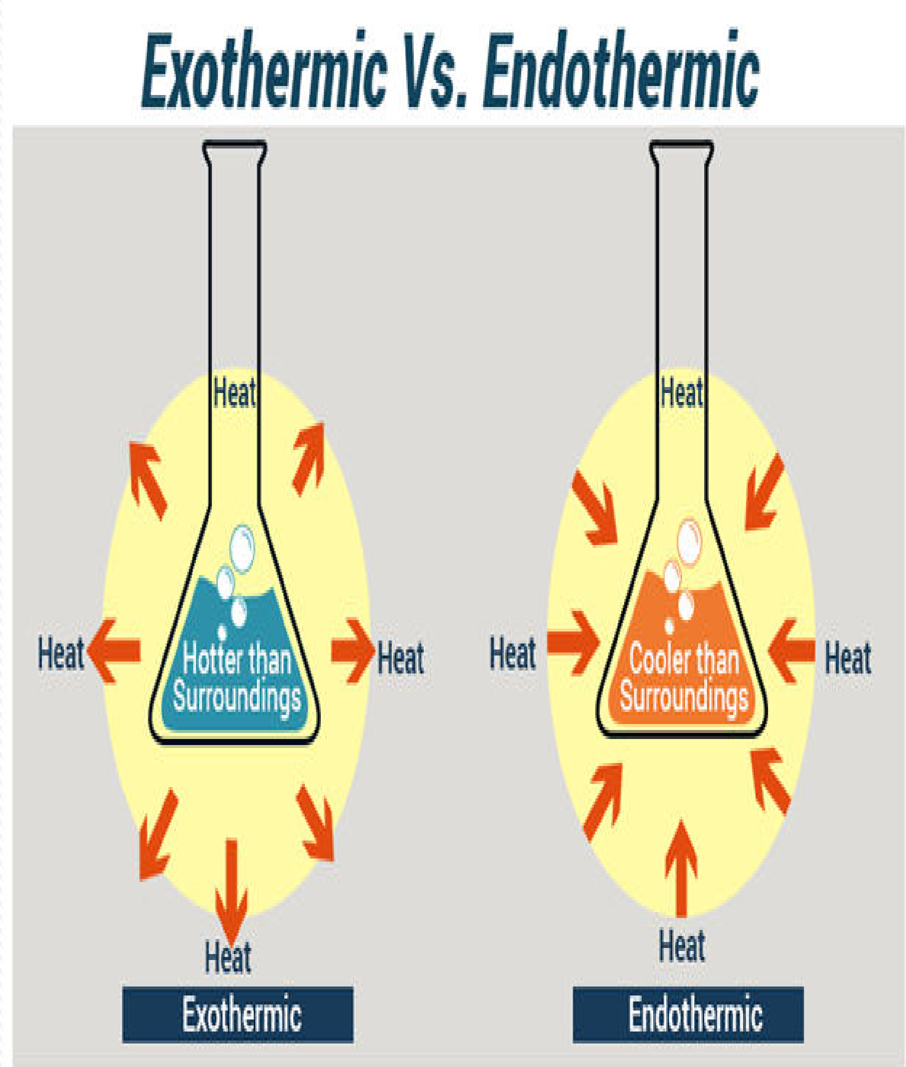
Energy Is Never Destructed

(Soul is energy never destructed and it is Immortal)

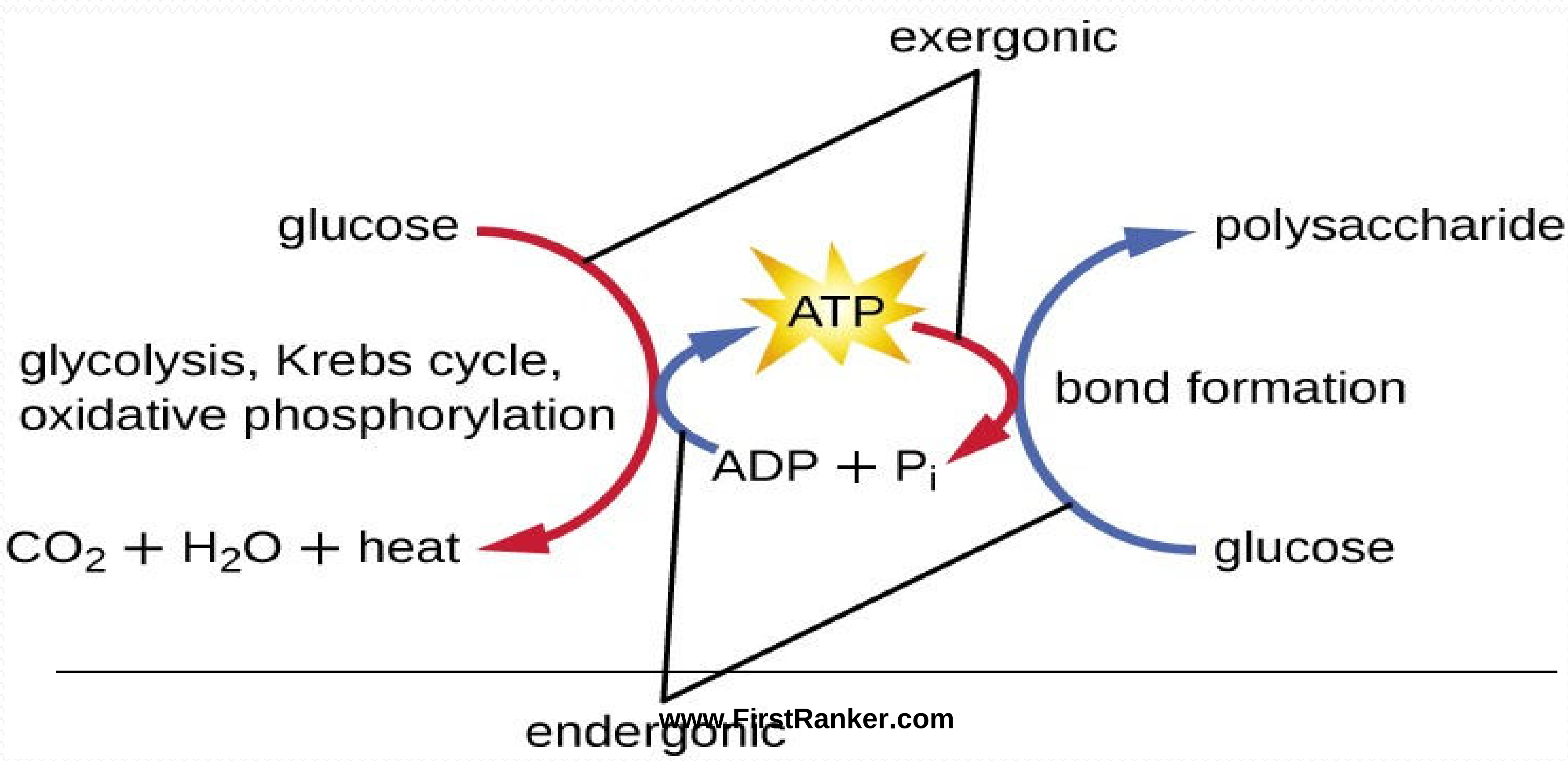
- **Total energy of a system, including its surroundings, remains constant**
- Energy is **neither lost nor gained** during any change
- May be **transformed** into another form of energy
- May be **transferred from one part of system** to another or

Conditions Of Bioenergetics

- Isothermic (mostly)
- Endothermic/ Endergonic/Anabolic
- Exothermic/Exergonic /Catabolic



PROCEED BY COUPLING OF EXERGONIC(Catabolic) PROCESSES



High Energy Compounds Of Human Body

- High energy compounds are **energy rich compounds.**
- Possess **high energy bonds** in its structures.
- **Cleavage** of these **high energy bonds liberate more energy than that of ATP hydrolysis.**

S.No	Examples Of High Energy Compounds	Free Energy Released On Hydrolysis. Cal/mol
1	Phospho Enol Pyruvate	-14.8
2	Carbamoyl Phosphate	- 12.3
3	Cyclic AMP	-12.0
4	1,3 Bis Phospho Glycerate	-11.8

S.No	Examples Of High Energy Compounds	Free Energy Released On Hydrolysis. Cal/mol
5	Creatine Phosphate	-10.3
6	S Adenosine Methionine (SAM)	-10.0
7	Succinyl CoA	-7.7
8	Acetyl CoA	-7.7
9	ATP	-7.3

TABLE 11–1 Standard Free Energy of Hydrolysis of Some Organophosphates of Biochemical Importance

Compound	ΔG°' kJ/mol	ΔG°' kcal/mol
Phosphoenolpyruvate	−61.9	−14.8
Carbamoyl phosphate	−51.4	−12.3
1,3-Bisphosphoglycerate (to 3-phosphoglycerate)	−49.3	−11.8
Creatine phosphate	−43.1	−10.3
ATP → AMP + PP_i	−32.2	−7.7
ATP → ADP + P_i	−30.5	−7.3
Glucose-1-phosphate	−20.9	−5.0
PP_i	−19.2	−4.6
Fructose-6-phosphate	−15.9	−3.8
Glucose-6-phosphate	−13.8	−3.3
Glycerol-3-phosphate	−9.2	−2.2

Significance Of High Energy Compounds

OR

Fates Of High Energy Compound In Catabolic And Anabolic Pathways

- **During Catabolic pathways/reaction**

- **High energy compounds** follow **substrate level phosphorylation reaction.**

- High energy compounds **cleave high energy bond** to generate **high energy used for phosphorylation of ADP with pi at reaction level.**

- **Generate ATP at substrate/reaction level.**

Substrate Level Phosphorylation

- **Mode of generation of ATP at substrate level**

- Involves **cleavage of high energy bond present in high energy compound**

- Bond **energy released is used for Phosphorylation reaction**

- **Generates ATP directly and instantly at reaction level without involvement of ETC**

Examples Of High Energy Compounds Undergoing Substrate Level Phosphorylation.

S.No	High Energy Compound	Enzyme Catalyzing	Product Obtained	High energy Phosphate Compound Generated	Metabolic Pathway Involved
1	1,3 Bis Phospho Glycerate	Phospho Glycerate Kinase	3 Phospho Glycerate	ATP	Glycolysis
2	Phospho Enol Pyruvate	Pyruvate Kinase	Enol Pyruvate	ATP	Glycolysis
3	Succinyl CoA	Succinate Thio Kinase	Succinate	GTP	Krebs/TCA Cycle

- **During Anabolic pathways/reaction**

- High energy compounds follow **condensation** or **bond building reactions.**

- High energy compound cleave to generate energy

- Energy used for building C-C bonds.

HIGH-ENERGY PHOSPHATES PLAY A CENTRAL ROLE IN ENERGY CAPTURE AND TRANSFER

High Energy Compounds Generated In Catabolic Pathways Are Utilized In Anabolic Reactions

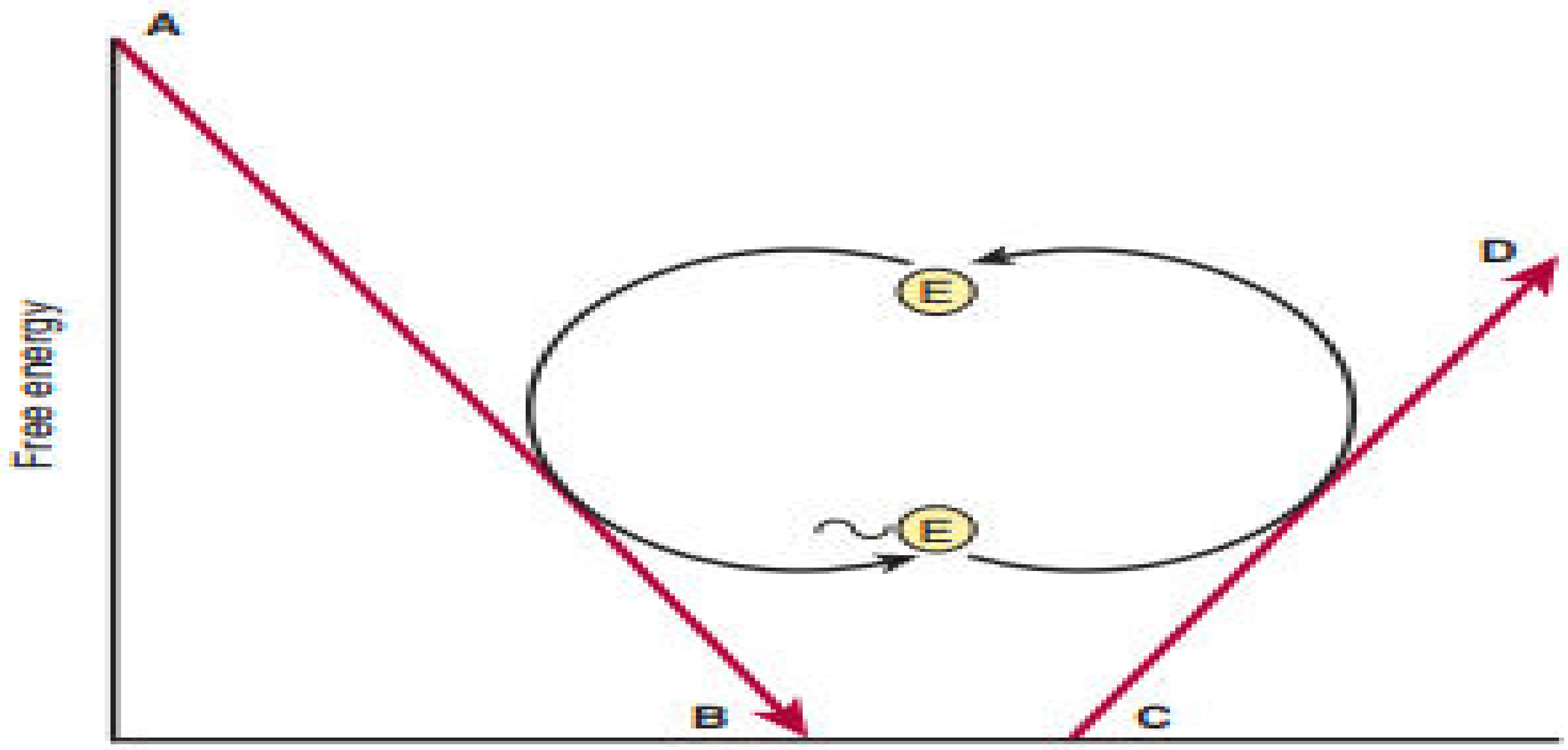


FIGURE 11–3 Transfer of free energy from an exergonic to an endergonic reaction via a high-energy intermediate compound (~Ⓔ).

HIGH-ENERGY PHOSPHATES ACT AS "ENERGY CURRENCY" OF CELL

Free Energy of hydrolysis Of High Energy Phosphate Bonds has Important Bioenergetics Significance

Adenylate Kinase (Myokinase) Interconverts Adenine Nucleotides

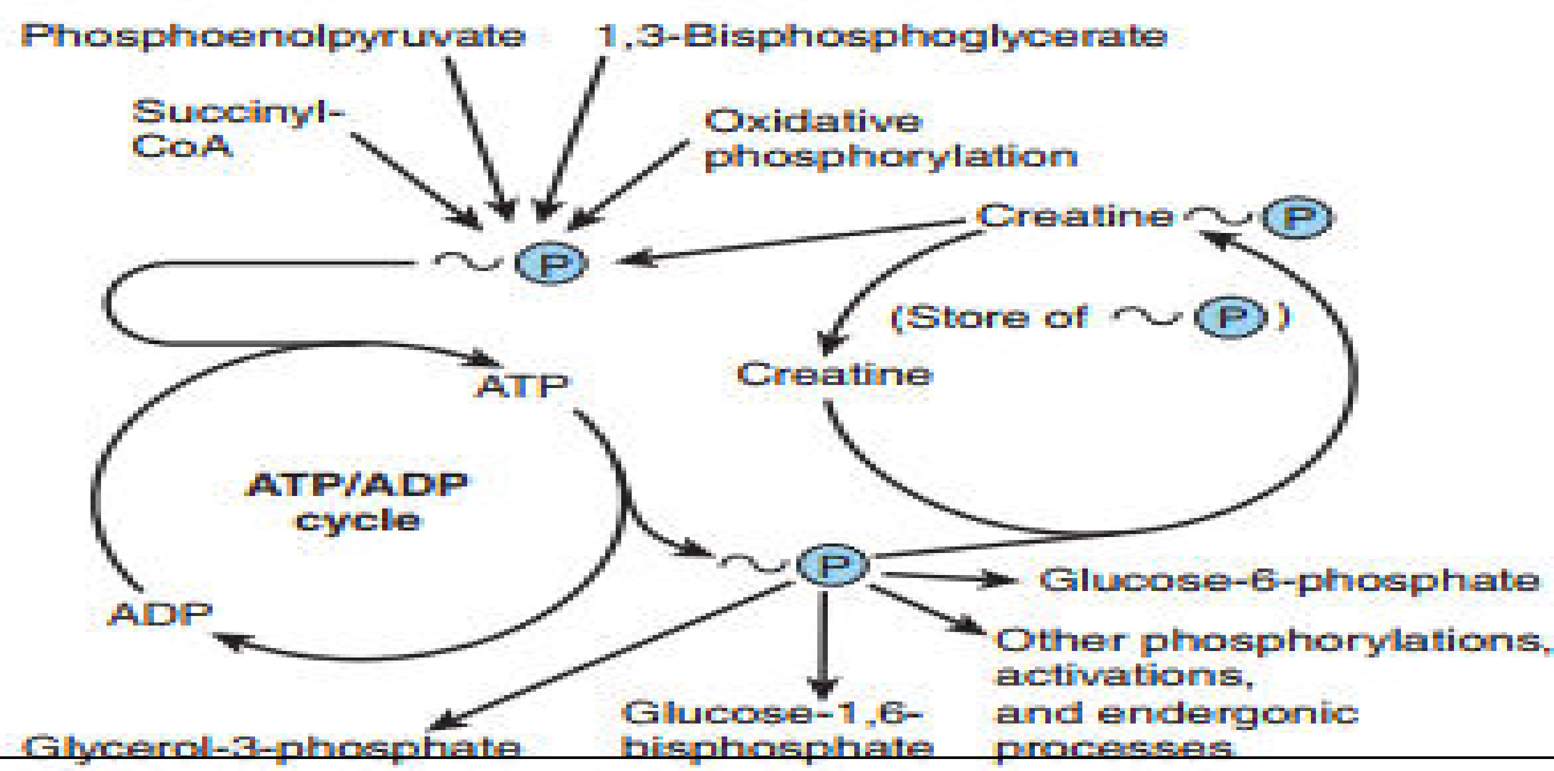


FIGURE 11–7 Role of ATP/ADP cycle in transfer of high-energy phosphate.

Important Features Of ATP

- Contains **three high energy phosphate bonds**
- Drive **endergonic reactions**
- It is **chemical energy currency** of body
- Functions in body as a **complex with Mg2+**
- Biosynthesized by **ATP synthase**
- Couples thermodynamically **Unfavorable reactions to Favorable One**
- ATP synthesis is inhibited by Uncouplers

What Is Biological Oxidation?

- Biological oxidations :
  - **Oxidation reactions/Process**
  - Occurring in living **cells.**

Importance/Features Of Biological Oxidation

- **Biological Oxidation Reactions/Process :**

  - **Involves Oxygen**
  - **Associated with metabolism**
  - **Generates ATP**
  - **Vital for functioning of cells**
  - **Survival and existence of human body.**

Definition Of Oxidation Reactions

- **Oxidation reactions** are **biochemical reactions** where there is either:
  - **Removal / Loss of Hydrogen (Dehydrogenation)**
  - **Removal or Loss of Electrons**
  - **Addition of Oxygen (Oxygenation)**

Feature Of Biological Oxidation

- Oxidation of a molecule (electron donor) is always accompanied by reduction of a second molecule (electron acceptor)

- Most **predominant type** of **Oxidation reaction in body** is:
    - **Dehydrogenation Reaction**
    - **Catalyzed by Dehydrogenases**

- **Dehydrogenases** catalyzes to **remove Hydrogen** from substrates.

- Which are **temporarily accepted** by **Coenzymes.**

Coenzymes
and
Enzymes

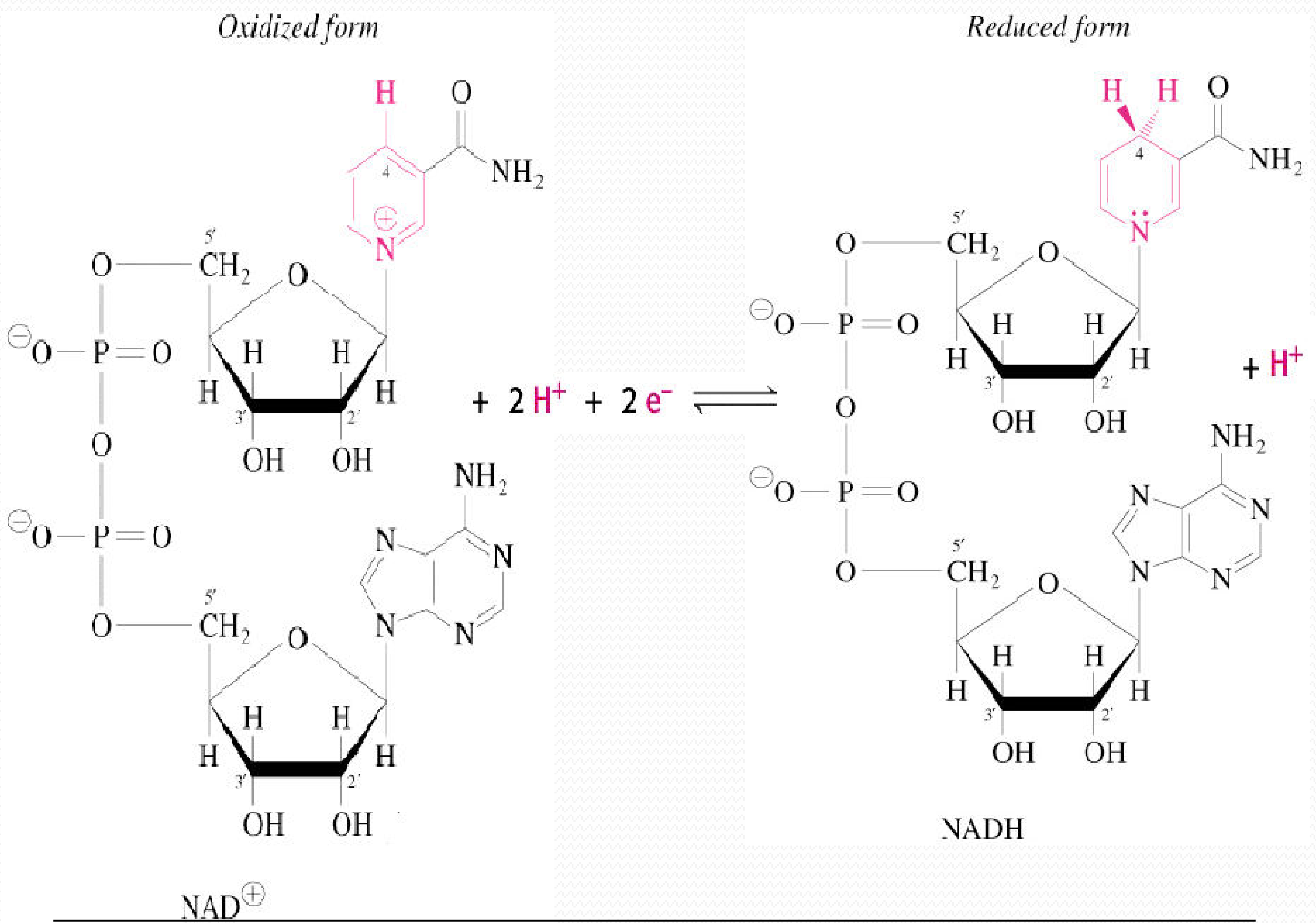
of
Biological Oxidation
Reactions

Coenzymes
and
Inorganic Cofactors
Of
Biological Oxidation
Reactions

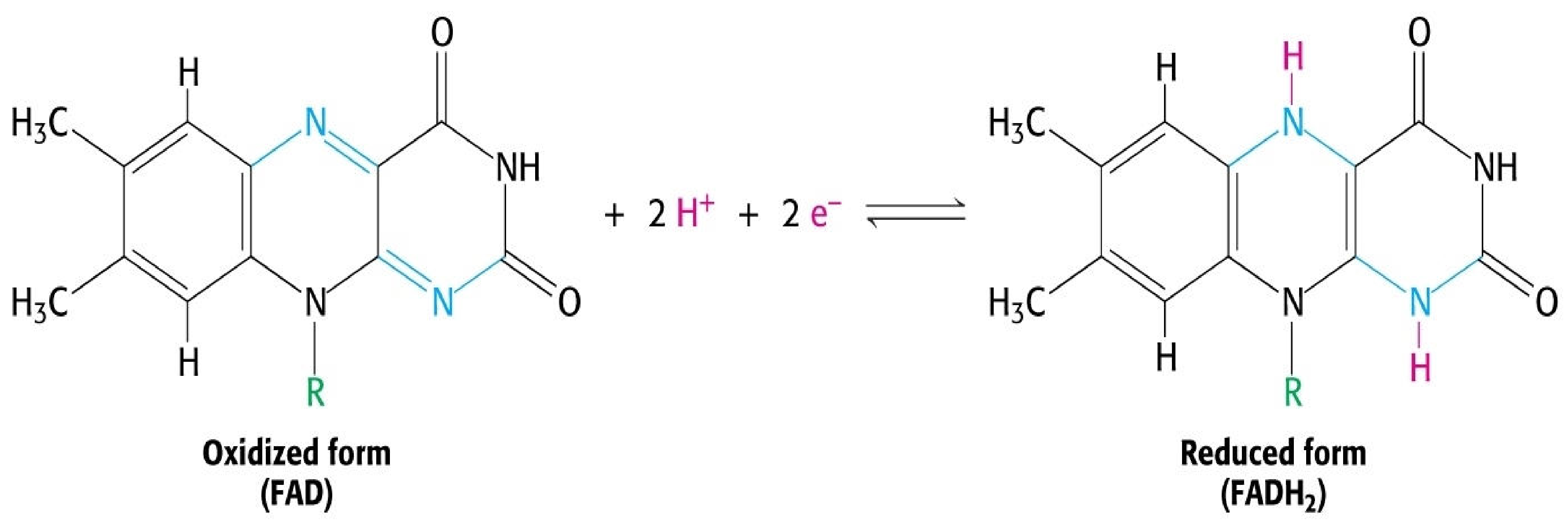
- FMN
- FAD
- NAD^+
- $NADP^+$
- THBP (Tetra Hydro Biopterin)
- Cu^{++}
- Fe^{+++}

- **Oxidized Coenzymes** **involved in Oxidation/Dehydrogenation reactions.**
  - **NAD^+**
  - **$NADP^+$**
  - **FAD**
  - **FMN**

- **Oxidized Coenzymes** temporarily accept the hydrogen from substrates and get transformed to **reduced coenzymes.**
  - **$NADH+H^+$**
  - **$FADH_2$**
  - **$NADPH+H^+$**
  - **$FMNH_2$**



The reduced and oxidized forms of NAD



The reduced and oxidized forms of FAD

5 Enzymes of Biological Oxidation

1. **AEROBIC DEHYDROGENASES**
2. **ANAEROBIC DEHYDROGENASES**
3. **OXYGENASES**
4. **OXIDASES**
5. **HYDROPEROXIDASES**

- All 5 Enzymes of Biological Oxidation reactions are classified in

Class I

Oxido Reductases

AEROBIC DEHYDROGENASES

- **Aerobic Dehydrogenases** are **Flavoproteins**
- Enzymes **covalently bound** to **coenzymes FMN or FAD**

MH_2 **Aerobic DH** → **M**

FMN → $FMNH_2$ **(Auto oxidizable)**

$H_2 + O_2$ → H_2O_2 —**Catalase**→ $H_2O + O_2$

- FMN/FAD are acceptors of removed Hydrogen

- Reduced Coenzymes ($FMNH_2/FADH_2$) formed are **auto oxidizable**

- Reduced coenzymes get **reoxidized at reaction level.**

- **Oxygen** gets **directly involved** at reaction level to **reoxidize the reduced coenzymes.**

- H_2O_2 is a byproduct of **Aerobic Dehyrogenase** activity.

- **Catalase** then detoxify the **H_2O_2 to H_2O and O_2**.

Specific Examples Of Aerobic Dehydrogenases

- **L Amino acid Oxidase** (Oxidative Deamination of A.A)
- **Xanthine Oxidase** (Purine Catabolism)
- **Glucose Oxidase** (Glucose Oxidation to Gluconic acid)
- **Aldehyde Dehydrogenase** (Alcohol Metabolism)

ANAEROBIC DEHYDROGENASES

- Anaerobic Dehydrogenases catalyzes to **remove hydrogen from substrates.**

- With the help of coenzymes **NAD^+/$NADP^+$/FAD.**

DEHYDROGENASES CANNOT USE OXYGEN AS A HYDROGEN ACCEPTOR

MXH_2 → (Anaerobic Dehydrogenase) → **MX**

NAD^+ → **NADH+ H^+** (Non auto oxidizable)

↓

Enter Electron Transport Chain For its reoxidation

- Coenzymes temporarily accept the hydrogen from substrates and get reduced to
  - NADH+ H^+
  - $FADH_2$
  - NADPH+H^+
  - $FMNH_2$

- Reduced coenzymes formed in Anaerobic Dehydrogenase reactions are :
  - Non autoxidizable/not reoxidized at reaction level.

- **Reduced coenzymes $NADH+H^+$ and $FADH_2$** formed at Anaerobic Dehydrogenase reaction

- Has to **enter ETC for its reoxidation.**

- **Oxygen** is **involved indirectly** at an **end of ETC** as **electron and proton acceptor .**

- **Metabolic water** is an end product of ETC.

Remember

- Reduced coenzyme **NADPH+H⁺ do not enter ETC**
- **NADPH+H⁺** is utilized as **reducing equivalent** for **reduction reactions** catalyzed by **Reductases.**

NAD^+ Dependent Anaerobic Dehydrogenases

Enzymes	Pathway /Reaction
Glyceraldehyde -3-PO4 Dehydrogenase	Glycolysis
Pyruvate Dehydrogenase	PDH Complex
Isocitrate Dehydrogenase	TCA cycle
α Ketoglutarate Dehydrogenase	TCA cycle
Malate Dehydrogenase	TCA cycle
Lactate Dehydrogenase	Pyruvate/Lactate metabolism
Glutamate Dehydrogenase	Glutamate metabolism
β Hydroxy Acyl Dehydrogenase	Beta Oxidation of Fatty acids

$NADP^+$ Dependent Dehydrogenases

- **Glucose -6-Phosphate Dehydrogenase**
  (HMP Shunt)
- **Phospho Gluconate Dehydrogenase**
  (HMP Shunt)
- Note **NADPH+H^+does not enter ETC** for its reoxidation instead they are involved in reduction reactions.

FAD Dependent Anaerobic Dehydrogenases

- **Succinate Dehydrogenase**
  (TCA Cycle)
- **Acyl CoA Dehydrogenase**
  (β Oxidation Of Fatty Acids)

FMN Dependent Anaerobic Dehydrogenase

- **NADH Dehydrogenase (Warburg's Yellow Enzyme)**

  First Component of ETC/ Complex I of ETC

OXYGENASES

- Oxygenases **add Oxygen** atom from molecular oxygen (O_2) **into substrate**.

- Form Oxidized Products

OXYGENASES CATALYZE DIRECT TRANSFER AND INCORPORATION OF OXYGEN INTO A SUBSTRATE MOLECULE

Mono Oxygenases

- Mono Oxygenases add **one oxygen atom** from molecular oxygen to the substrate.
- Forms Hydroxyl group (-OH)
- **Monoxygenases are** also termed as **Hydroxylases** or **Mixed Function Oxidase.**

$AH + O_2 + BH_2$ —Mono Oxygenase→ $AOH + B + H_2O$

Tyrosine + O_2 + THBP —Tyrosine Hydroxylase→ DOPA + DHBP + H_2O

Examples Of Mono Oxygenases

- **Phenylalanine Hydroxylase**
  (Phenylalanine to Tyrosine)
- **Tryptophan Hydroxylase**
  (Tryptophan to 5HydroxyTryptophan)
- **25 Hydroxylase**
  (Vitamin D - Cholecalciferol activation)
- **1 α Hydroxylase**
  (Vitamin D - Cholecalciferol activation)

Di Oxygenases

- Dioxygenases are **true Oxygenases**
- Incorporates **two Oxygen atoms from O2.**

A+ O2 —Dioxygenase→ AO2

Examples Of Dioxygenases

- **Tryptophan Di Oxygenase/ Tryptophan Pyrrolase**

  (Tryptophan NFormyl Kynurenine)
- **PHPP Dioxygenase**
- **Cysteine Dioxygenase**
- **Homogentisate Oxidase**

  (Homogentisate to 4 Maleyl Acetoacetate)

Cytochromes P450 Are Monooxygenases Important in Steroid Metabolism & for Detoxification of Many Drugs

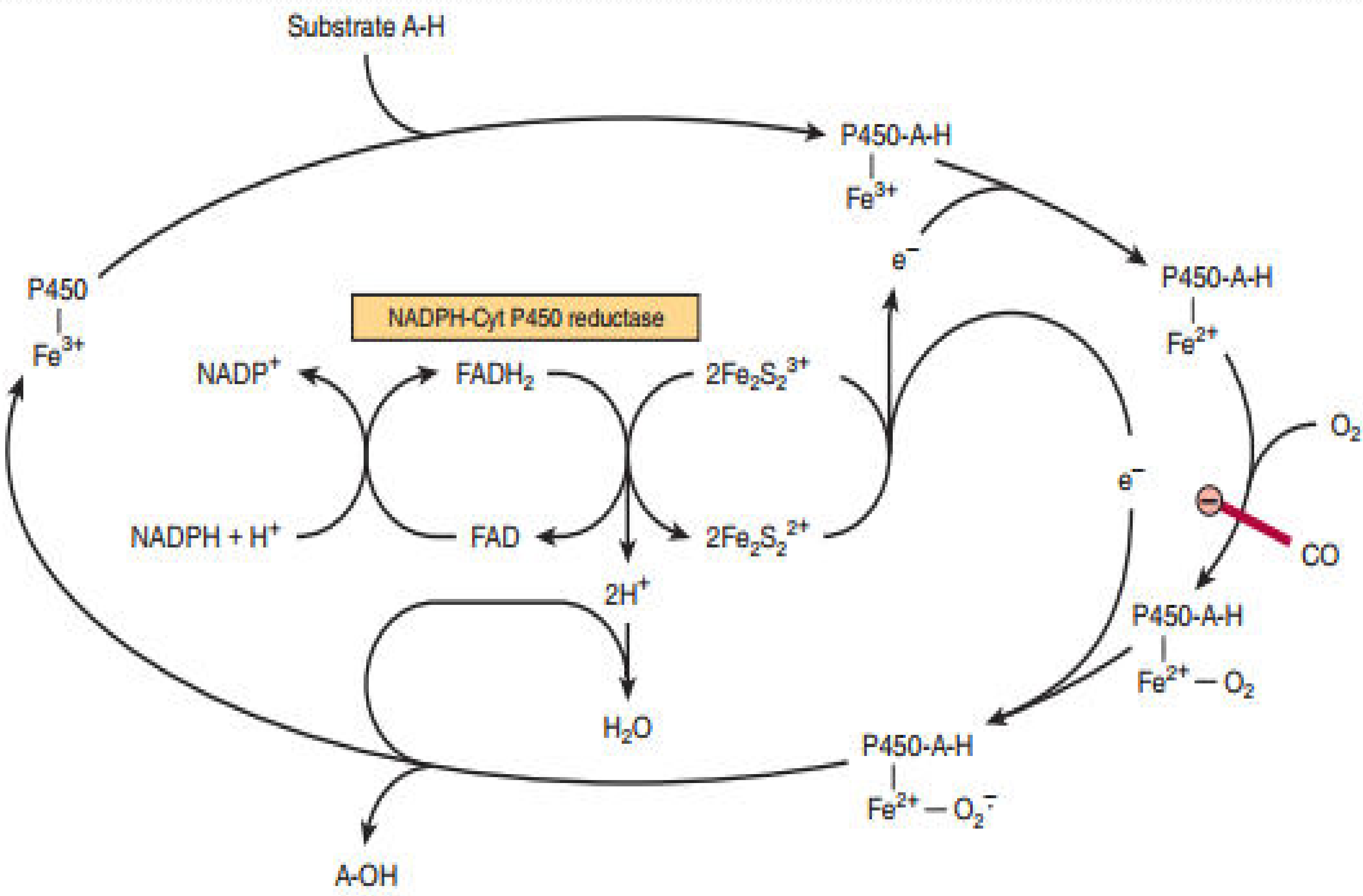


FIGURE 12–6 Cytochrome P450 hydroxylase cycle. The system shown is typical of steroid hydroxylases of the adrenal cortex. Liver microsomal cytochrome P450 hydroxylase does not require the iron-sulfur protein Fe_2S_2. Carbon monoxide (CO) inhibits the indicated step.

Oxidases

- Oxidases involve **activated molecular Oxygen** as **Hydrogen** (electron and proton) **acceptor.**

- Oxidases Reduce Oxygen to form Water (H_2O)

OXIDASES USE OXYGEN

AS A HYDROGEN ACCEPTOR

AH_2 + ½ O_2 → Oxidase → A+ H_2O

Tyrosine+ O_2 → Tyrosinase -Cu^{++} → DOPA + H_2O

Examples Of Oxidases

- **Cytochrome Oxidase-** Classic Example (**Hemoprotein** ETC enzyme)
- **Ascorbate Oxidase**
- **Mono Amine Oxidase**
- **Catechol Oxidase**

HYDROPEROXIDASES USE HYDROGEN PEROXIDE OR AN ORGANIC PEROXIDE AS SUBSTRATE

- Hydroperoxidases detoxify **Hydrogen Peroxide** in body.

- **H_2O_2** is a **substrate/reactant** for **Hydroperoxidases.**

- Hydroperoxidases are **Hemoproteins**.
- Contains **loosely bound Heme as prosthetic group.**
- Hydroperoxidases **prevent accumulation of H_2O_2** in cells.
- H_2O_2 if accumulated in cells is toxic
  - Leads to disruption of membranes(Hemolysis).
  - Increases risk of cancer and atherosclerosis.

Specific Examples Of Hydroperoxidases

- Peroxidases
- Catalase

Peroxidases Reduce Peroxides Using Various Electron Acceptors

- **Indirectly react with H_2O_2**
- **Glutathione Peroxidase** (In R.B.C's)
- **Leukocyte Peroxidase** (In W.B.C's)

$$H_2O_2 + AH_2 \xrightarrow{\text{PEROXIDASE}} 2H_2O + A$$

$$H_2O_2 + 2\,GSH \xrightarrow{\text{Glutathione Peroxidase}} 2H_2O + GSSG$$

(Reduced active Form) (Oxidized inactive Form)

- **Catalase**
- **Directly reacts with H_2O_2.**
- Associated with Aerobic Dehydrogenase catalyzed reaction.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Biological Oxidation Process

Electron Transport Chain (ETC)

Oxidative Phosphorylation

Synonyms Of ETC

1. Electron Transport Chain (ETC)
2. Oxidative Phosphorylation
3. Electron Transport System (ETS)
4. Fate of Reduced Coenzymes of $FADH_2$ and $NADH+H^+$
5. Respiratory Chain
6. Internal/Cellular Respiration
7. Tertiary metabolism
8. Final Oxidative Pathway

What is Electron Transport Chain?

Electron Transport chain

- Biological oxidation process **very vital for human being survival**
- **Truly Aerobic** in nature(indispensable on O2)
- Located and operated at **inner membrane of Mitochondria**
- **Alternate Oxidation and Reduction** Reactions carried out in process

What is Oxidative Phosphorylation?

- **Oxidation process** (ETC) is tightly **coupled with Phosphorylation** of ADP with pi to **generate ATP.**

- **Illustrated as Sun and Day Light**

- Oxidative Phosphorylation is a **major mode of ATP generation in human body**

What is Fate of ETC/ Oxidative Phosphorylation ?

REOXIDIZES REDUCING EQUIVALENTS ($NADH+ H^+$ and $FADH2$)

GENERATED DURING ANAEROBIC DEHYDROGENASE REACTION

- **Electron Transport Chain On Operation**

- **Transports Electrons and Protons**

- Through **series of ETC components**

- **Finally H_2 is received by activated molecular Oxygen (1/2 O_2)**

- **Generates significant byproduct ATP and metabolic water at end of process**

Condition In which ETC Operates

- ETC operates in **truly aerobic condition.**
- **Oxygen unloaded** at cellular level by **HbO_2**

- Gets **utilized at** an end of **ETC** process. **(Respiratory Chain)**

Site Of
Electron Transport Chain

OR

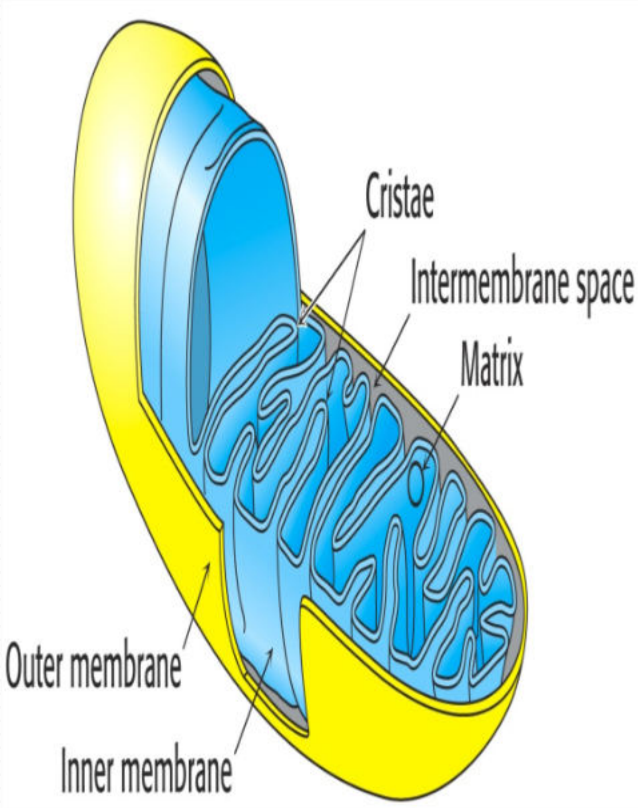
Oxidative Phosphorylation

- **ETC is located and operated in all cells which contain Mitochondria (Power house of Cell)**

(Except mature Erythrocytes which are devoid of mitochondria)

Location of Mitochondrial ETC Complexes

- **Inner membrane of Mitochondria**
- **Rich In Cardiolipin**



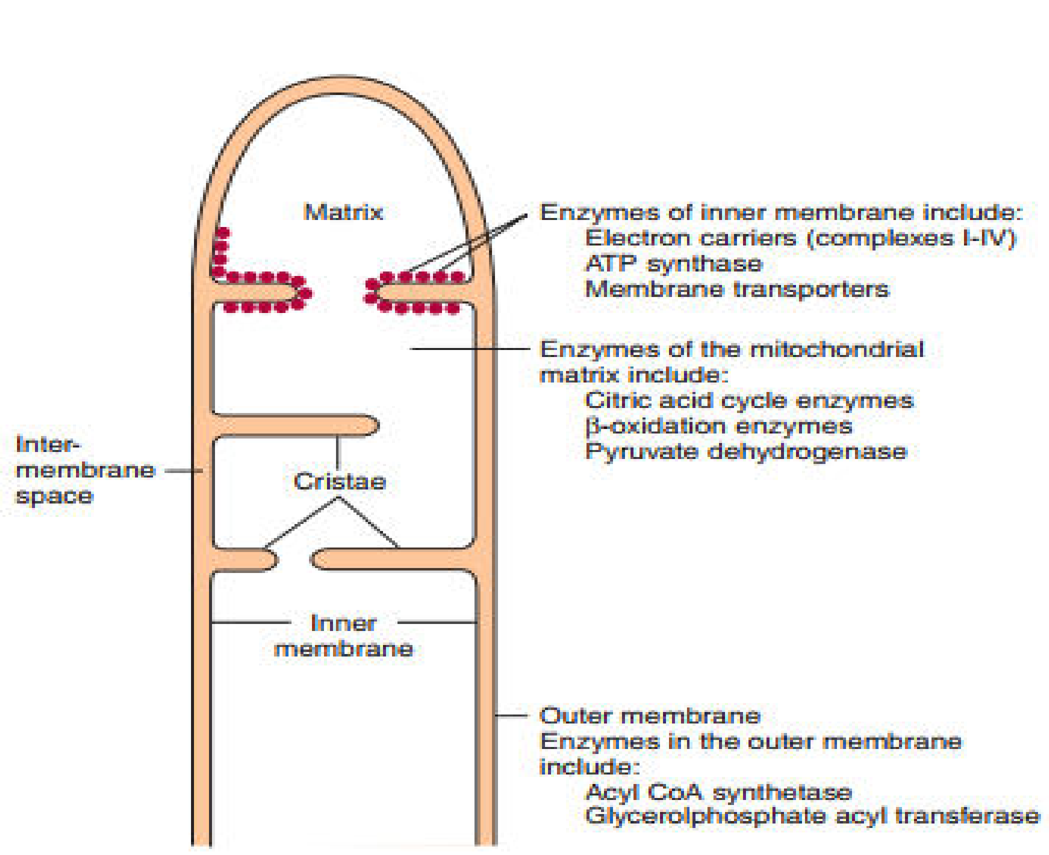


FIGURE 13–1 **Structure of the mitochondrial membranes.** Note that the inner membrane contains many folds or cristae.

- Components and Enzymes of **ETC are arranged** towards inner surface of **inner membrane of mitochondria as:**
  - **Vectorial conformation**
  - **Increased order of positive redox potential**

Number of Mitochondria Vary in Different cells

Number of Mitochondria changes from cell to cell , tissue to tissue, organ to organ, organism to organism

Factors Responsible For Number of Mitochondria in Cell

- **Type of cell, organ** and **its function**

- **Metabolic status** of an individual

- **Physical activity** of an individual

- **How much energy cell needs** to produce?

  - High number of Mitochondria present in **Heart, Rod cells, Sperm, ciliated** cells

  - **Muscle cells** for example, contain more **number** of mitochondria **compared to Kidney cells**.

  - **Marathon runners have more number** of mitochondria in their leg muscle cells than **people with desk jobs**

Components Of ETC

Series Of Protein Complexes

Flavoproteins & Iron-Sulfur Proteins (Fe-S),Cytochromes are Components of Respiratory Chain Complexes

1.Flavo Protein- (First Component)

NADH Dehydrogenase-FMN and FeS centers(Warburg's Yellow Enzyme)

2. Coenzyme Q/ Ubiquinone

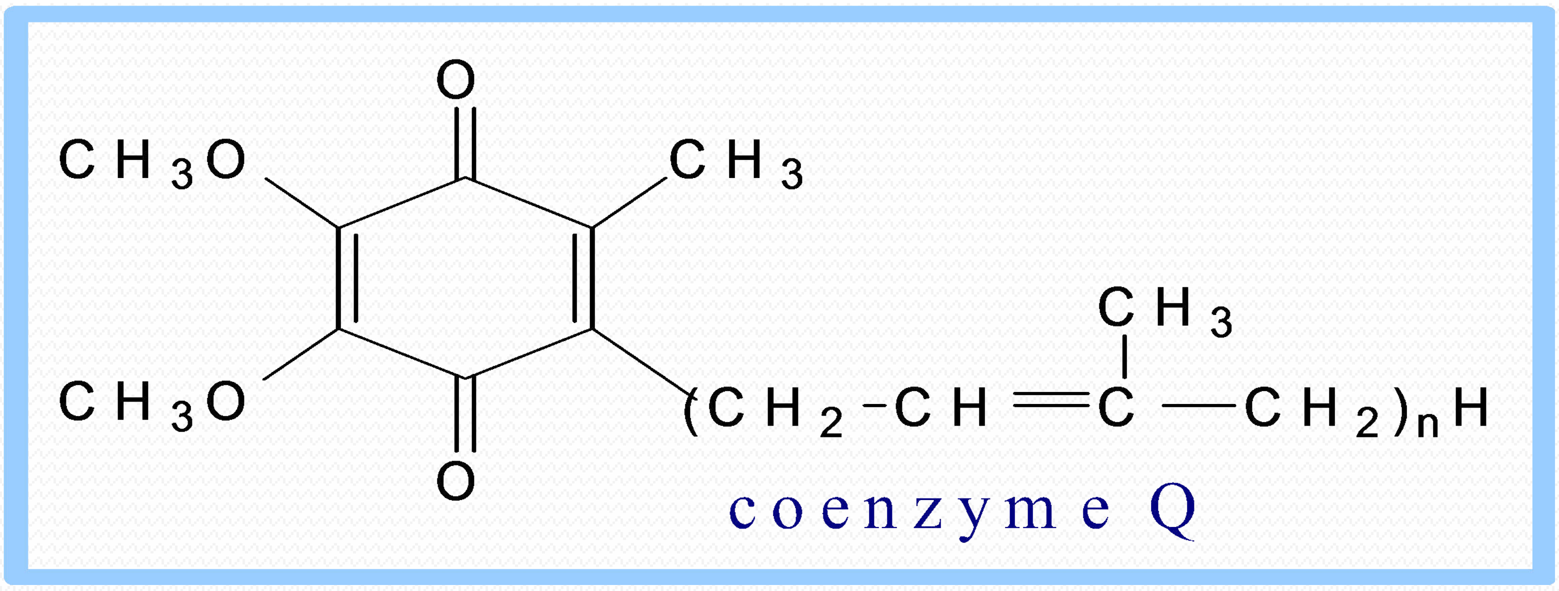
3. Series of Cytochromes-

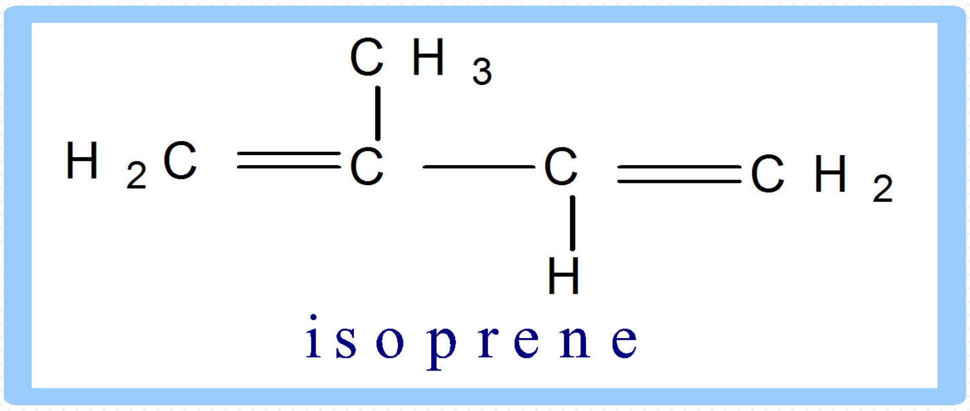
Cytochrome b-Cytochrome c1- Cytochrome c- Cytochrome aa3

Coenzyme Q / Ubiquinone

- **Coenzyme Q** (CoQ)/ **Ubiquinone)** is **located in lipid core** of mitochondrial membrane.
- It is a **Quinone derivative**
- **Lipophilic** dissolves in hydrocarbon core of a membrane.

- **Coenzyme Q** is very **hydrophobic**.





- Coenzyme Q has a long **Poly isoprenoid tail**, with multiple units of isoprene.

- In human cells, most often n = 10

- **Q_{10} isoprenoid tail** is longer than width of a bilayer.

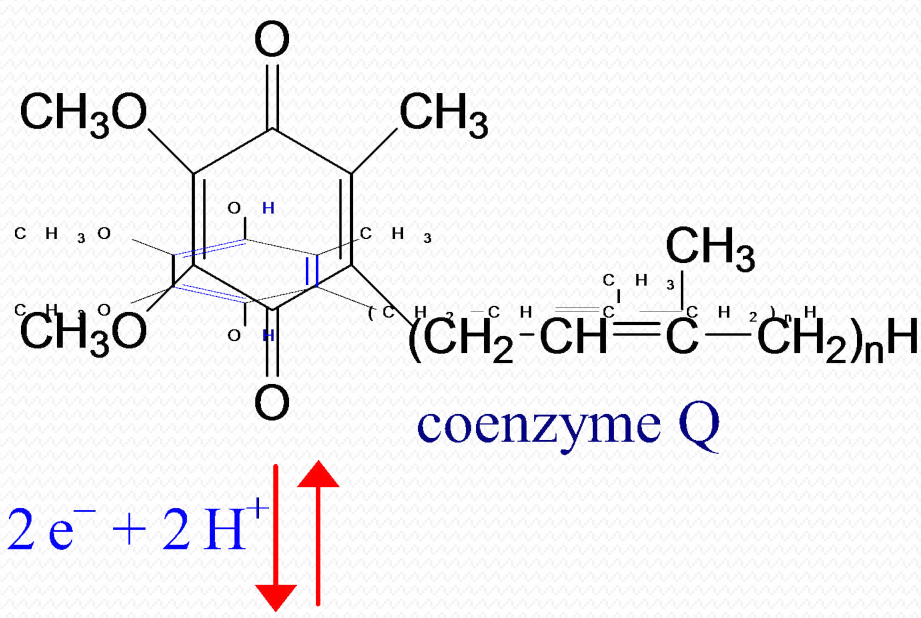
- **Coenzyme Q** functions as a **mobile e^- carrier** within mitochondrial inner membrane.

- Its role in trans-membrane **H^+ transport** coupled to e^- transfer (**Q Cycle**).

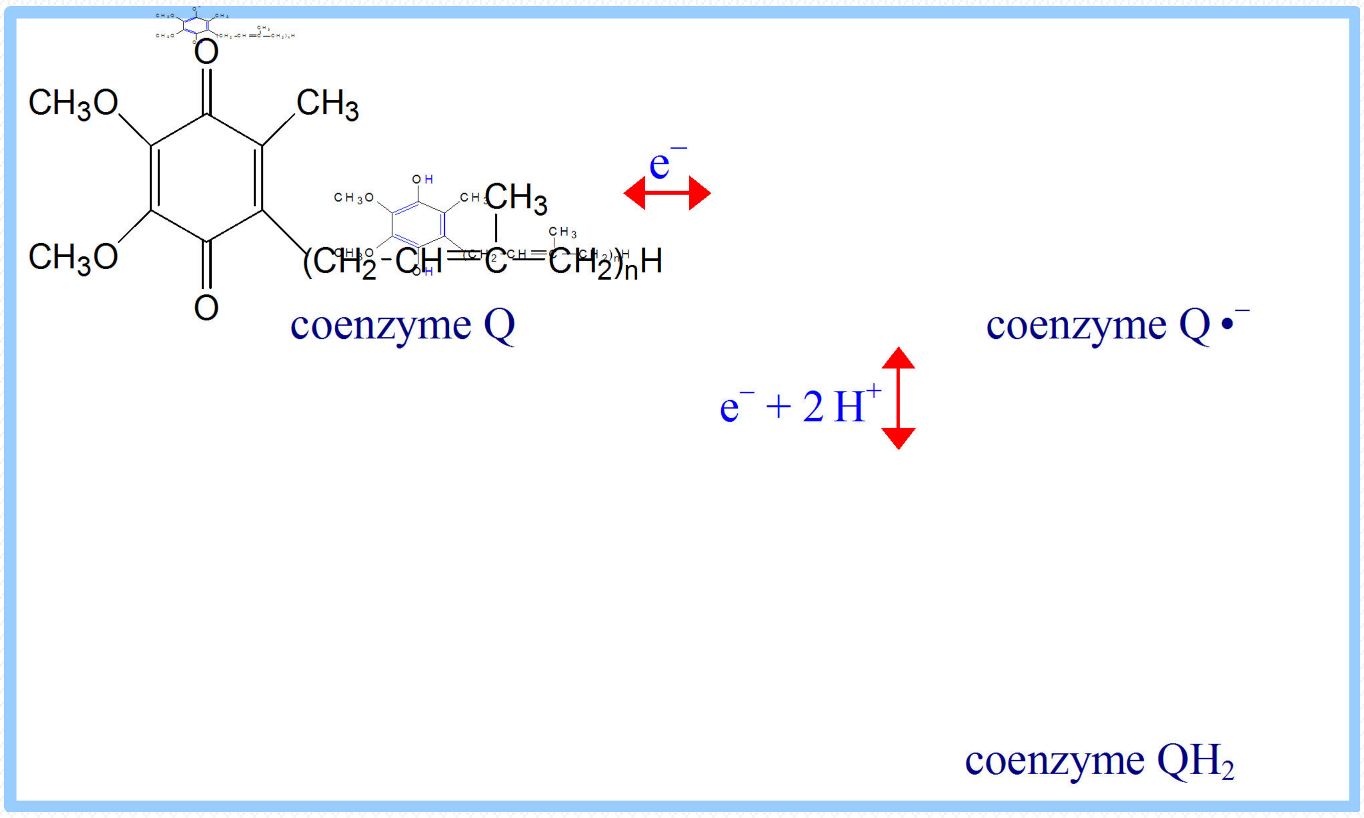
Coenzyme Q
Accepts
Both Protons and Electrons

Quinone ring of coenzyme Q can be reduced to **Quinol** in a 2e⁻ reaction:

$Q + 2\,e^- + 2\,H^+ \leftrightarrow QH_2$.



coenzyme QH_2



When bound to special sites in respiratory complexes, **CoQ** can accept **1 e⁻** to form a **semiquinone radical** (**Q·⁻**).

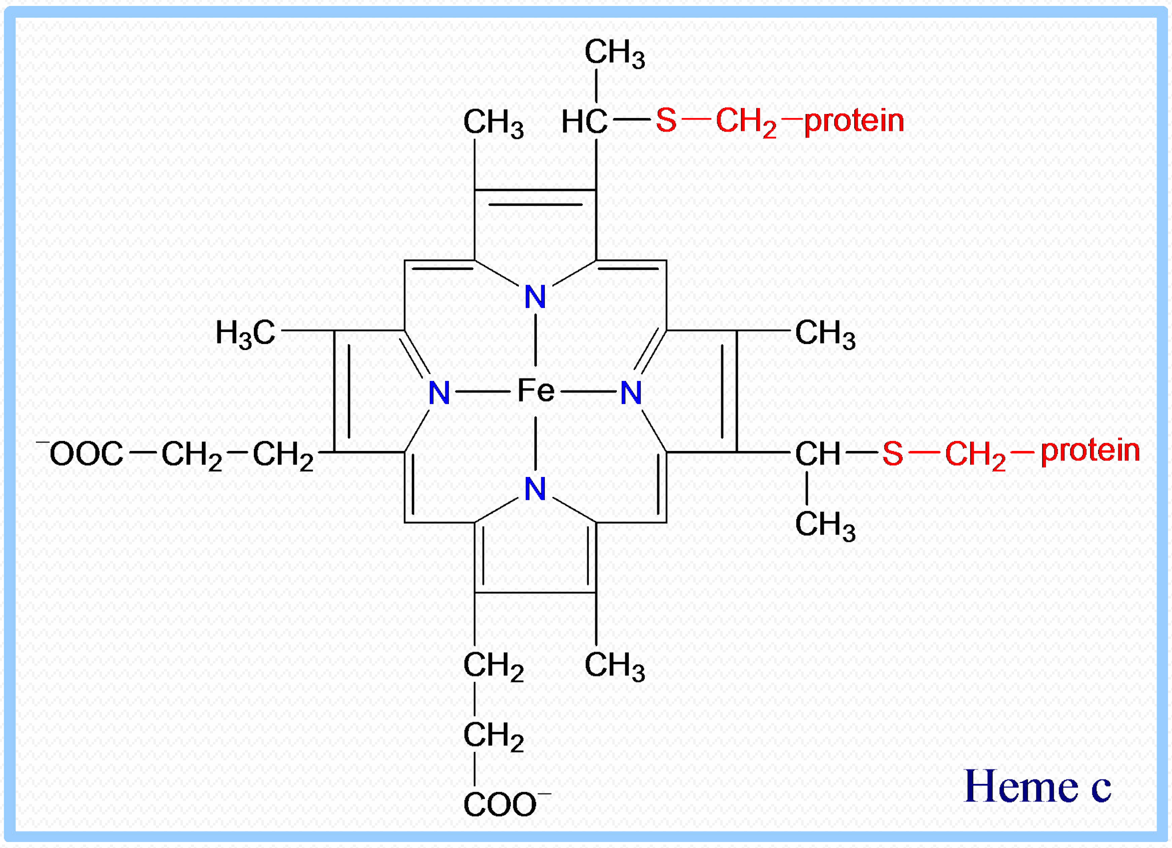
Thus CoQ, like FMN, can mediate between 1 e⁻ & 2 e⁻ donors/acceptors.

Cytochromes

- **Cytochromes** are Hemoproteins **conjugated proteins in ETC**
- **Carrier of electrons**
- Contain **heme as prosthetic group**

Cytochrome Heme

- Cytochrome Heme Iron is in transitional state
- **Carries only electrons**
- **$Fe(III) + e^- \leftrightarrows Fe(II)$**
- Only *one* electron is transferred at a time.



- Cytochrome heme iron can undergo **1 e⁻** transition between ferric and ferrous states:
- **Fe^{+++} + e^{-} ←→ Fe^{++}**

(oxidized) (reduced)

Cytochromes May Also Be Regarded as Dehydrogenases

- Series of *Cytochromes* ***b, c_1, c, aa_3*** relay electrons (one at a time, in this order

- **Cytochrome c** is a small, water soluble protein with a single heme group.

- Cytochromes a & a_3 are often referred to as **Cytochrome Oxidase /complex IV**

- Cytochrome aa3 has Fe and Cu.

- All **Cytochromes** except Cytochrome Oxidase are **Anaerobic Dehydrogenase activity**.

- Cytochromes absorb light at characteristic wavelengths.

- **Absorbance changes** upon oxidation/reduction of Heme Iron

Components of Respiratory Chain are Contained in Protein Complexes Embedded in Inner Mitochondrial Membrane

Five Complexes of Oxidative Phosphorylation

Complexes of Oxidative Phosphorylation

- There exists **5 complexes**

- **Processing Oxidative Phosphorylation to generate ATP**

- Complexes are combination of one or two components

- **Complex I- NADH CoQ Reductase**
  NADH Dehydrogenase FMN and FeS centre
- **Complex II – Succinate CoQ Reductase**
  Succinate Dehydrogenase FAD and FeS centre
- **Complex III–CoQ Cytochrome C Reductase**
  Cytochrome b – Cytochrome c1
- **Complex IV- Cytochrome Oxidase**
  Cytochrome aa3
- **Complex V – ATP Synthetase**
  Fo and F1 of ATP Synthase

Composition of Oxidative Phosphorylation Complexes

Complex	Name	No. of Proteins	Prosthetic Groups
Complex I	NADH –CoQ Reductase	46	FMN, 9 Fe-S centers
Complex II	Succinate-CoQ Reductase	5	FAD, cyt b_{560}, 3 Fe-S centrs.
Complex III	CoQ-cyt c Reductase	11	cyt b_H, cyt b_L, cyt c_1, Fe-S_{Rieske}
Complex IV	Cytochrome Oxidase	13	cyt a, cyt a_3, Cu_A, Cu_B

ETC Components Associated With Multiple Iron Sulfur Centers

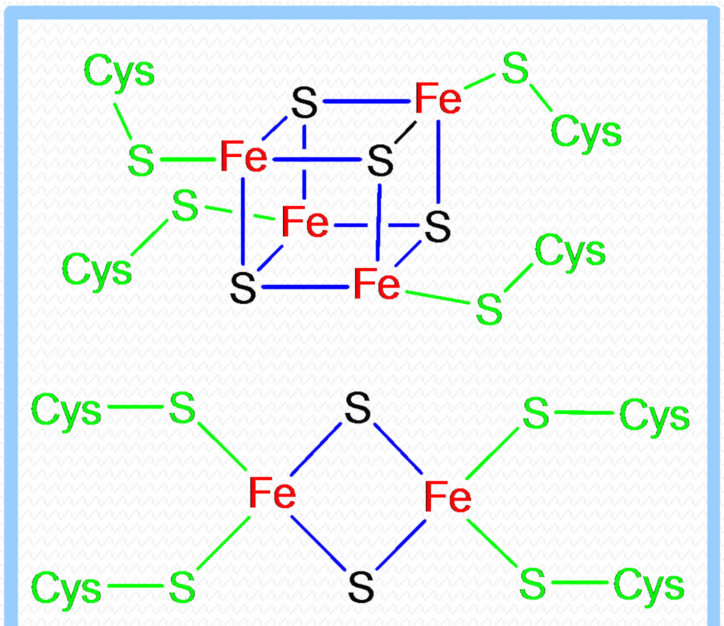
Iron exists in Transitional State Responsible for Oxidation and Reduction Reactions

Complex I,II and III contains Iron Sulfur Centers

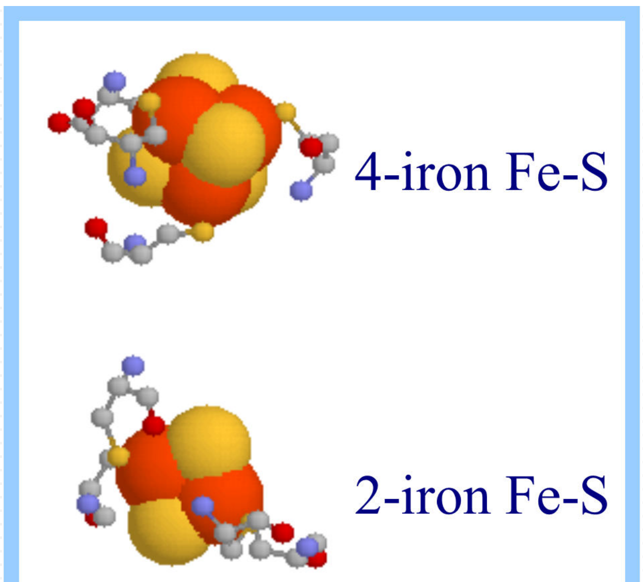
Complex IV and V do not Contain Iron Sulfur Centers

ETC Components With Iron Sulfur Centers

- NADH Dehydrogenase
- Coenzyme Q-Cytochrome Reductase
- Succinate –Coenzyme Q Reductase



Iron-Sulfur Centers



Two iron-sulfur centers from complex I

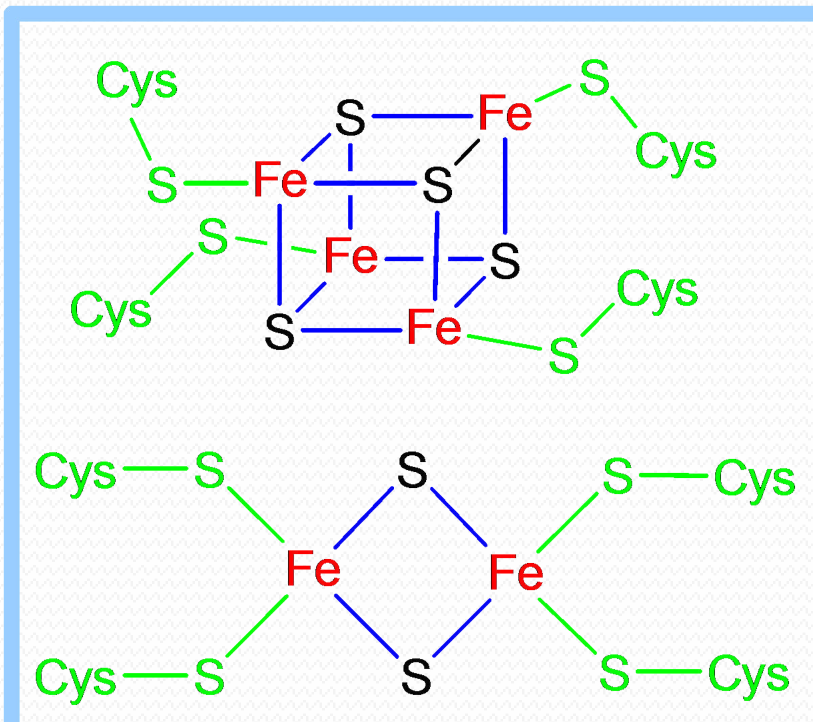
Iron-sulfur centers (Fe-S) are prosthetic groups containing **2, 3, 4 or 8 iron atoms** complexed to elemental & Cysteine **S**.

4-Fe centers have a tetrahedral structure, with **Fe** & **S** atoms alternating as vertices of a cube.

Cysteine residues provide **S** ligands to the iron, while also holding these prosthetic groups in place within the protein

Electron transfer proteins may contain multiple Fe-S centers.

Iron-sulfur centers transfer only one electron, even if they contain two or more iron atoms, because of close proximity of iron atoms.

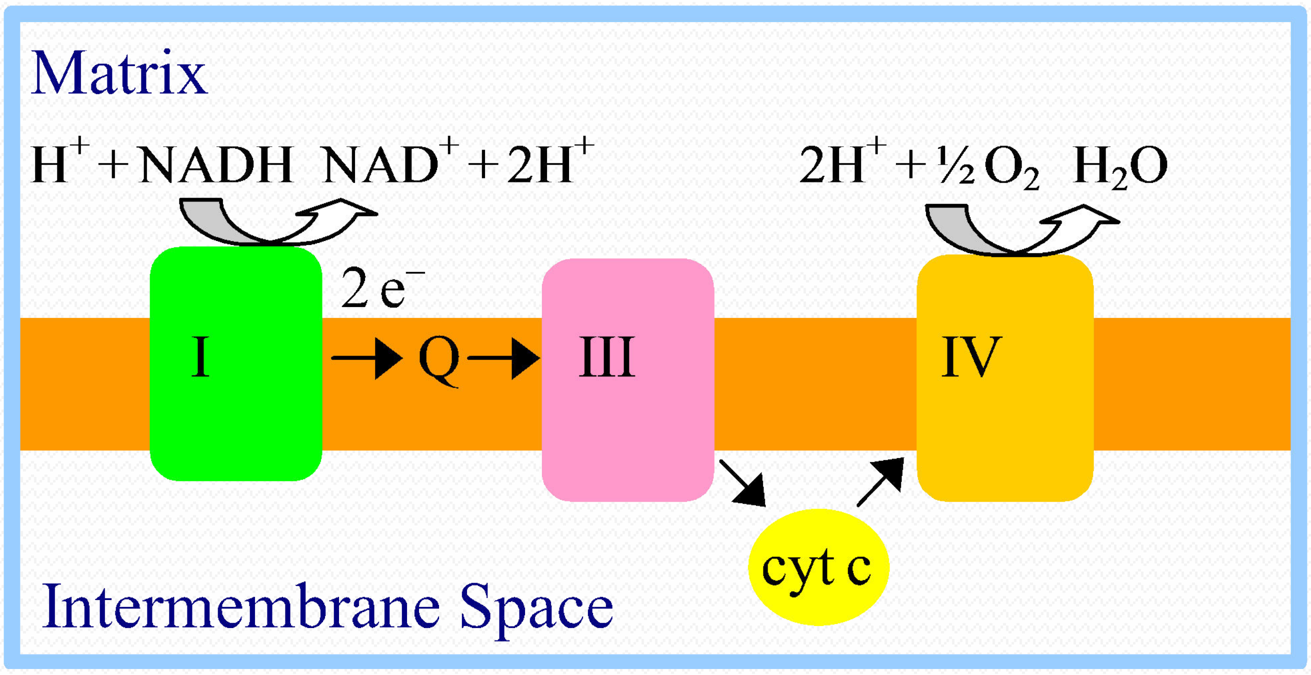


Iron-Sulfur Centers

$$Fe^{+++} \text{ (oxidized)} + 1\, e^{-} \leftrightarrow Fe^{++} \text{ (reduced)}$$

COMPLEX IV

- **Cytochrome a-a3/ Cytochrome Oxidase** large protein
- Both a and a_3 contain heme and Cu
- Does not contain Fe –S clusters
- a_3 Cu binds to oxygen and donates electrons to oxygen
- **Cytochrome a3** - only component of ETC that can interact with O_2



Cytochrome Oxidase (complex IV) carries out following irreversible reaction:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

Four electrons are transferred into complex one at a time from Cytochrome c.

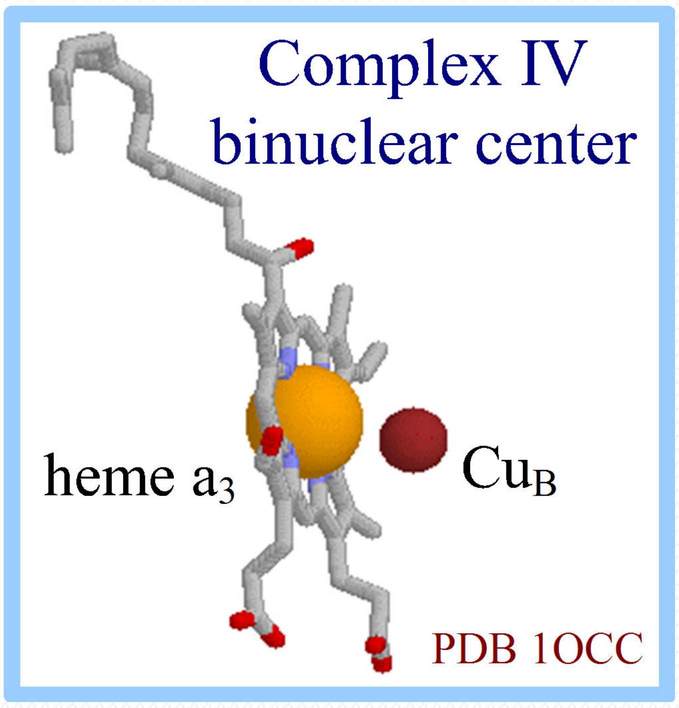
- Complex IV/Cytochrome Oxidase reduces molecular Oxygen to water.

Cytochrome Oxidase

e^- from cyt c to a

Cu(II) ⇆ Cu(I)

Heme A and Cu act together to transfer electrons to oxygen



Metal centers of cytochrome oxidase (complex IV):
heme a & heme a_3,
Cu_A (2 adjacent Cu atoms) & Cu_B.

O_2 reacts at a **binuclear center** consisting of heme a_3 and Cu_B.

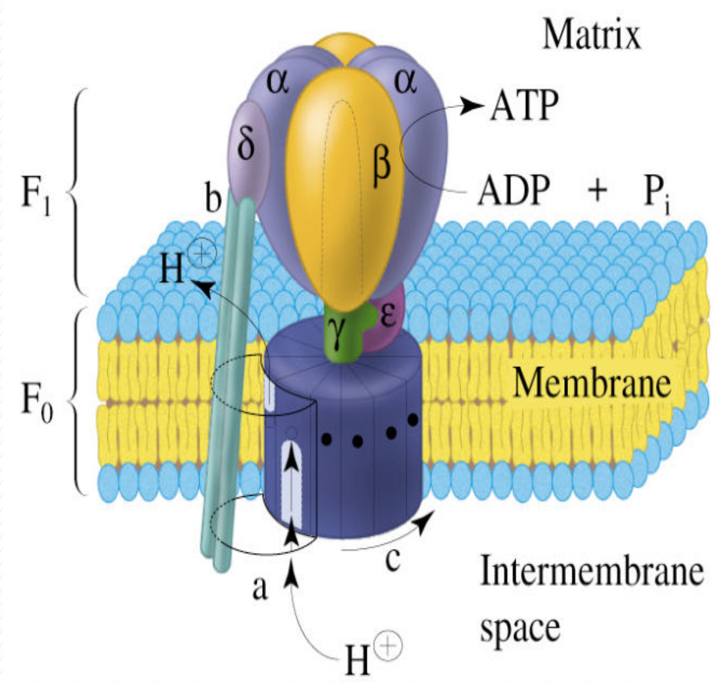
- An **Iron-Copper Center** in Cytochrome Oxidase Catalyzes Efficient O2 Reduction

Complex V
ATP Synthase

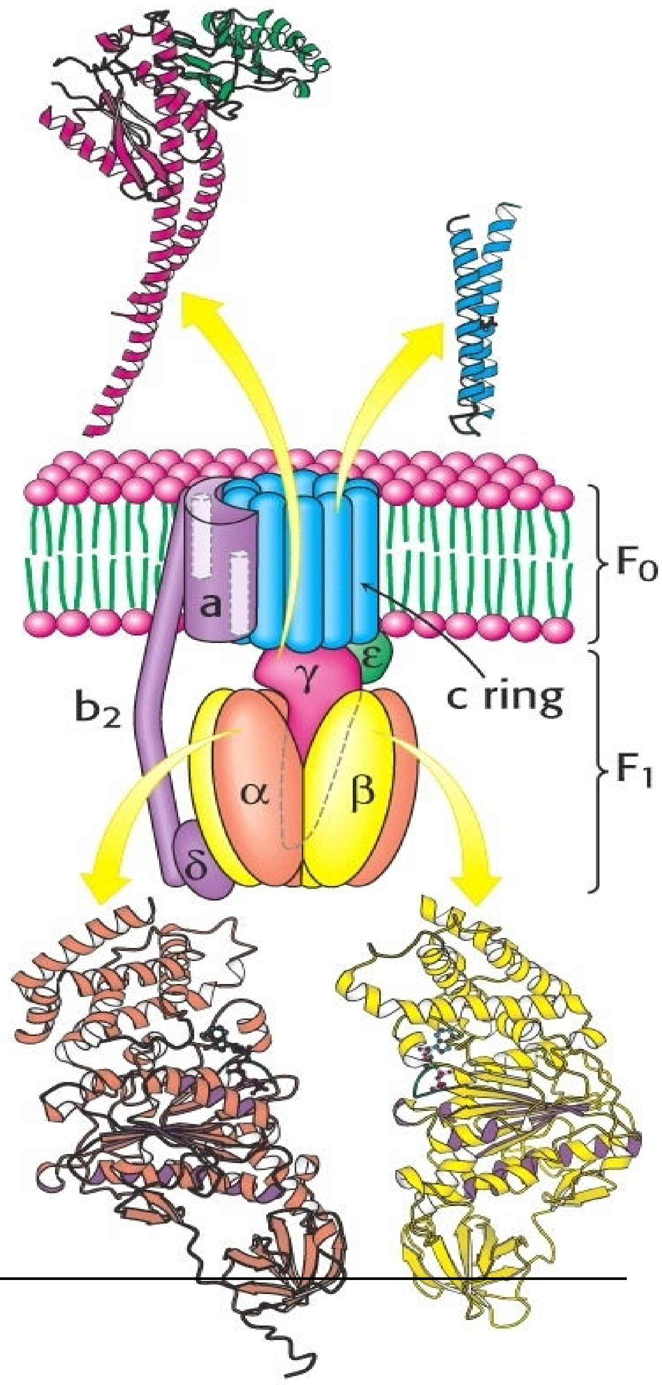
Two units, F_o and F_1
("knob-and-stalk"; "ball on a stick")

F_1 contains the catalytic subunits where ADP and P_i are brought together for combination.

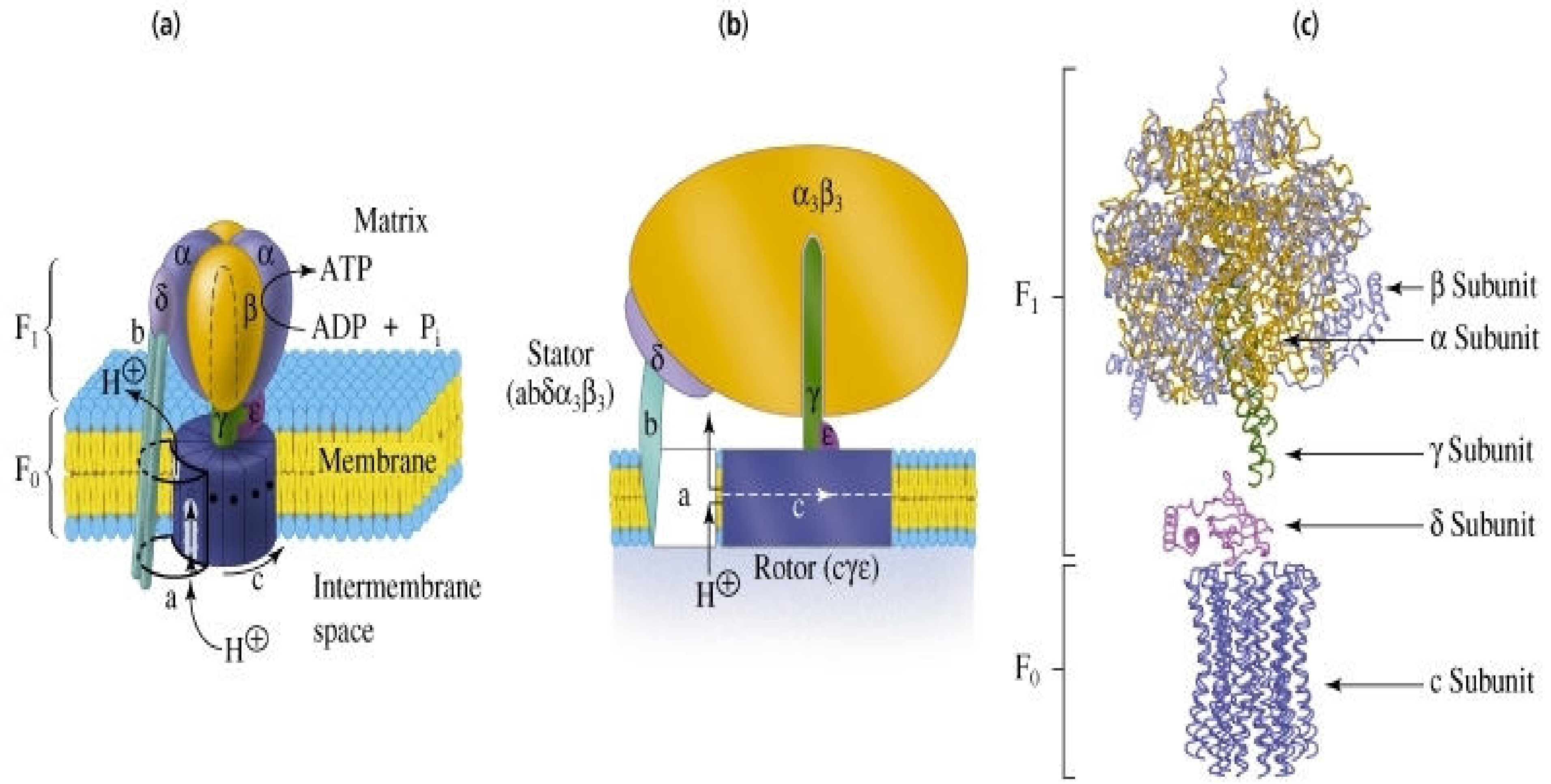
F_0 spans the membrane and serves as a proton channel.



- F_1 contains 5 types of polypeptide chains - $\alpha_3\beta_3\gamma\delta\varepsilon$
- F_o - $a_1b_2c_{10-14}$ (c subunits form cylindrical, membrane-bound base)
- F_o and F_1 are connected by a $\gamma\varepsilon$ stalk and by exterior column (a_1b_2 and δ)
- Proton channel is – between c ring and a subunit.



Complex V ATP Synthase



How do ATPase and ATP Synthase Differ?

- **ATPase** is an enzyme that **hydrolyze ATP** to form ADP
- **ATP synthase synthesize ATP**
- **Both** enzyme **found in mitochondr**

F- ATPase/ATP Synthase

- F-ATPase belong to superfamily of related ATP Synthases

F-ATPase is a Rotating Motor

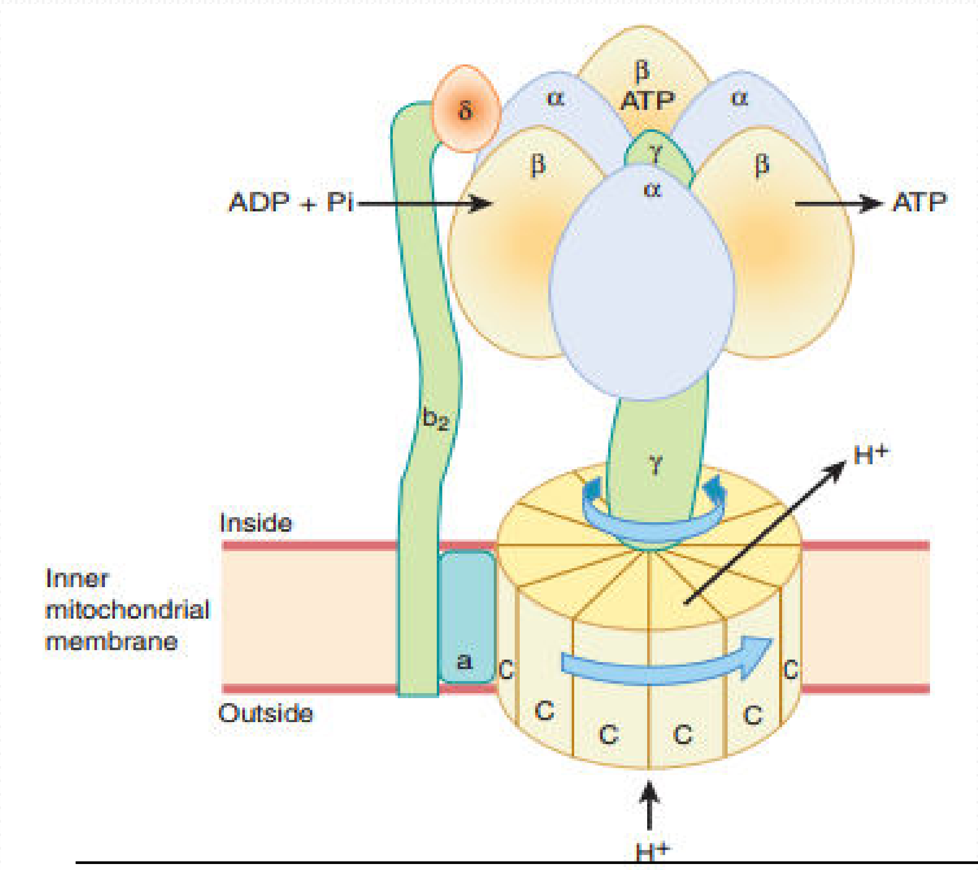
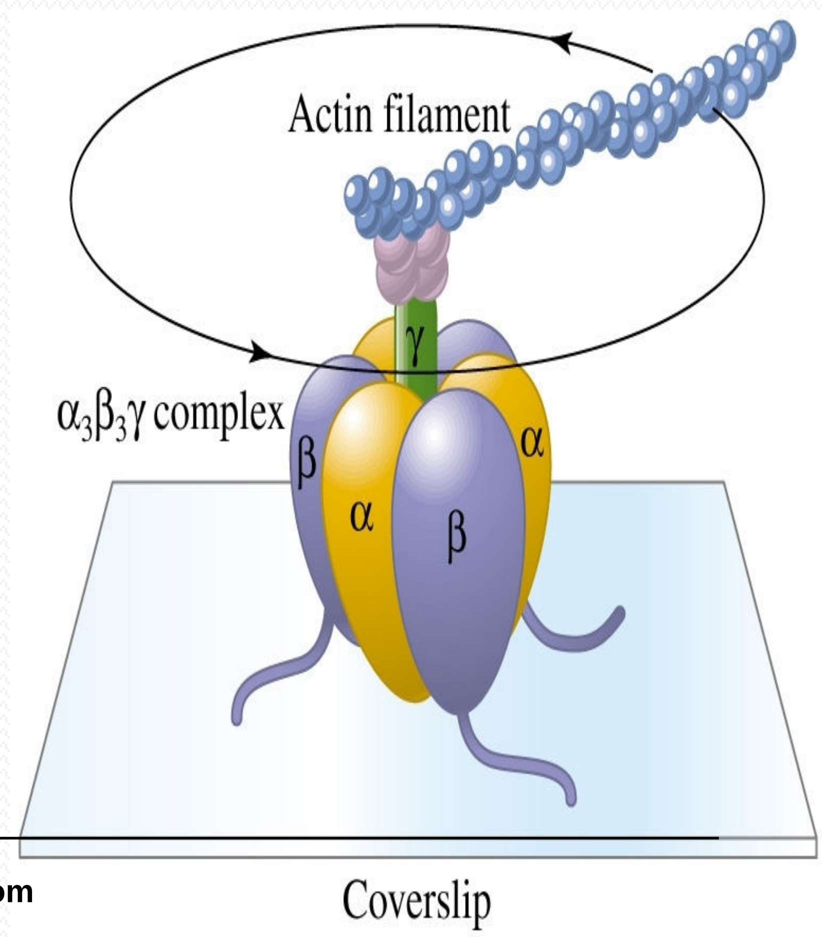


FIGURE 13–8 Mechanism of ATP production by ATPase



Mechanism Of Oxidative Phosphorylation

Salient Features/Required Criteria's Of ETC/Oxidative Phosphorylation OR Criteria's Required For Oxidative Phosphorylation

1
Arrangement Of Electron Transport Chain Components In Increased Order Of Positive Redox Potential

Redox Potentials & Redox Couples

- **FREE ENERGY CHANGES CAN BE EXPRESSED IN TERMS OF REDOX POTENTIAL**

- Redox Potential is a measure of **tendency** of a **redox couple** to **accept or donate electrons** under standard condition.

- Components that have **most negative redox potentials**
- Have **weakest affinity for electrons**
- **Hence has capacity to donate its electrons.**

- Redox couple with **most positive redox potentials** have
- **Strongest affinity for electrons** therefore
- Possess **strongest tendency to accept electrons.**

- During E.T.C there is transfer of reducing equivalents
- From **low redox potential** to **high redox potential.**

- This **exhibit free energy change** there by **liberating heat energy**

- Electrons move spontaneously from one component of ETC to another with a

- **low redox potential** (a low affinity for electrons) to a component with a

- **high redox potential** (a high affinity for electrons)

- In ETC electrons move from a carrier with

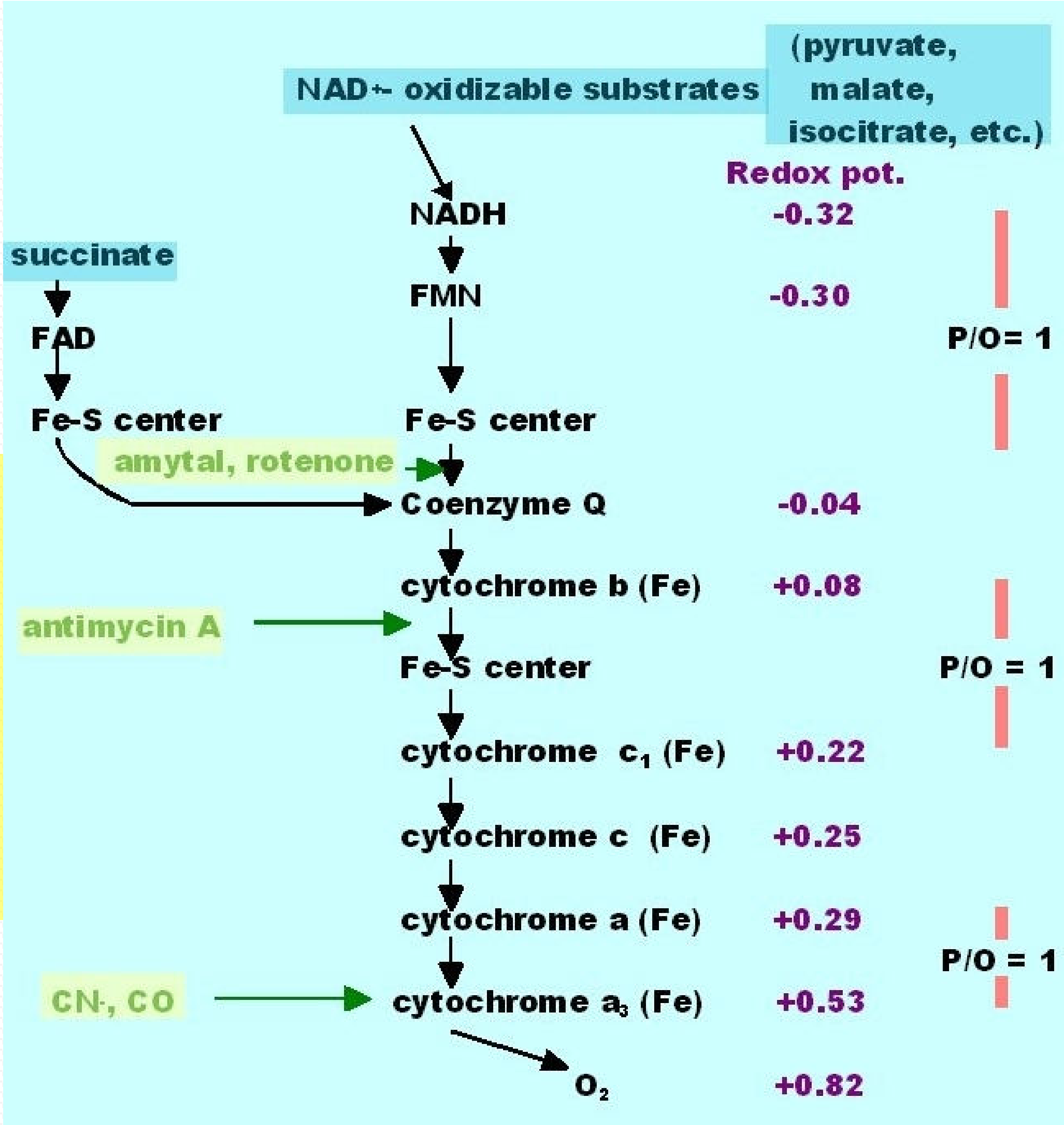
- **Low redox potential**
  (high tendency to **donate electrons**) toward carriers

- **Higher redox potential**
  (high tendency to **accept electrons**)

Redox Couple

- Components of ETC has capacity to exist in **oxidant and reduced forms.**
- This pair is known as **redox couple**
  - **$CoQ/CoQH_2$**
  - **Cyt b Fe^{+++}/Cyt b Fe^{++}**

Sequence of Respiratory Electron Carriers



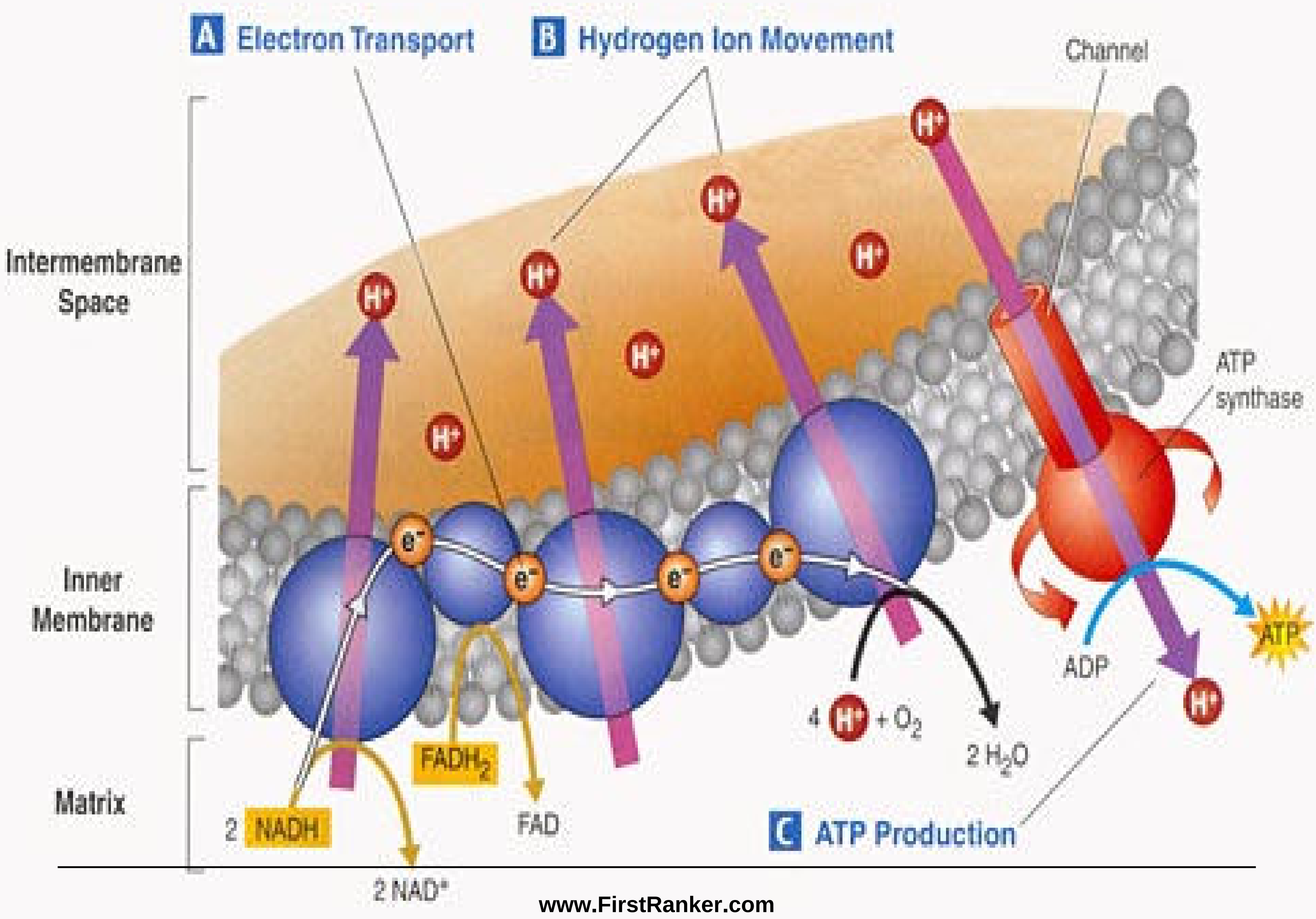
Inhibitors in green

2
Development Of

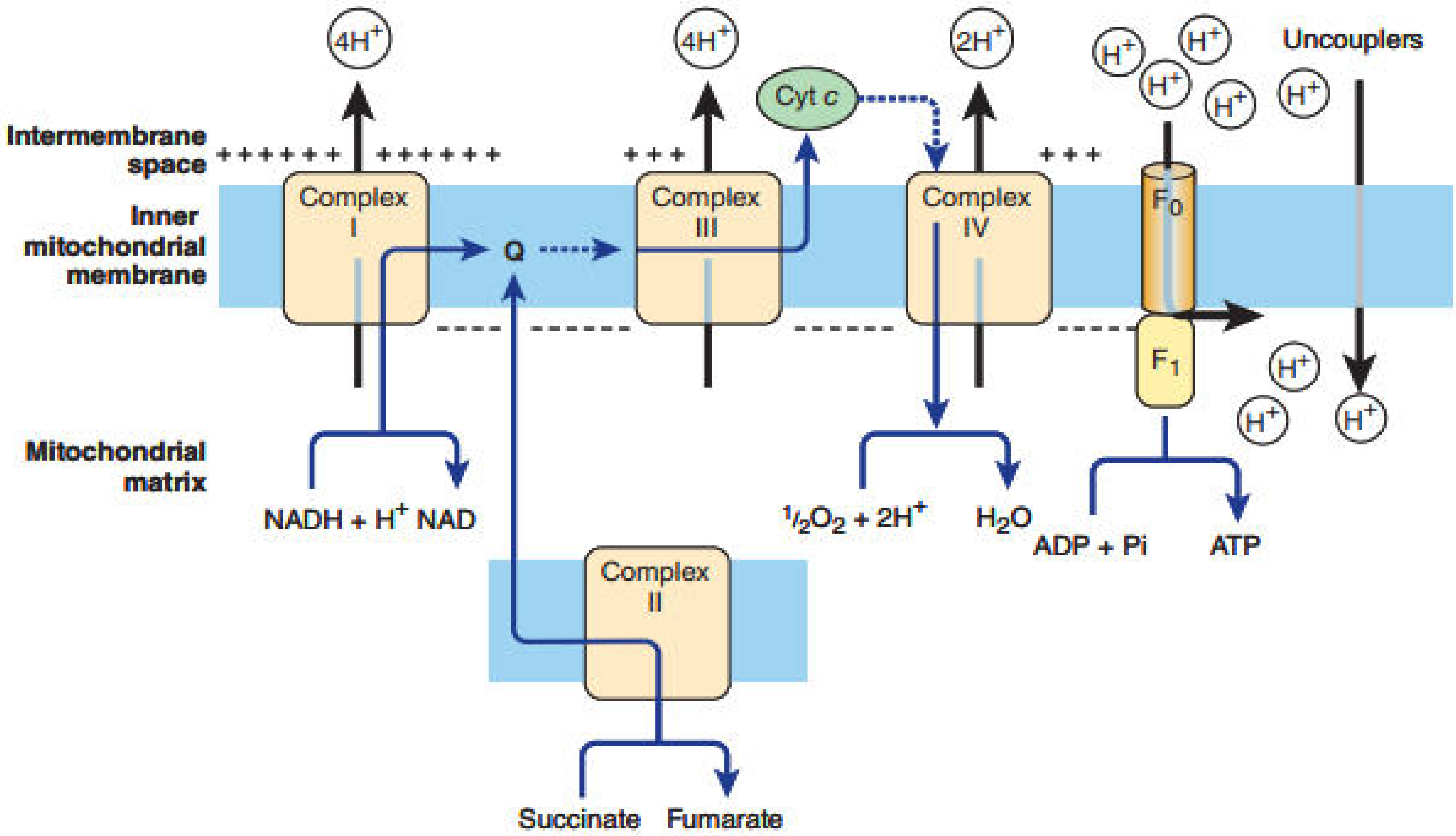
Proton Gradient And Proton Motive Force

In Intermembrane Space

Complex I,III and IV
Pumps Protons From Matrix side to Intermembrane Space and generates
Proton Motive Force



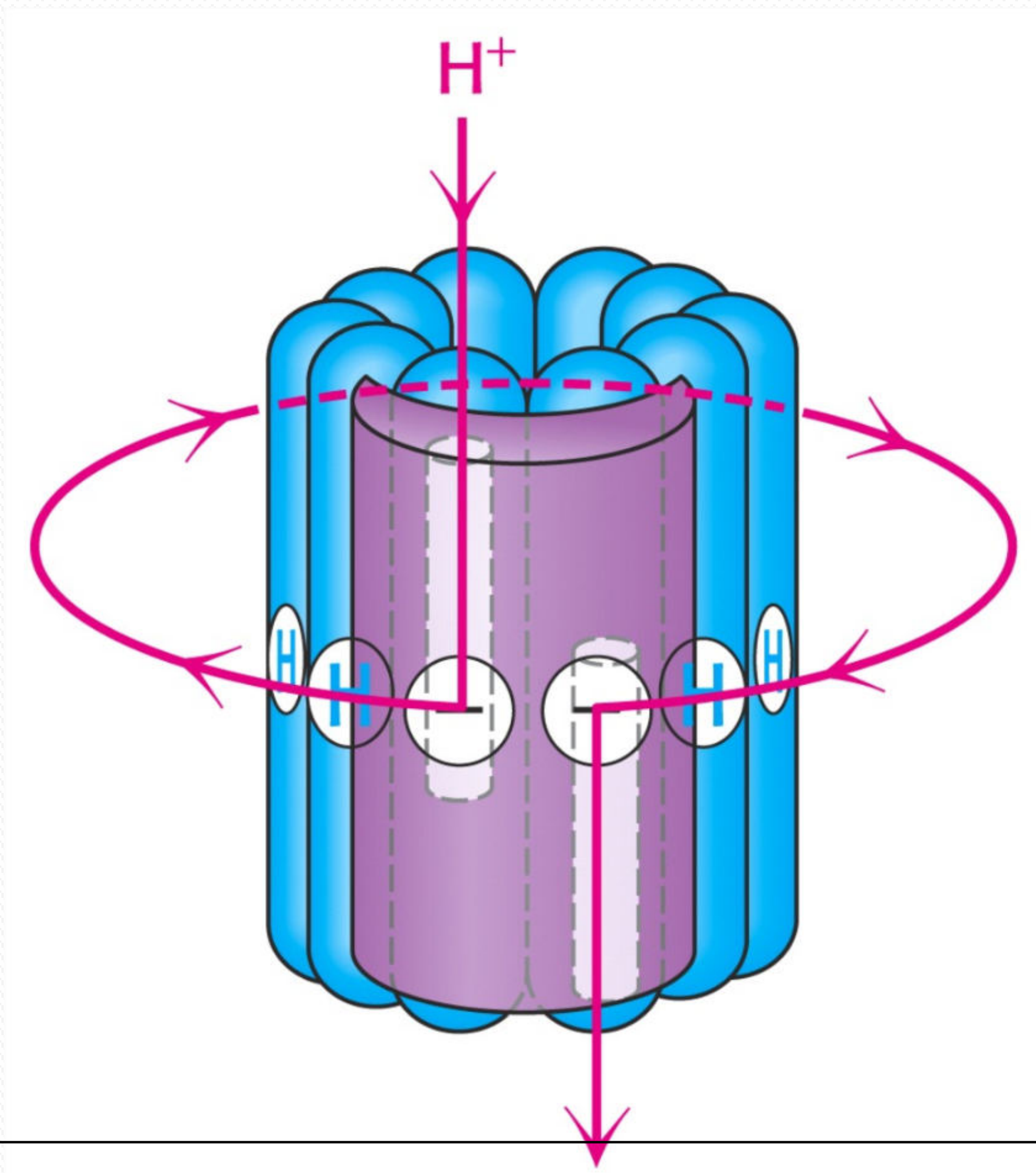
Complex I,III and IV Serve as Proton Channels

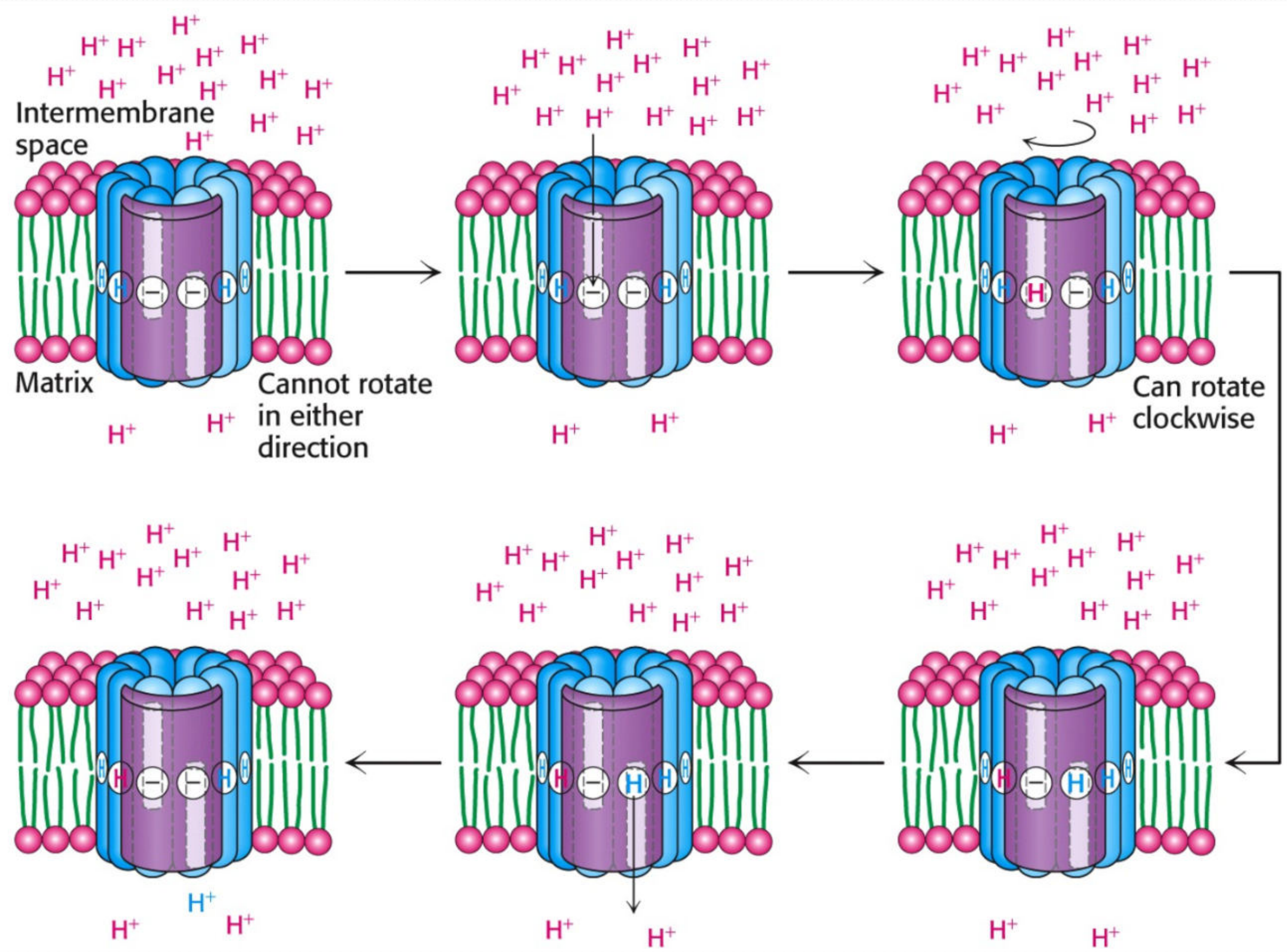


- Complex **I ,III and IV** act as a **Proton Pump.**
- Pump out protons from matrix side to **inter membrane space** of mitochondria.
- Develop a **proton gradient** in inter membrane space.
- This supports the mechanism of Oxidative Phosphorylation.

- **A Large Drop in Redox Potential across each of the three Respiratory Enzyme Complexes (I,III,IV).**

- Provides the **Energy for H^+ Pumping**





3

Free Energy Change Occurs Due To Transport OF Proton Pumping and Electron Exchange During Oxidative Phosphorylation

4

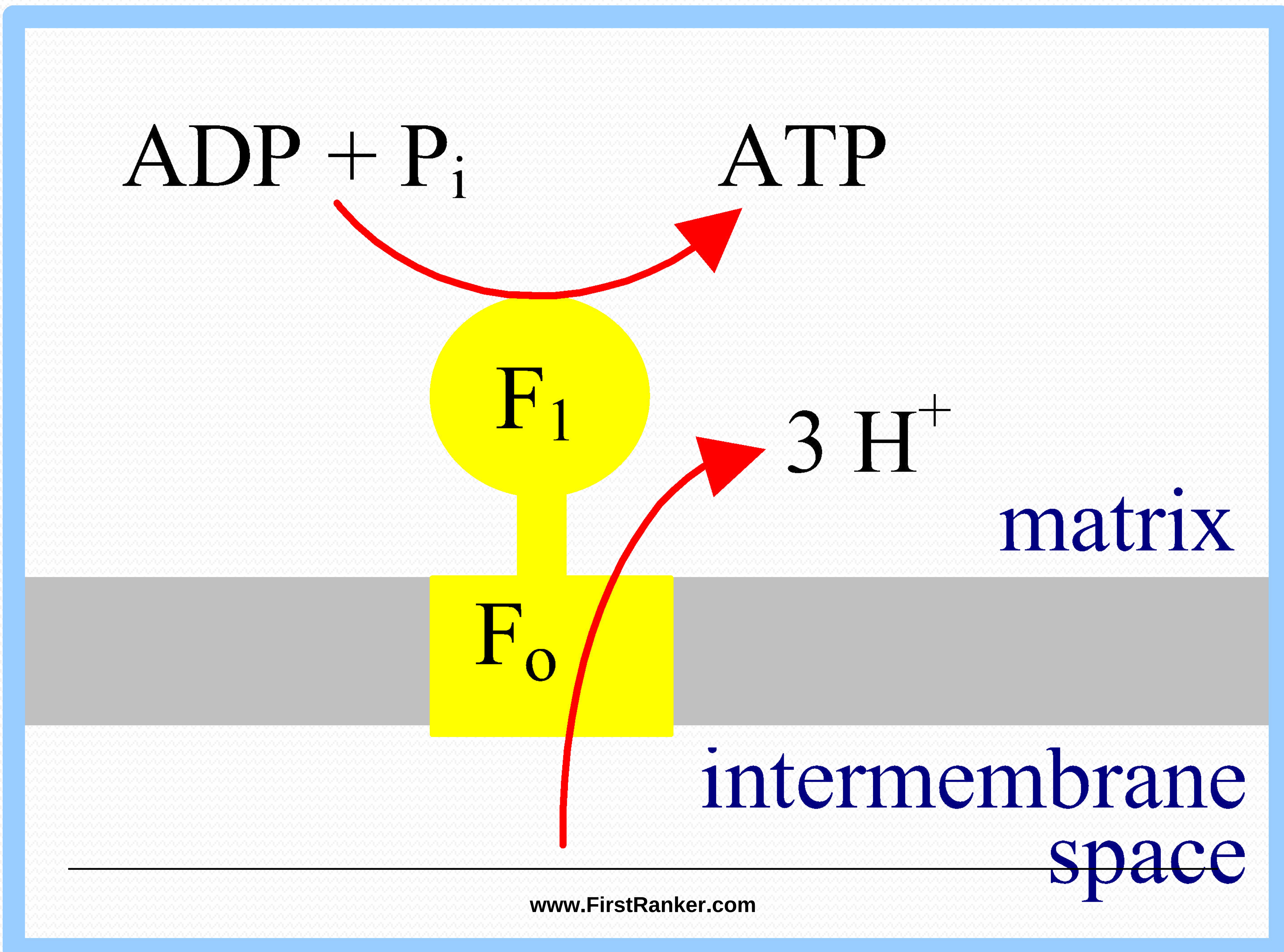
Heat Energy Generated At Certain Specific Sites During Oxidation Is Transformed By Chemical Phosphorylation Reaction of ADP and pi to form ATP

5

ATP Synthase /Complex V

Activation for Phosphorylation Reaction

- **Proton gradient runs downhill** through ATP Synthase to **drive synthesis of ATP**



- **F_1F_o of ATP Synthase catalyzes phosphorylation reaction for ATP synthesis**

- **Transport of H^+** from intermembrane space to into the mitochondrial matrix **through ATP Synthase is mandatory.**

- Transport of **at least 3 H^+ per ATP is required through ATP Synthase for its activation and catalysis.**

- Thus heat energy is transformed to chemical form of energy (ATP) in Oxidative Phosphorylation.

6
Oxygen is Terminal Acceptor of Protons and electrons During Oxidative Phosphorylation To Generate Metabolic Water

- **Oxygen** has **highest** (most positive) **standard redox potential**

- Most likely to accept electrons from other carriers.

- Electrons ultimately reduce Oxygen to water (metabolic water)
  - $2\ H^{+} + 2\ e^{-} + ½\ O_2 \dashrightarrow H_2O$

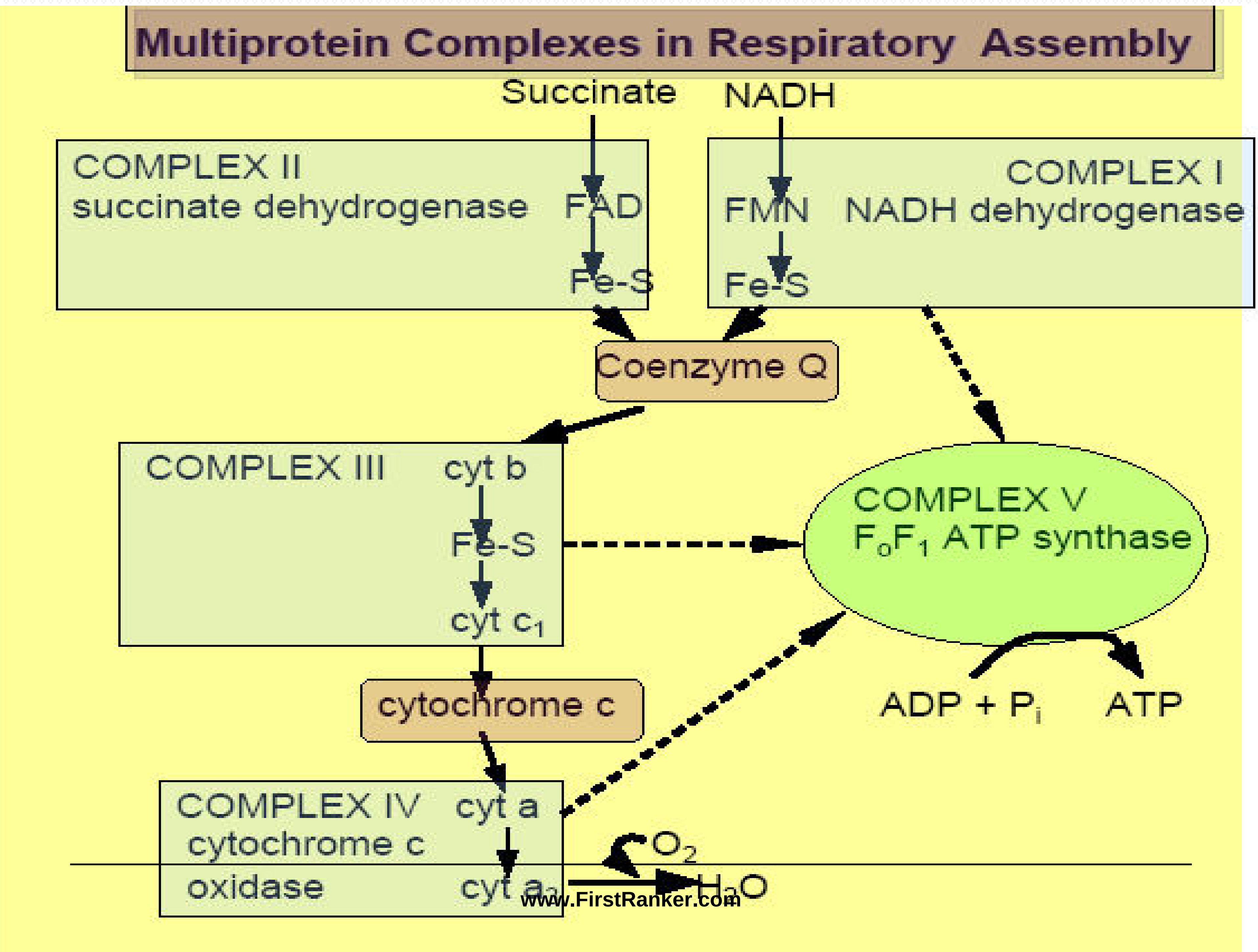
- At end of E.T.C by catalytic activity of **Cytochrome Oxidase**

- **Protons** released at Coenzyme Q and **electrons** transported by Cytochromes are
- **Accepted** by activated molecular **oxygen (1/2 O2)** to form metabolic water.

- **Cytochrome oxidase** controls rate of O_2 uptake which
- Means this **enzyme determines how rapidly we breathe.**

- Respired Oxygen transported by Hb unloaded at tissue/ cellular level is utilized during E.T.C.

7
Coenzyme Q Accepts Electrons Via Complexes I & II



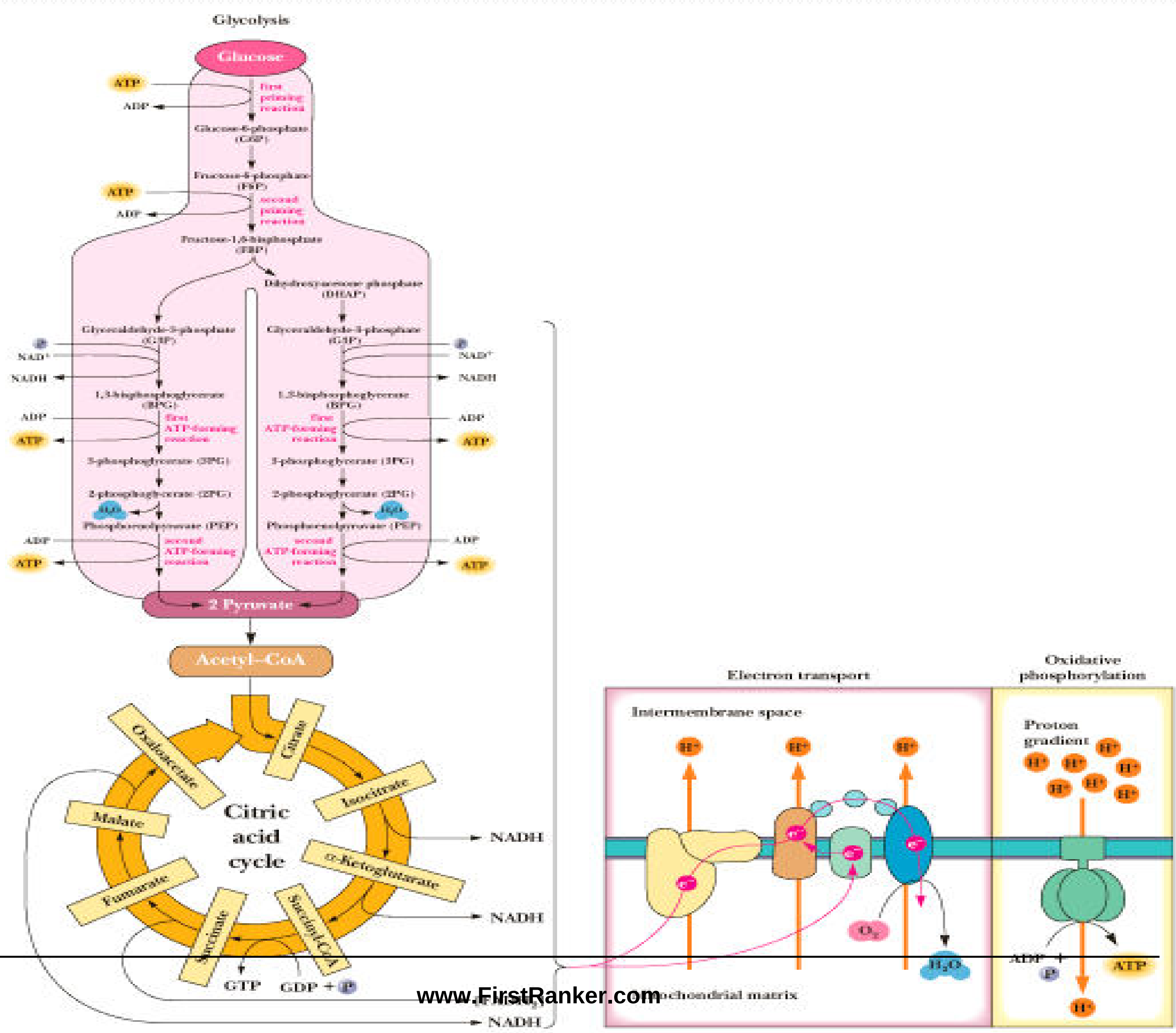
Point To Note

- In ETC electrons flow from
- Most electro negative potential NADH+H^+ (-0.32) to most **electro positive potential** (+0.82) ½ O_2 .

HOW ETC /Oxidative Phosphorylation Operates ?

Most Oxidative
Metabolic Pathways
(TCA and Beta Oxidation Of Fatty acids)

Located In Mitochondrial Matrix
Generate Reduced Coenzymes



- Reduced coenzymes **$NADH+H^+/FADH_2$**

- **Generated** during **Anaerobic Dehydrogenase reactions** of **Carbohydrates, Lipids metabolic pathways.**

- **Get reoxidized on entering E.T.C**

- **Reduced coenzymes $NADH+H^+$ and $FADH_2$ are formed in Mitochondrial matrix:**
  - **Oxidative Decarboxylation of Pyruvate** to Acetyl CoA by **PDH complex**.
  - **Oxidation of Acetyl CoA** by **TCA cycle**
  - **Beta Oxidation of fatty acids**

- $NADH+H^+$ and $FADH_2$ are **energy rich molecules**

- Contains a **pair of electrons having a high transfer potential.**

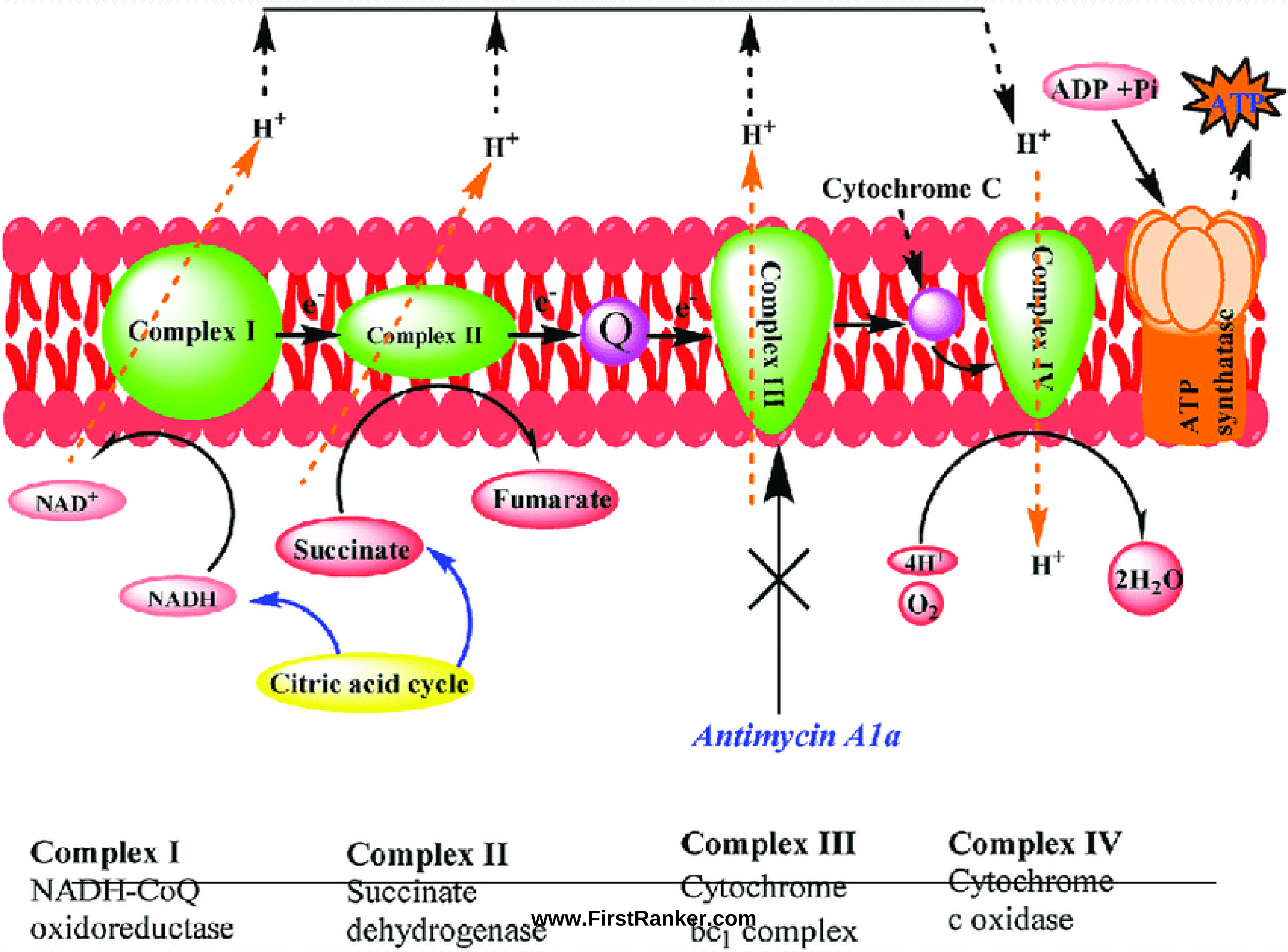
Entry Of $NADH+H^+$ in ETC

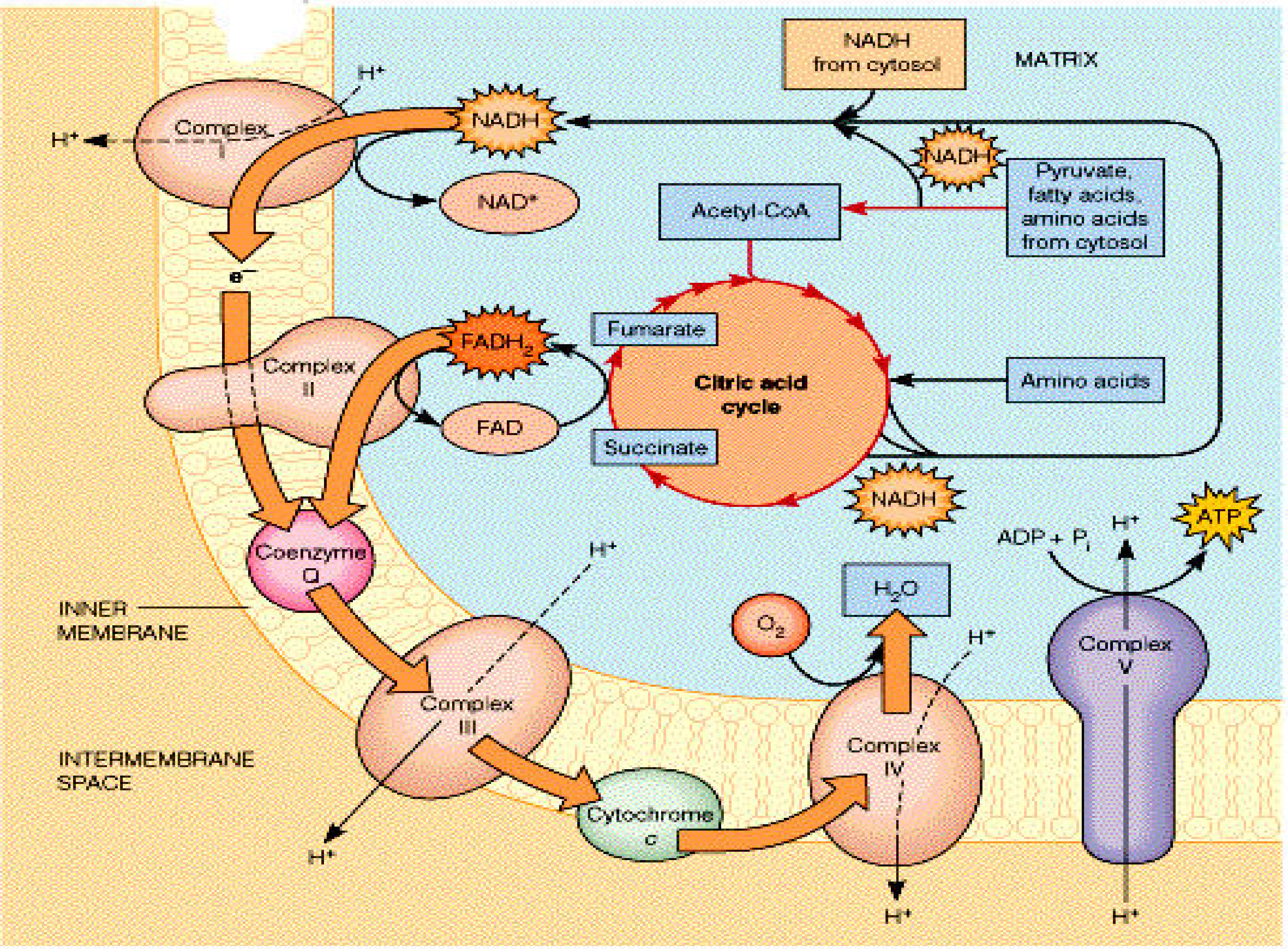
- When $NADH+H^+$ enters ETC reducing equivalents Protons and Electrons are taken up by **first component /Complex I (Flavoproteins)**

- Then from complex I the reducing equivalents are transferred to CoQ.

Entry Of FADH2 in ETC

- **FADH2 is generated** at Succinate Dehydrogenase reaction(**Complex II**)
- FADH2 enters ETC process and its reducing equivalents are **taken up by CoQ .**
- CoQH2 then here onwards **transfers only electrons to series of arranged Cytochromes** and **Protons are released in matrix.**





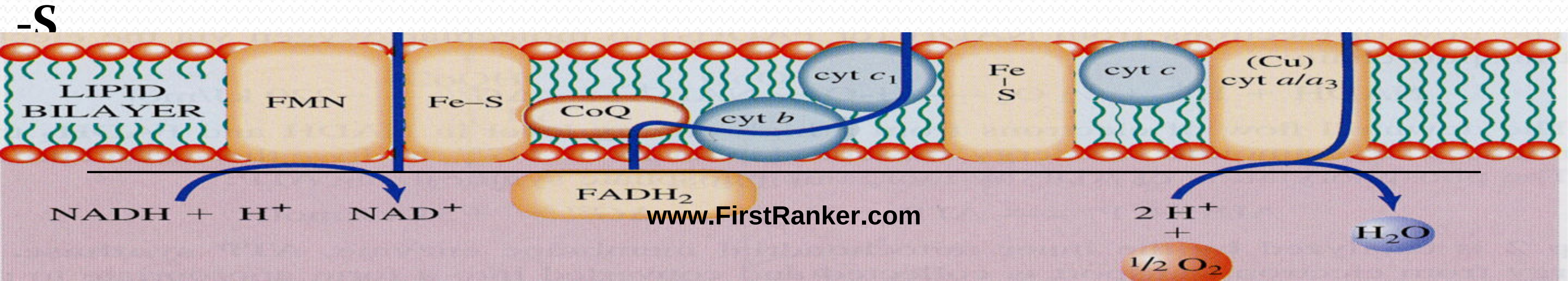
ELECTRON TRANSPORT CHAIN

Series of enzyme complexes (electron carriers) embedded in the inner mitochondrial membrane, which oxidize NADH+H⁺ and $FADH_2$ and transport electrons to oxygen is called Respiratory Electron-Transport Chain (ETC).

Sequence of Electron Carriers in ETC

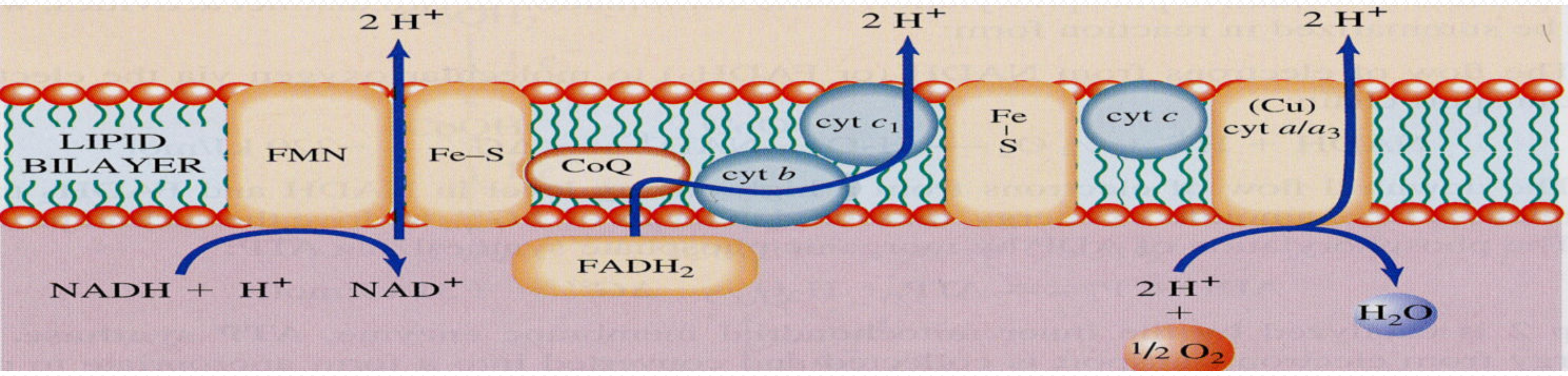
NADH → FMN-Fe-S → Co-Q Fe (cyt b) → cyt c_1 → cyt c → cyt a → cyt a_3 → O

Succinate → FAD Fe-S (→ Co-Q)



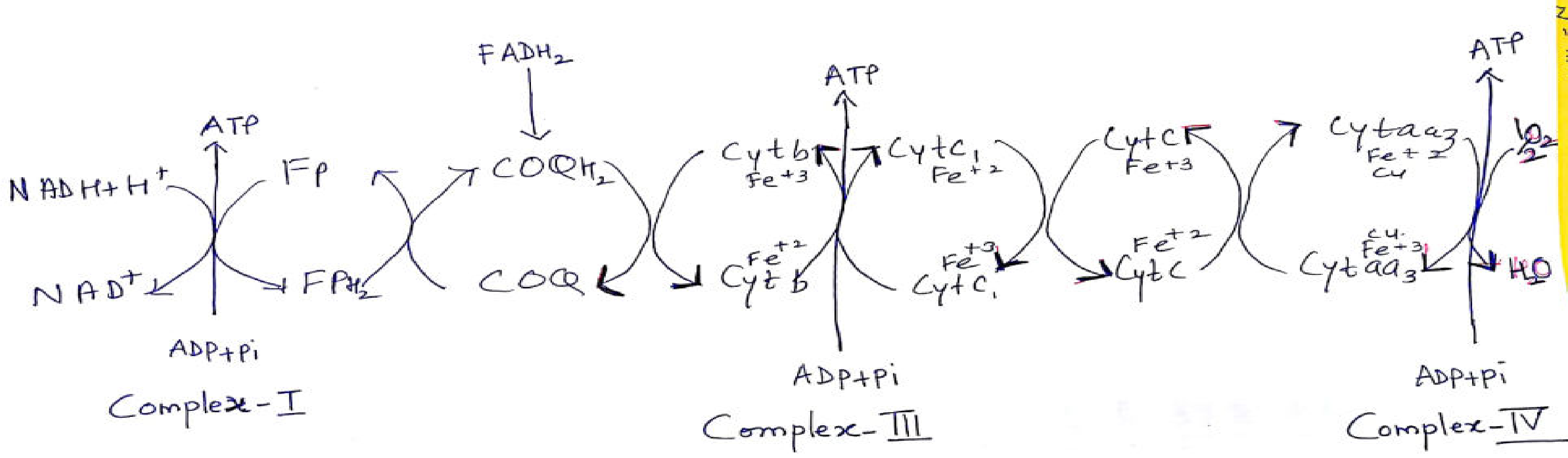
Electrons of NADH or $FADH_2$ are used to **reduce molecular oxygen to water.**

A large amount of free energy is liberated.



The electrons from NADH+H+ and $FADH_2$ **are not transported directly to O_2** but **are transferred through series of electron carriers** that undergo reversible reduction and oxidation.

ETC Process



A PROTON GRADIENT POWERS THE SYNTHESIS OF ATP

Transport of electrons from NADH or $FADH_2$ to O_2 via the electron-transport chain is exergonic process:

$NADH + ½O_2 + H^+ \Leftrightarrow H_2O + NAD^+$

$FADH_2 + ½O_2 \Leftrightarrow H_2O + FAD^+$

$\Delta G^{o\prime}$ = -52.6 kcal/mol for NADH

-36.3 kcal/mol for $FADH_2$

This process is coupled to the synthesis of ATP (endergonic process)

$ADP + P_i \Leftrightarrow ATP + H_2O$ $\Delta G^{o\prime}$=+7.3 kcal/mol

- In E.T.C **both Protons and Electrons** are **transferred up to Coenzyme Q** level.
- At coenzyme Q level protons ($2H^+$)are released in the medium.
- From **Coenzyme Q onwards only electrons are transferred** through a series of Cytochromes in E.T.C.

- Electrons get transfer through series of Cytochromes
- Cytochrome Fe is in transitional state (Ferric/Ferrous).

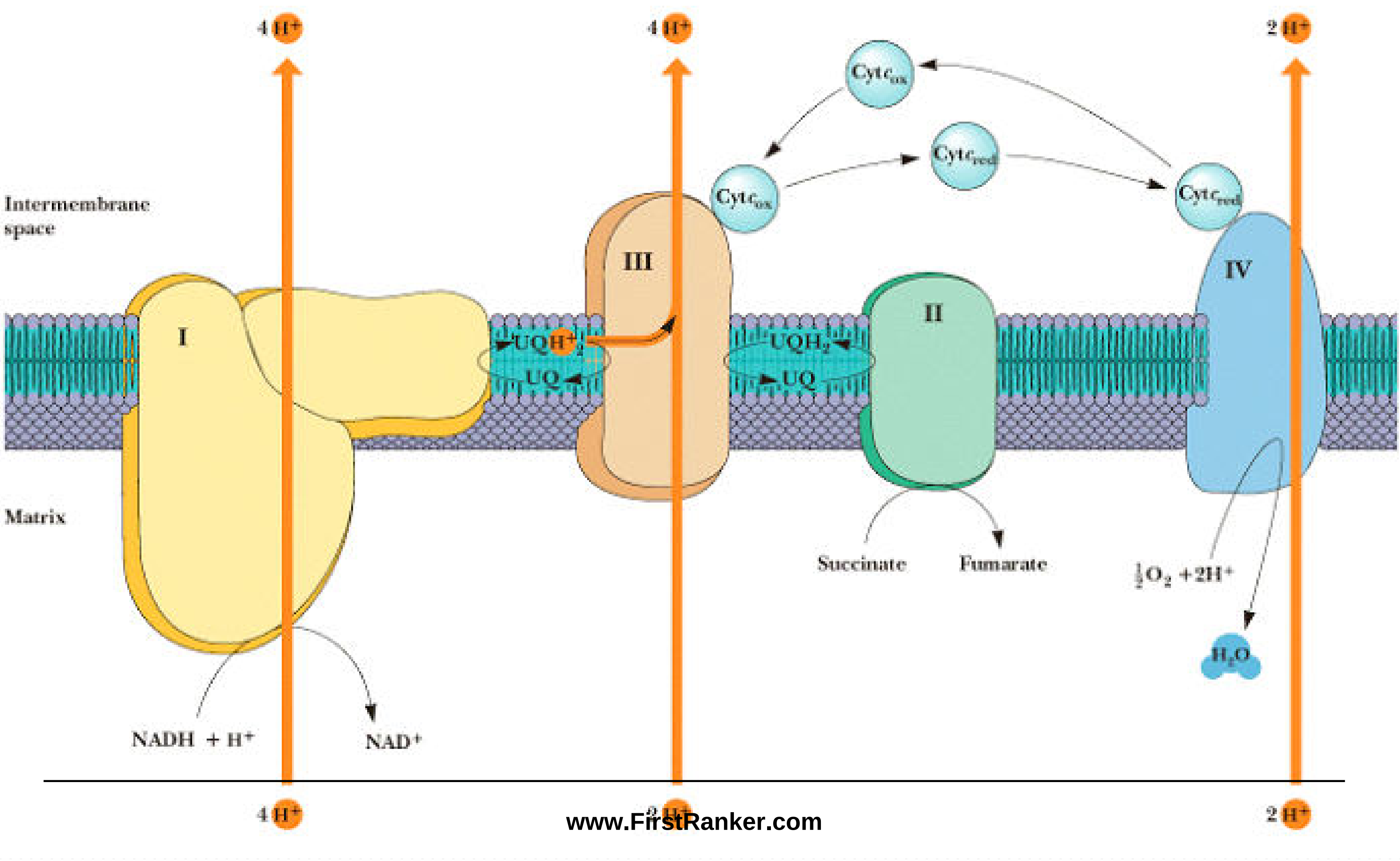
- In E.T.C there are **alternate reduction** and **oxidation** reactions.

- Flow of electrons through **ETC complexes leads to pumping** of **protons** out of the mitochondrial matrix in **intermembrane space**.

- This accumulation of protons generates a **pH/Proton gradient** and a transmembrane electrical potential that creates a **proton motive force**.

- **A Large Drop in Redox Potential across each of the three Respiratory Enzyme Complexes (I,III,IV).**
- Provides the **Energy for H^+ Pumping**

Electron Transport (Oxidative Process) is coupled to Phosphorylation



- **ATP is synthesized** when 3 **protons flow back from intermembrane space of mitochondria to mitochondrial matrix** through an **enzyme complex ATP synthase.**

- Oxidation of fuels and phosphorylation of ADP are coupled by a proton gradient across an inner mitochondrial membrane.

- Thus Oxidative phosphorylation is process in which **ATP is formed**

- As a result of **transfer of electrons from NADH or $FADH_2$ to O_2** by a **series of electron carriers.**

Mechanism Of Oxidative Phosphorylation

Oxidative Phosphorylation

- **Oxidation tightly coupled with Phosphorylation**

- **E.T.C** (Oxidation)Process coupled with **phosphorylation** of ADP+pi to generate ATP.

Hypothesis And Theories Mechanism Of Oxidative Phosphorylation

- **Chemical Coupling Hypothesis**

- **Conformational Coupling Hypothesis**

- **Chemiosmotic Theory**

Chemical Coupling Hypothesis:

- Put forward by **Edward Slater (1953)**

- Proposed **series of high energy phosphorylated intermediates** are produced during E.T.C operation.

- Which are **used to produce ATP.**

Conformational Coupling Hypothesis

- **Paul Boyer 1964**
- Mitochondrial Cristae undergo **conformational change in the components of E.T.C.**
- **E.T.C components attain high energy state** which are responsible for the ATP production.

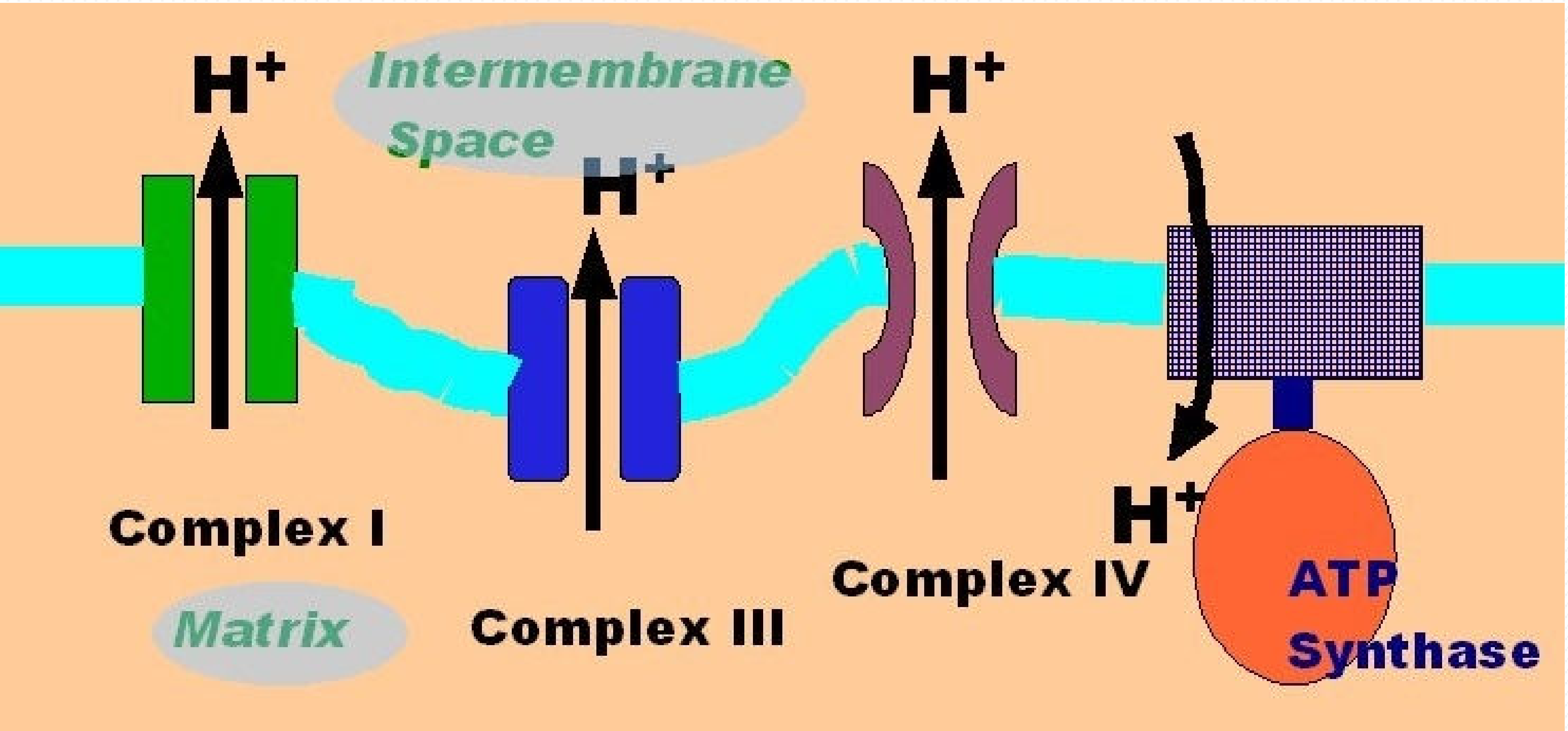
Chemiosmotic Theory

- Put forward by **Peter Mitchell (1961)** (Nobel Prize, 1978)
- **E.T.C process** and **ATP synthesis** is **coupled by a proton gradient developed in intermembrane space of mitochondria.**

Mitchell's Postulates for Chemiosmotic Theory

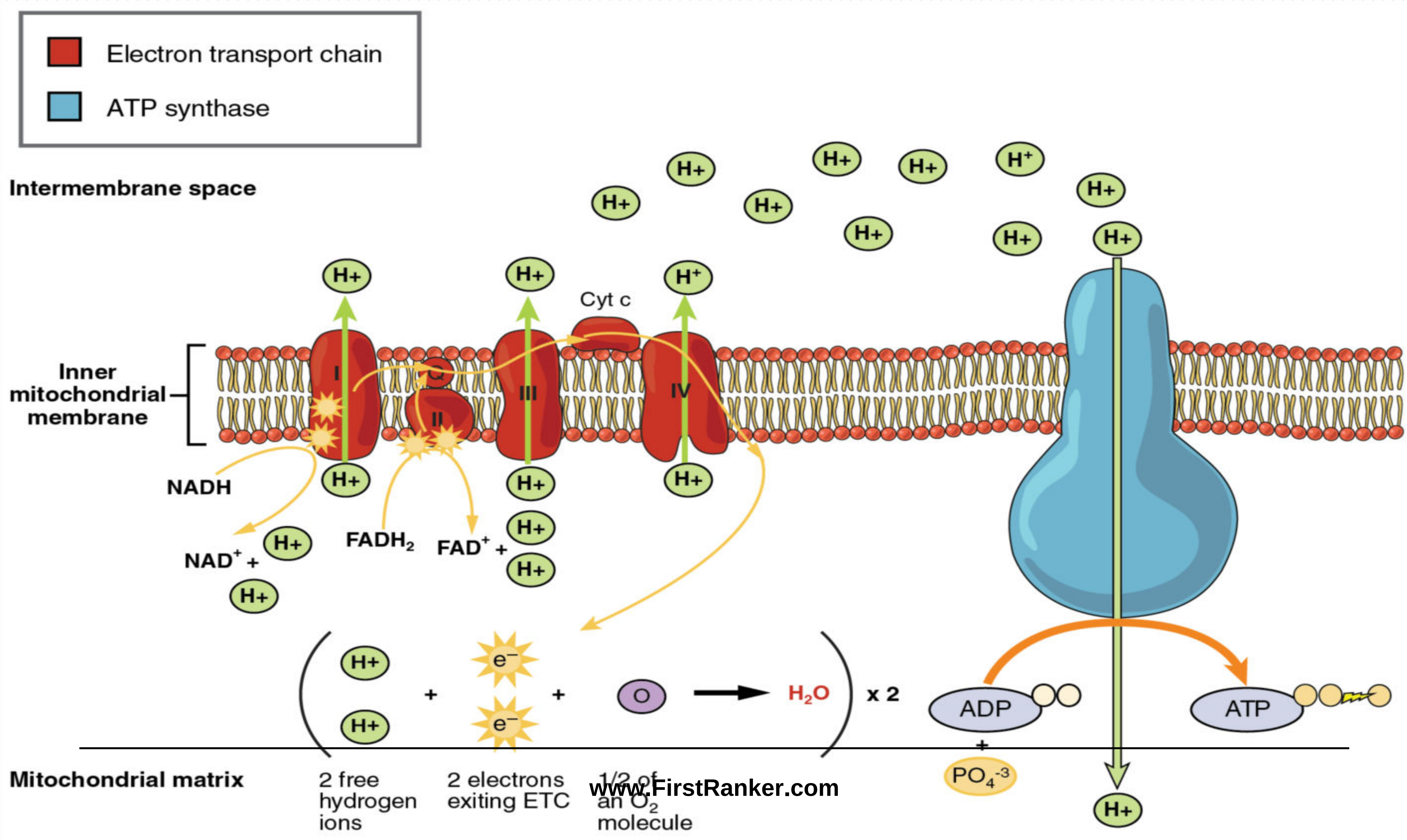
- **Intact inner mitochondrial membrane** is required
- Electrons are pumped through ETC **complexes I,III and IV**.
- Generates a **Proton gradient and in intermembrane space** of mitochondria.

Proton pumps are *Complexes* I, III and IV.



Protons return through ATP synthase

Oxidative Phosphorylation



- **Proton gradient** in inter membrane space creates **Proton Motive Force due to:**
  - **Proton gradient** have a **thermodynamic tendency**
  - **Proton gradient creates Electrochemical potential difference**
- Proton Motive Force **drives the Protons from mitochondrial intermembrane space back to matrix side**
- Through a **specific site of Fo and F1 particle of ATP Synthase**.

- **ATP Synthase catalyzes** the phosphorylation of ADP with pi

- In a reaction driven by **movement of H⁺** across the inner membrane back into the matrix **through it**.

- **Translocation of protons through ATP Synthase**
- **Stimulates and activates ATP Synthase**
- For catalytic action of phosphorylation- ADP with pi to form ATP.
- Supports mechanism of Oxidative Phosphorylation.

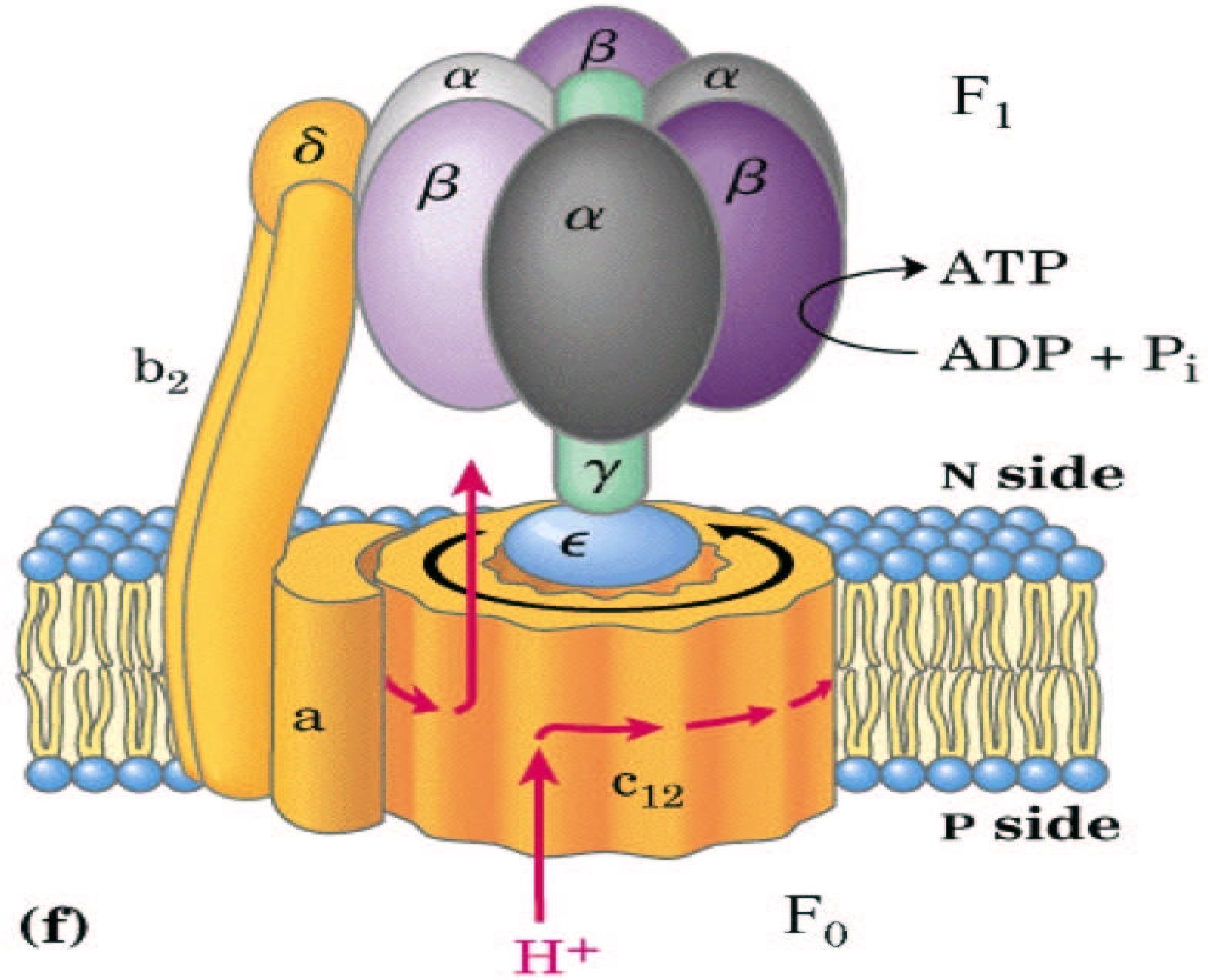
- **Flow of three H^+ through an ATP Synthase complex**

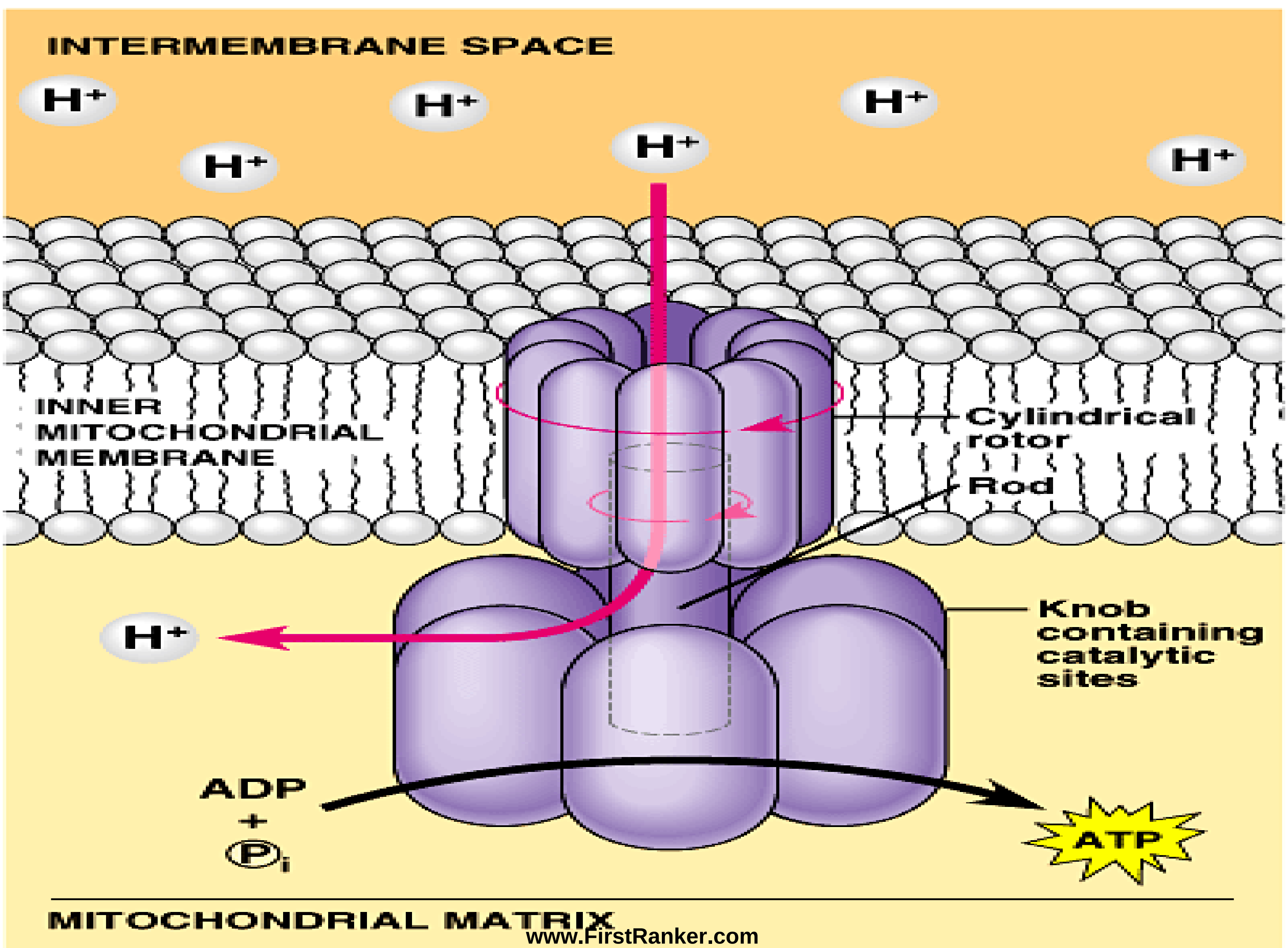
- Brings a **conformational change n domains of ATP Synthase**

- Which causes the **ATP synthase activate and catalyze phosphorylation reaction**

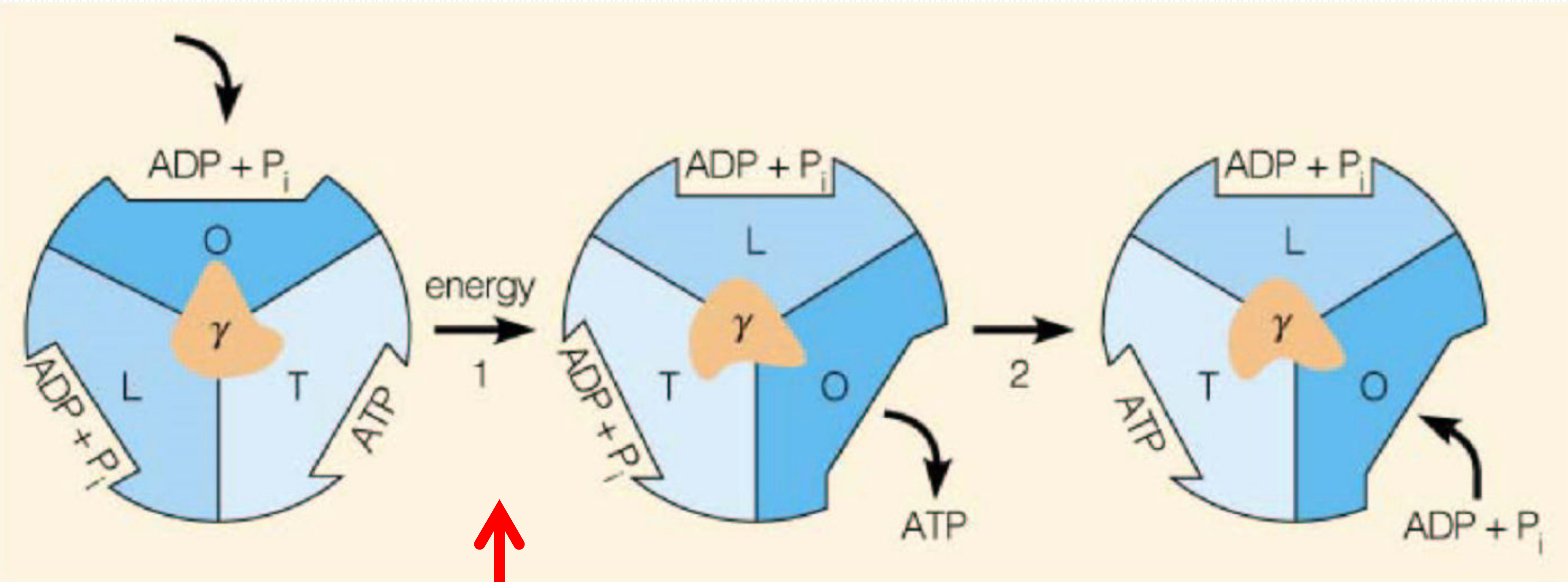
- To **synthesize ATP from ADP + Pi.**

ATP Synthase, a Molecular Mill.


F1
β
α
α
δ
β
α
β
ATP
ADP + Pi
b2
γ
N side
ϵ
a
c12
P side
(f)
H+
F0


INTERMEMBRANE SPACE
H+
H+
H+
H+
H+
H+
INNER
MITOCHONDRIAL
MEMBRANE
Cylindrical
rotor
Rod
H+
Knob
containing
catalytic
sites
ADP
+
Ⓟi
ATP
MITOCHONDRIAL MATRIX

ATP synthesis at F_1 results from **repetitive conformational changes as γ rotates**



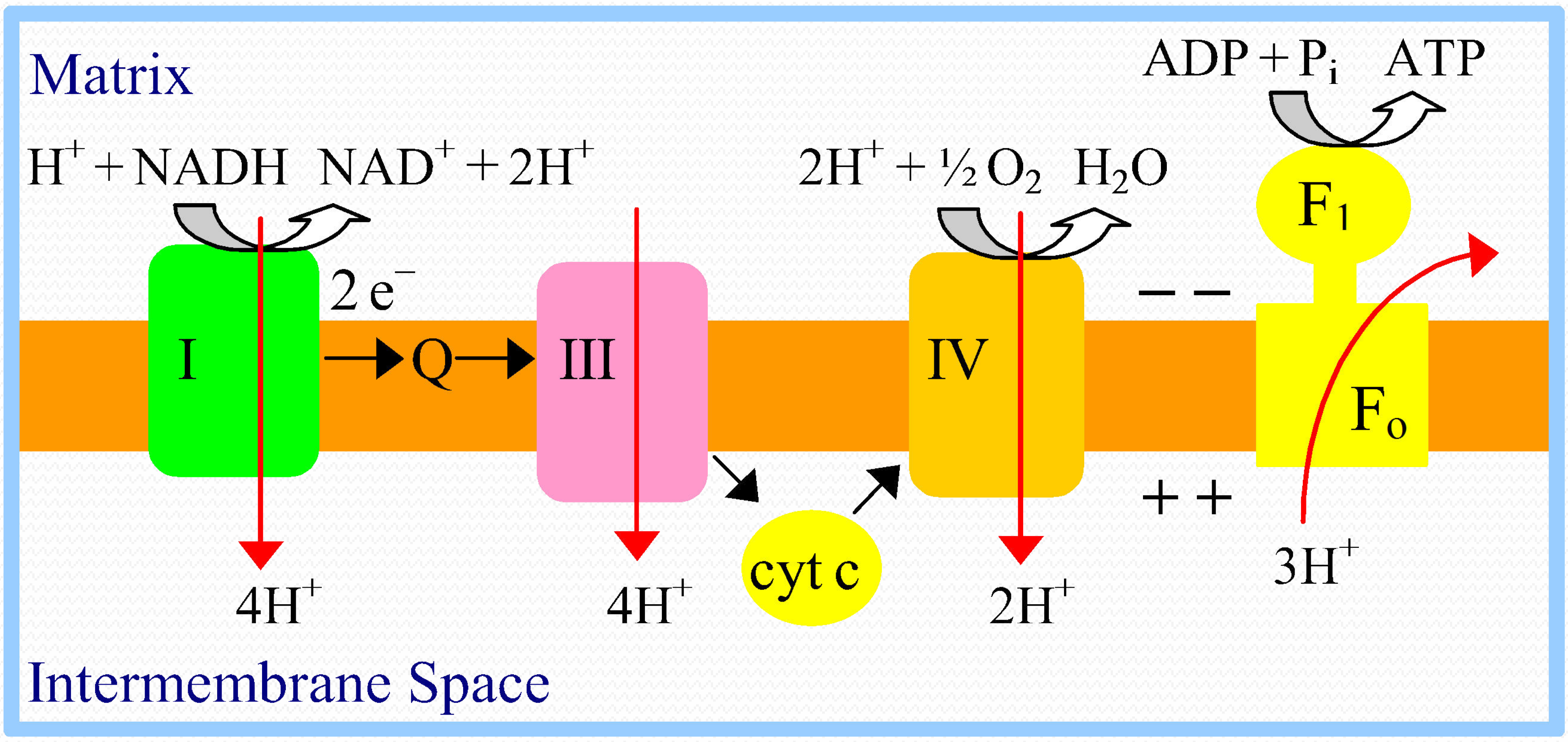
γ rotates 1/3 turn- energy for ATP *release*

- This process of producing ATP is known as oxidative phosphorylation.

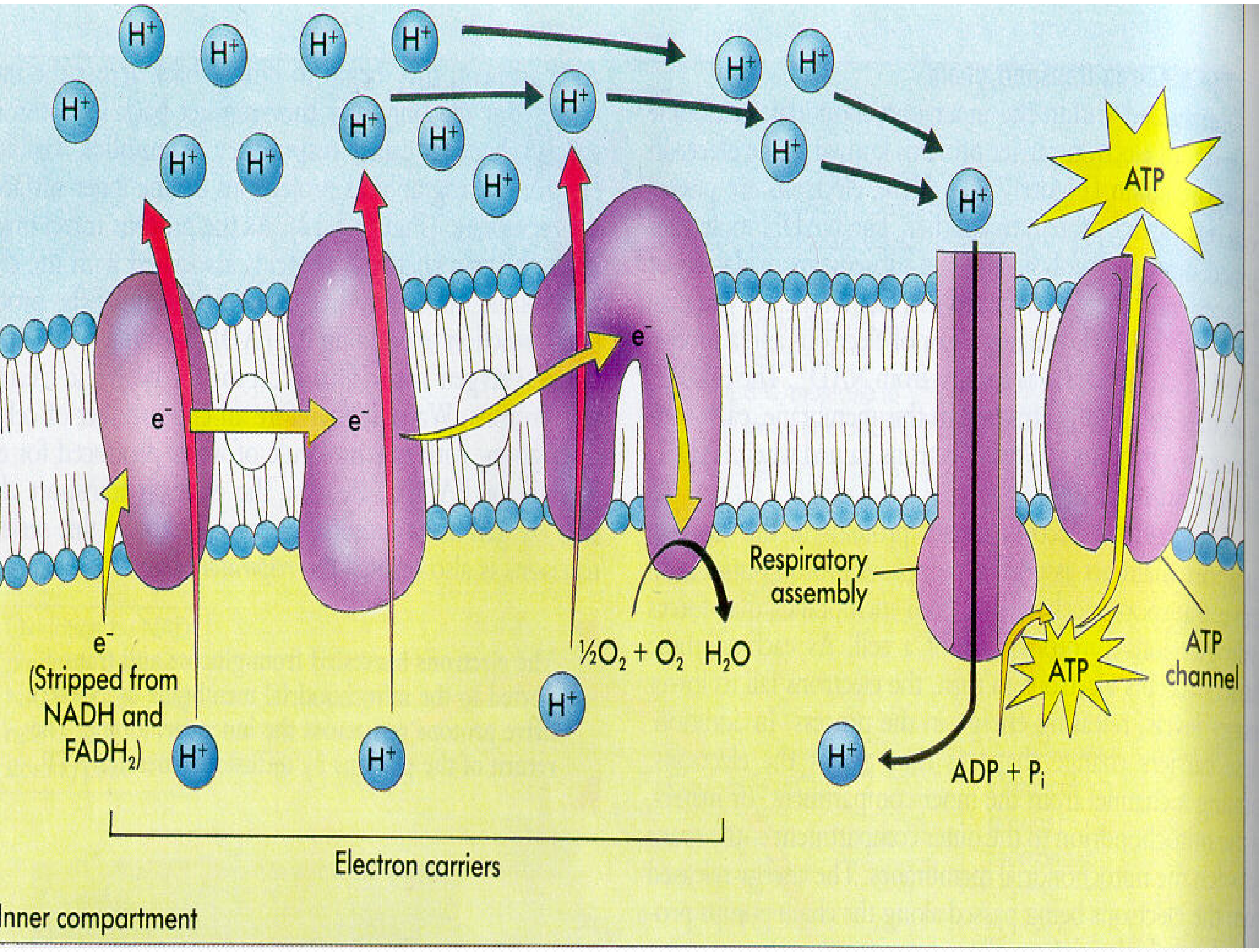
- Entire process of using **Proton gradient** and **proton motive force** to make ATP is called **Chemiosmosis**.

- During oxidative phosphorylation **total energy change is released in small increments.**

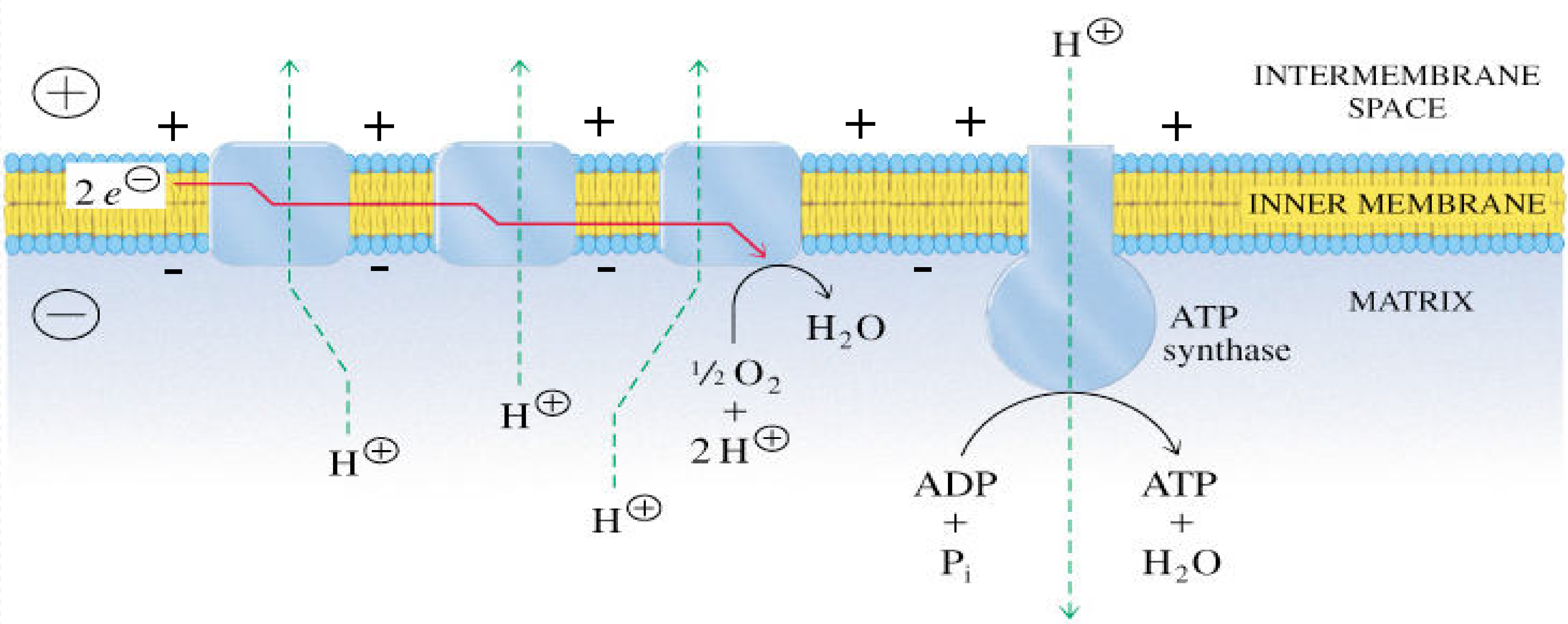
- So that energy can be trapped as chemical bond energy and form ATP.



Coupling of ATP synthesis to respiration is **indirect**, via a H^+ electrochemical gradient.



Overview of Oxidative Phosphorylation



As **electrons flow** through complexes of ETC, **protons are translocated** from matrix into the intermembrane space.

The free energy stored in the **proton concentration gradient** is tapped as protons reenter the matrix via **ATP synthase**.

As result **ATP is formed from ADP and** P_i.

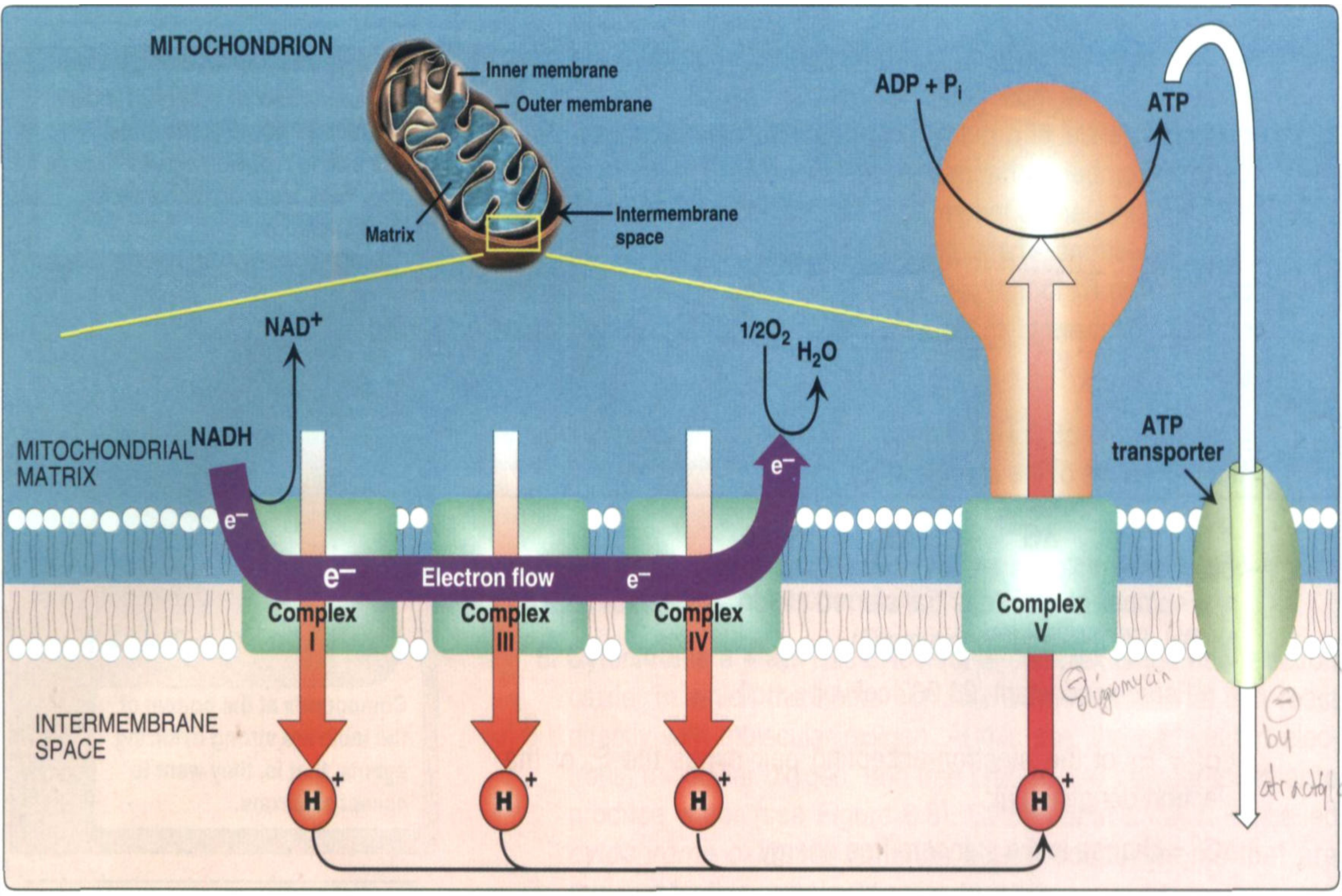


Figure 6.13
Electron transport chain shown coupled to the transport of protons. [Note: Complex II is not shown.]

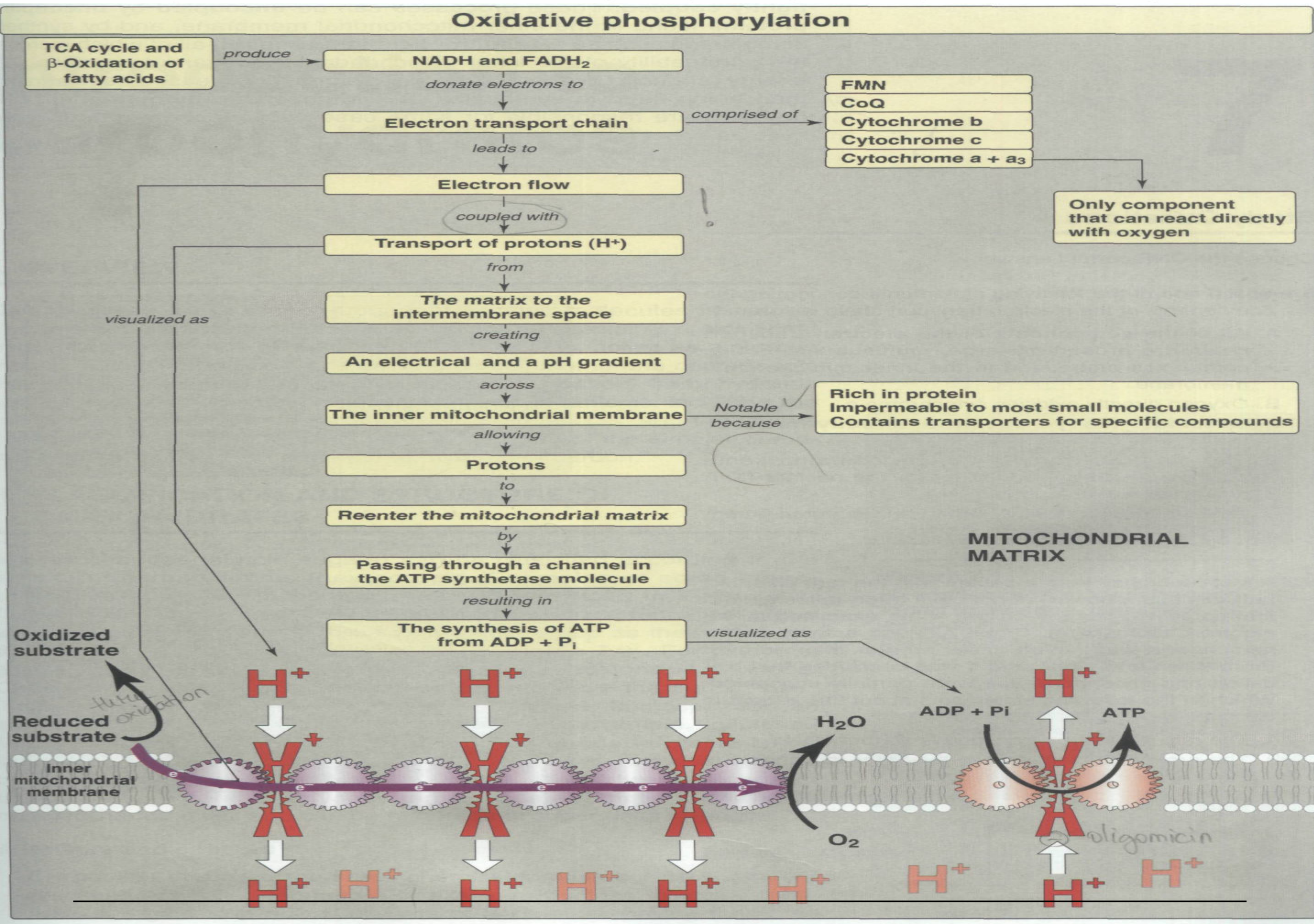
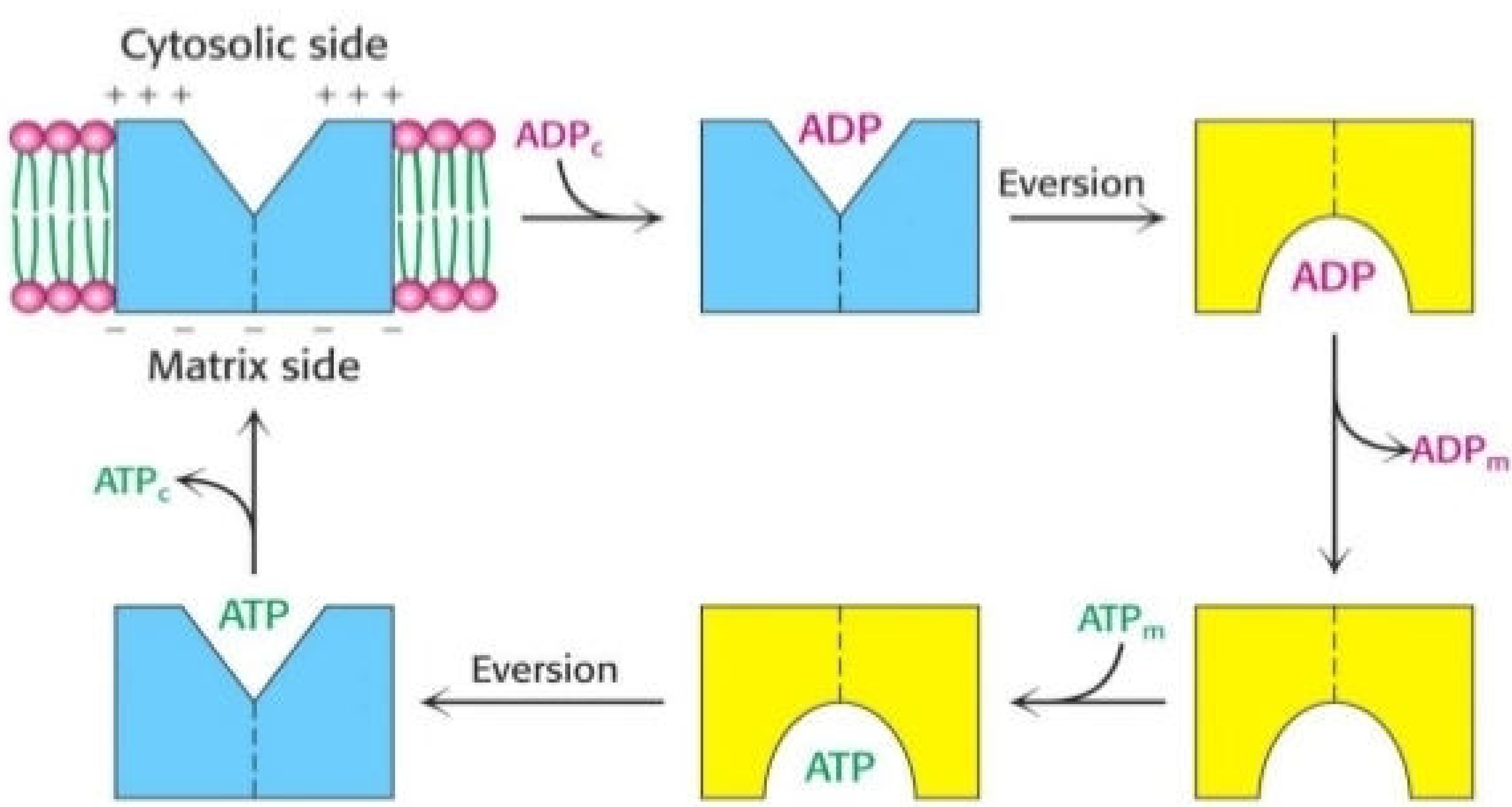


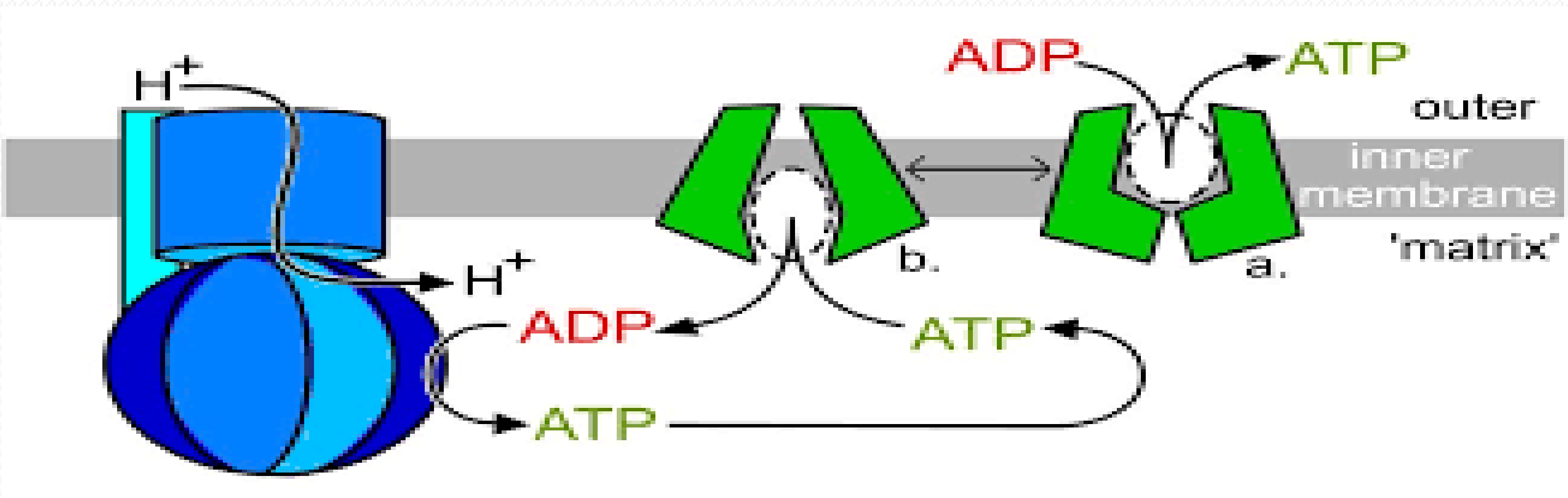
Figure 6.17
Summary of key concepts for oxidative phosphorylation. [Note: Electron flow and ATP synthesis are envisioned as sets of interlocking gears to emphase the idea of coupling.]

ATP Translocation From Mitochondria Through ATP/ADP Translocases

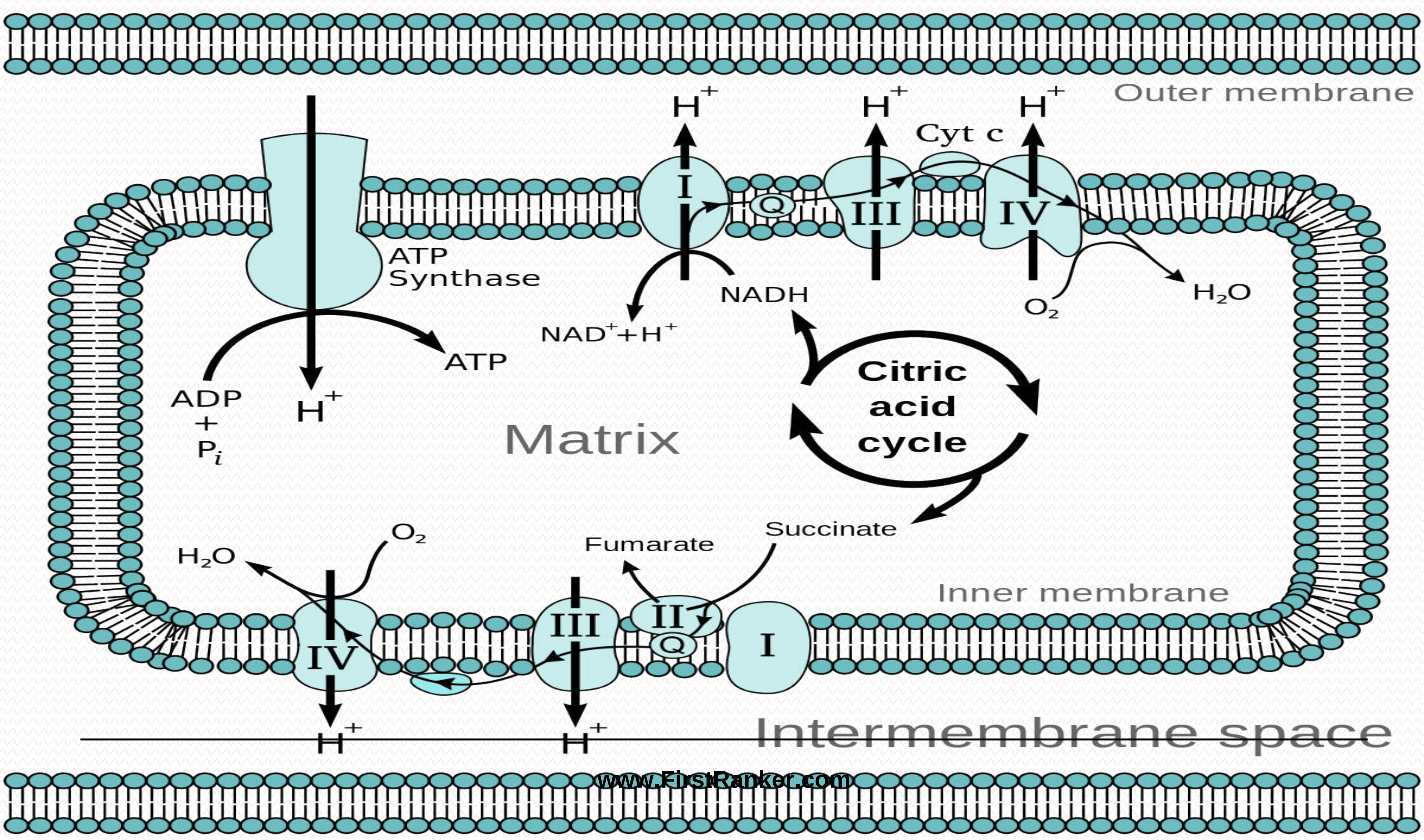
Movement of ATP/ADP Through translocase



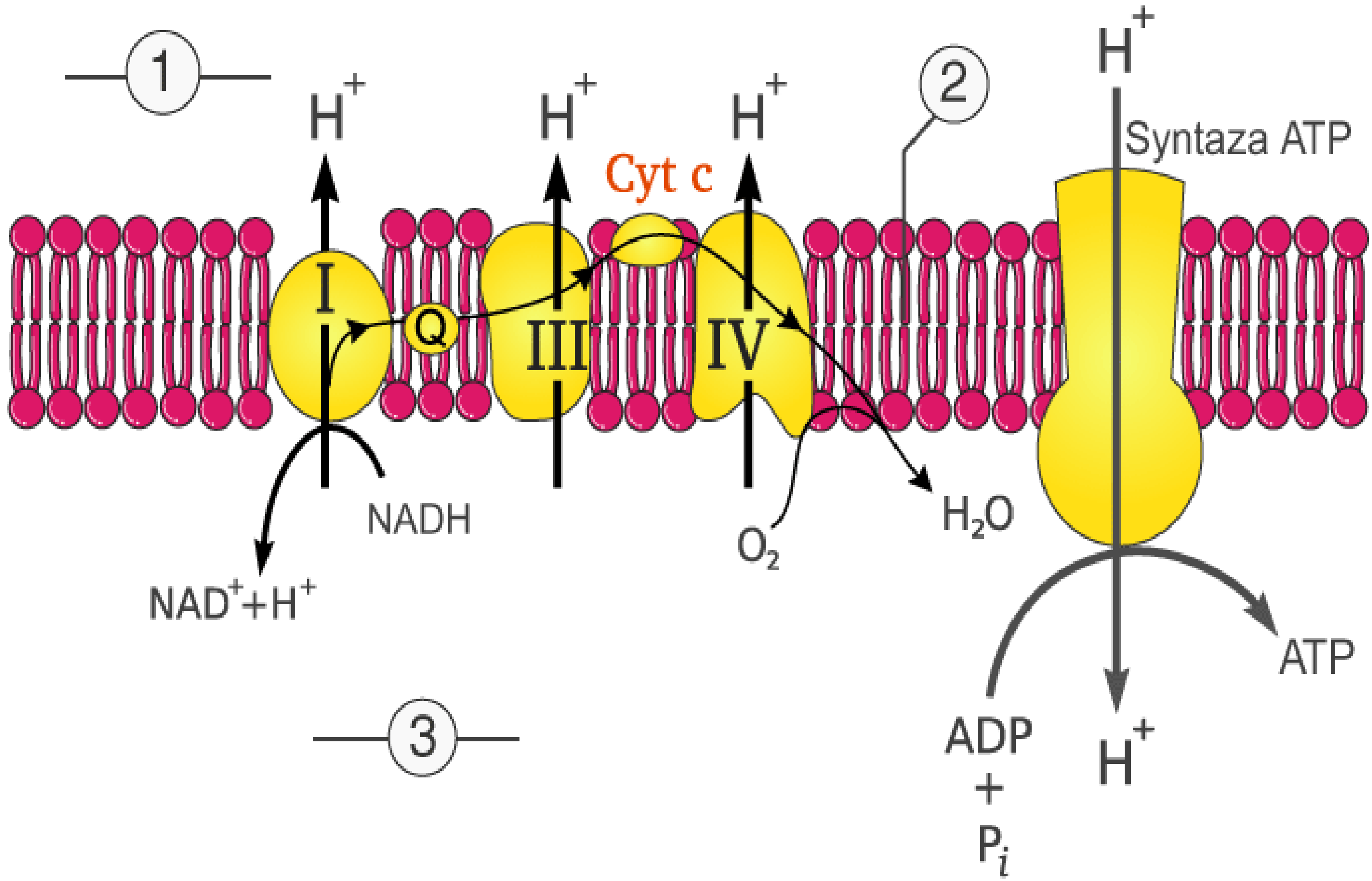
- **ATP molecules** produced in Oxidative Phosphorylation mechanism are
- **Transported out** of mitochondrial matrix through **specific transporters**



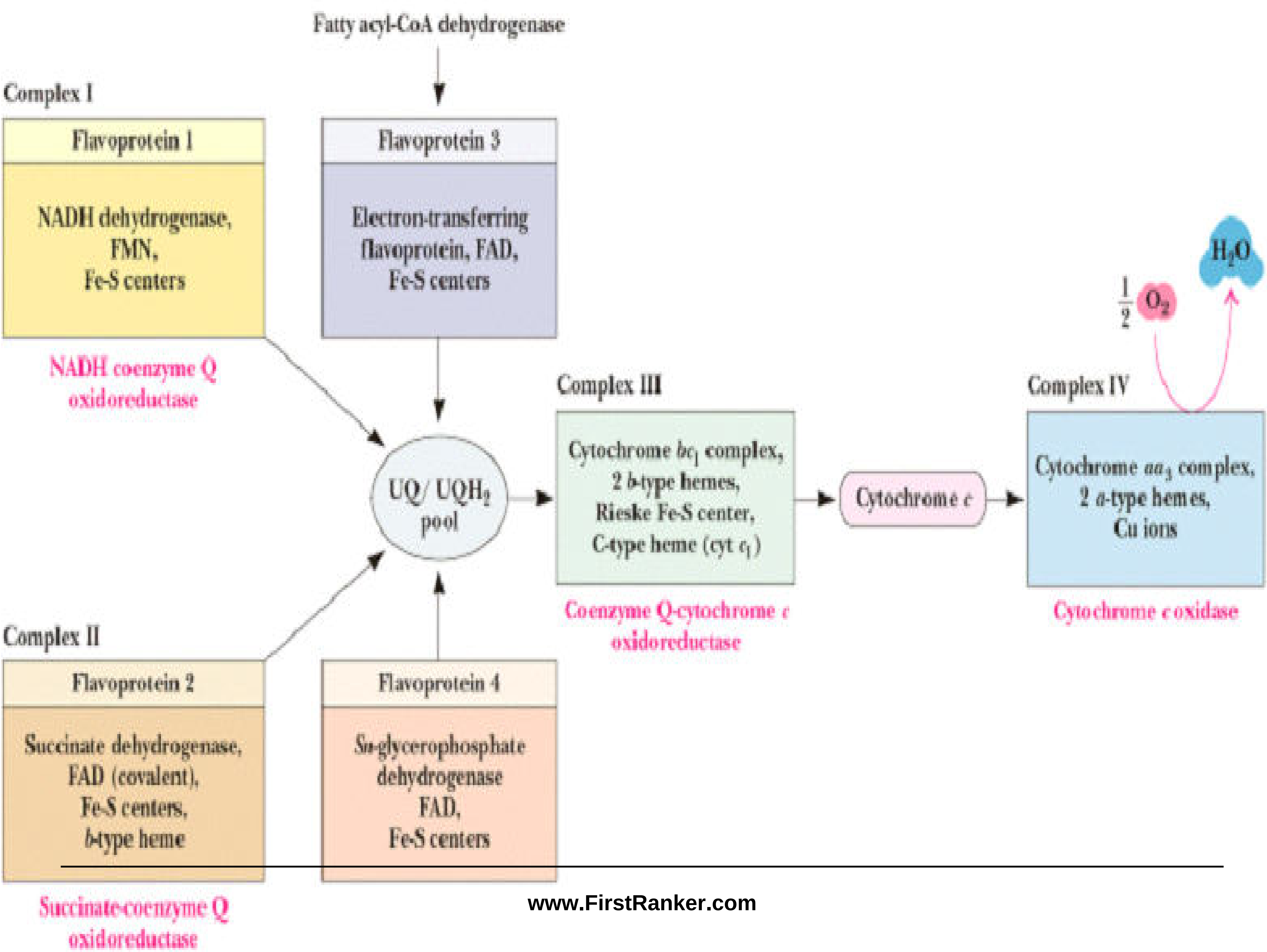
Operation Of ETC

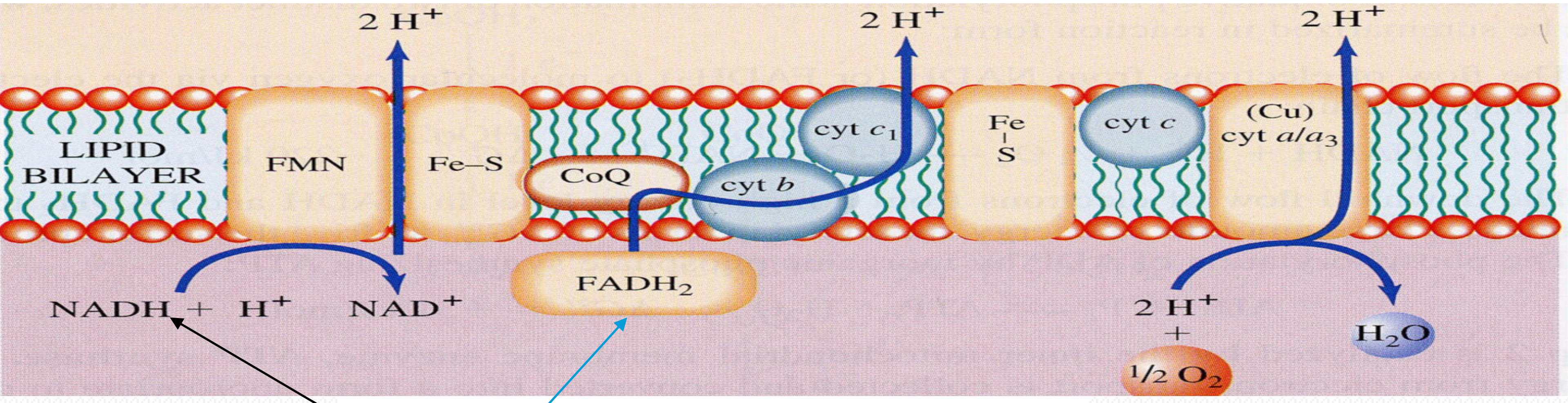


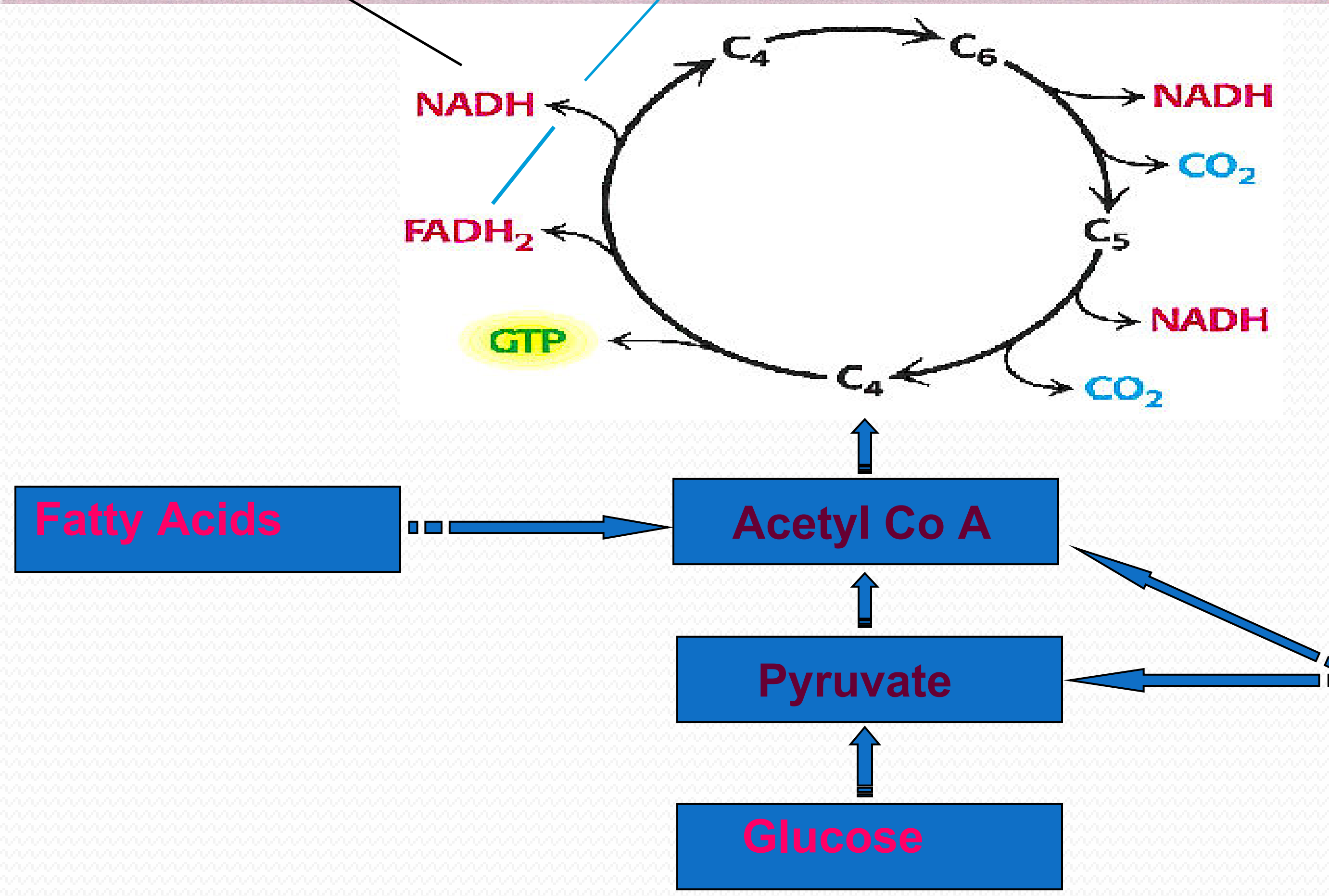
ELECTRON TRANSPORT CHAIN



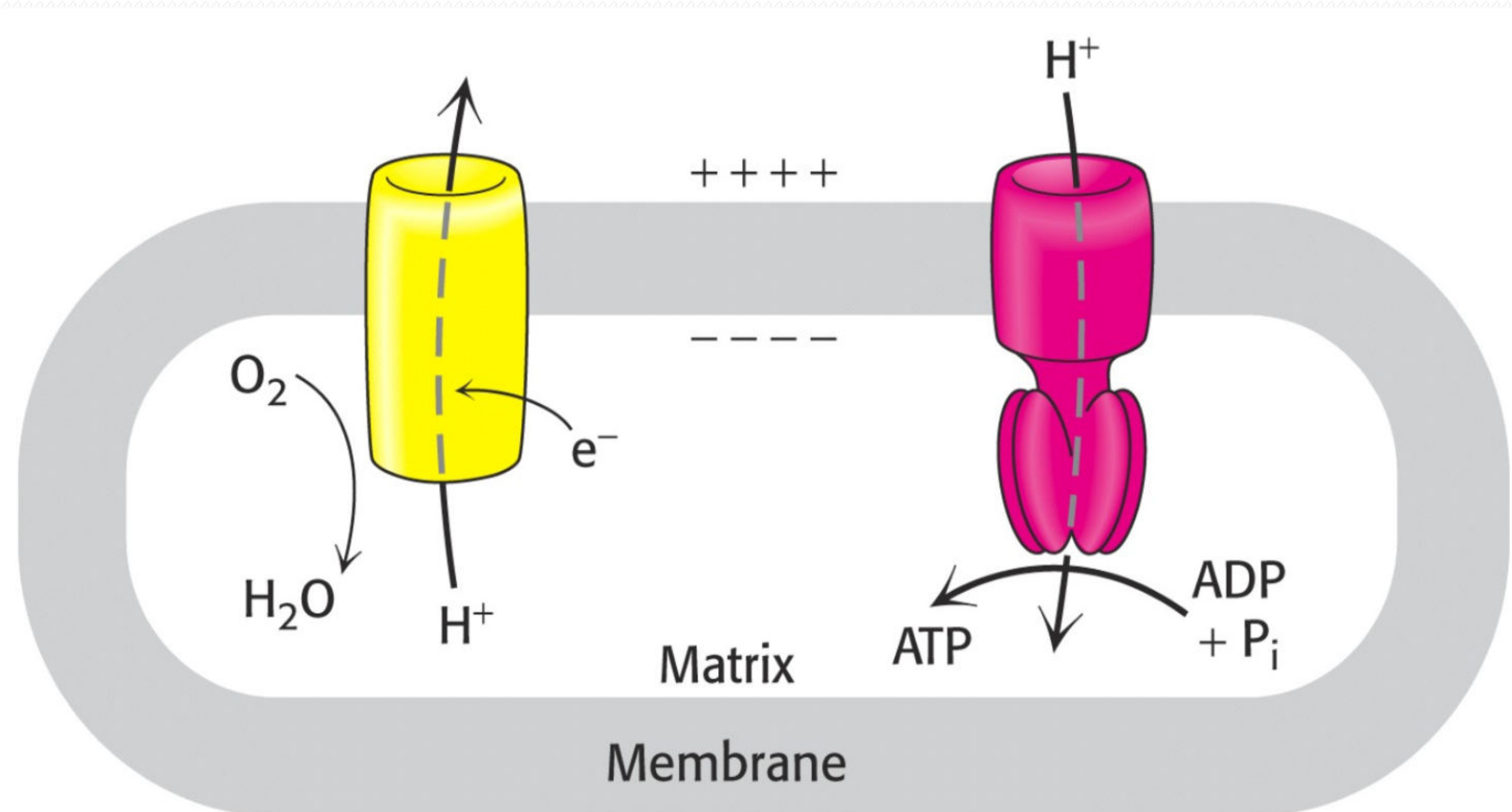
1 Intermembrane Space | 2 Inner Mitochondrial membrane | 3 Mitochondrial Matrix







Glycolysis, Fatty acid oxidation TCA cycle supplies NADH and $FADH_2$ to the Electron Transport Chain



WHY ETC OPERATES ?

- During E.T.C operation **total energy change is released in small increments**
- So that **energy can be trapped** as chemical bond energy to **form ATP.**

- When **two redox couples** of ETC **differ from each other** by **0.22 volts** in **standard redox potential**.
- At this site **free energy in the form of heat** released which is more than 7.3 Kcal.
- This **free heat energy** is **conserved** to **undergo Phosphorylation reaction** and **generate chemical form of energy-ATP.**

- Sites in E.T.C at which energy liberated is **less than 7.3 Kcal** is simply **dissipiated** in the **form of heat.**

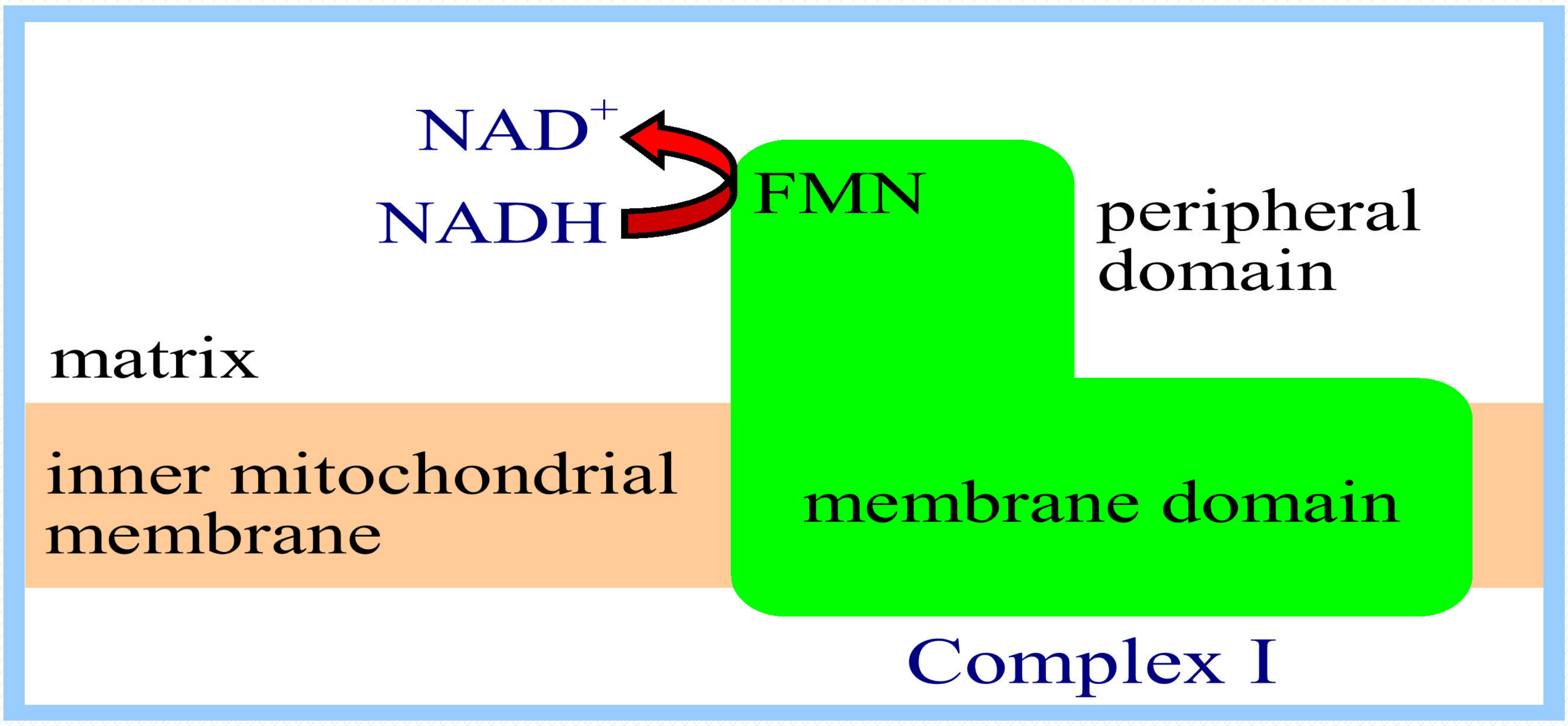
- Three sites in E.T.C (Complex I,III and IV) where heat energy liberated **more than 7.3 Kcal**

- **Utilized for phosphorylation reaction** of ADP with pi to **form ATP**.

- Electrons are transferred from **NADH+H⁺ → O_2** via multisubunit inner membrane **complexes I, III & IV**, plus **CoQ** & **Cytochrome c**.

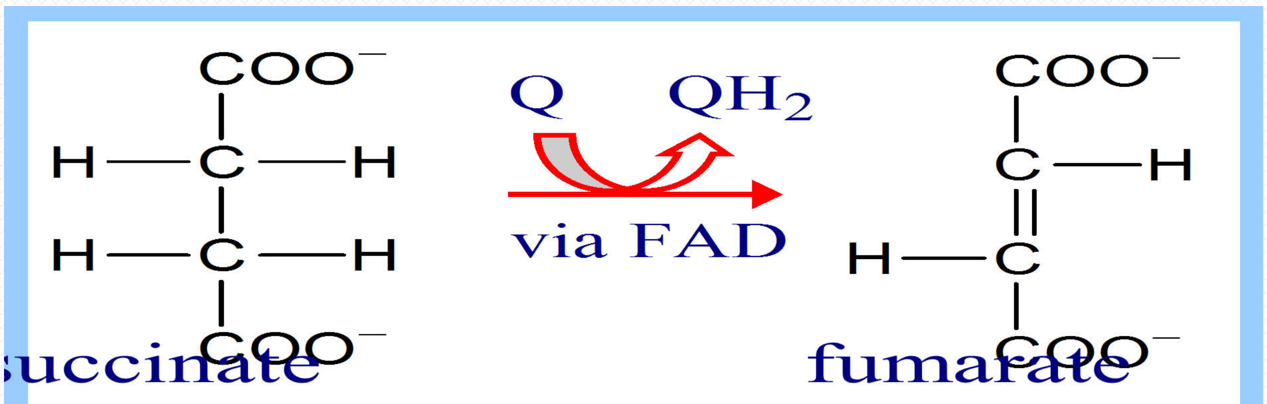
- Within each complex, electrons pass sequentially through a series of electron carriers.

Complex I catalyzes oxidation of NADH+H^+ with reduction of coenzyme Q:



NADH + H^+ + FP → NAD^+ + FPH_2

Coenzyme Q accepts 2 e^- and picks up 2 H^+ from FPH_2 to yield the fully reduced **QH_2**.



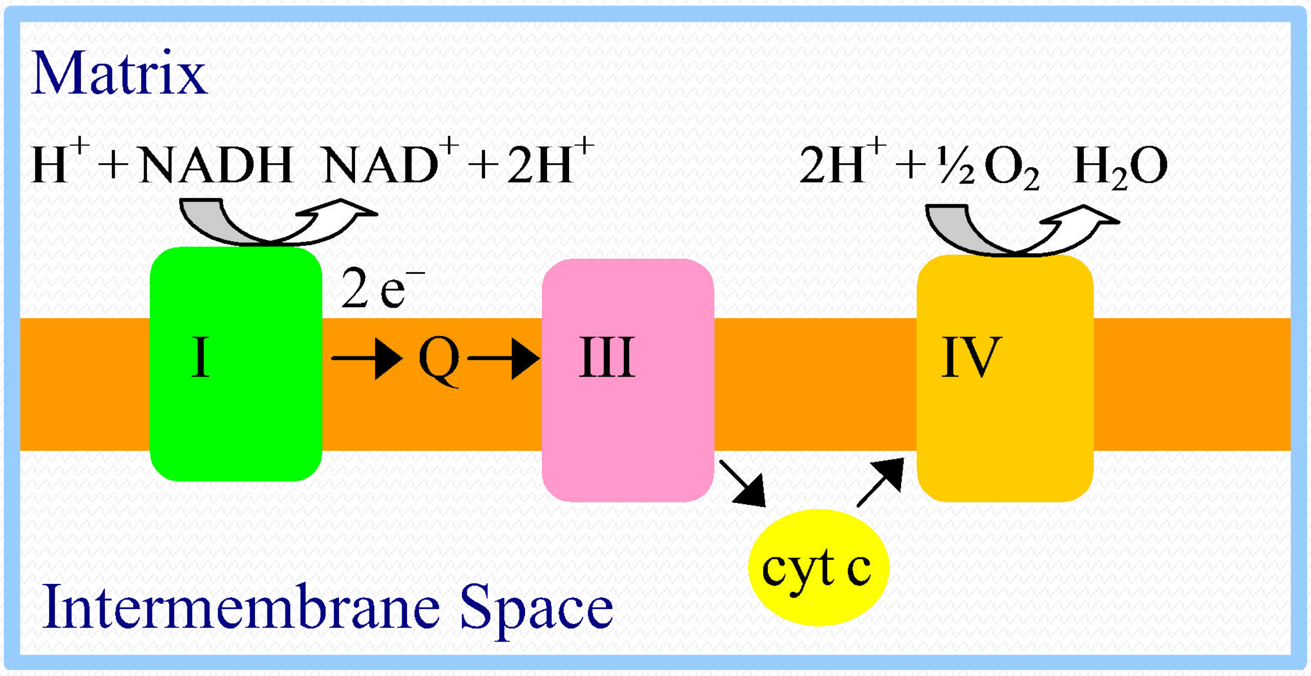
Succinate Dehydrogenase
(Complex II)

- **Succinate Dehydrogenase** of the Krebs Cycle is also called **complex II** or **Succinate-CoQ Reductase.**
- **FAD** is initial e^- acceptor.
- FAD is reduced to **$FADH_2$** during oxidation of Succinate to Fumarate.

- **$FADH_2$** generated by Succinate Dehydrogenase reaction gets **reoxidized** by **transfer** of **electrons** through a series of 3 iron-sulfur centers **to CoQ**, yielding Co**QH_2**.

- **QH_2** product may be reoxidized via **complex III.**

- Providing a pathway for transfer of electrons from Succinate into respiratory chain.



Complex III/ Cytochrome b-c1 complex accepts electrons from coenzyme **QH_2** that is generated by electron transfer in complexes I & II.

- **Cytochrome c** resides in intermembrane space.
- It alternately binds to complex III or IV during e$^-$ transfer.

Significance Of ETC

- **Reduced coenzymes gets reoxidized** to NAD^+ /FAD in ETC for its reutilization in metabolic oxidation reactions.
- Reduced coenzymes NADH+ H^+/$FADH_2$ give its reducing equivalents to E.T.C components and get reoxidized.
- **E.T.C generates chemical form of energy ATP** as a valuable by product.

P/O Ratio

- Ratio of ATPs formed per Oxygen reduced

OR

- Number of ATPs generated per Oxygen atom used in ETC process.

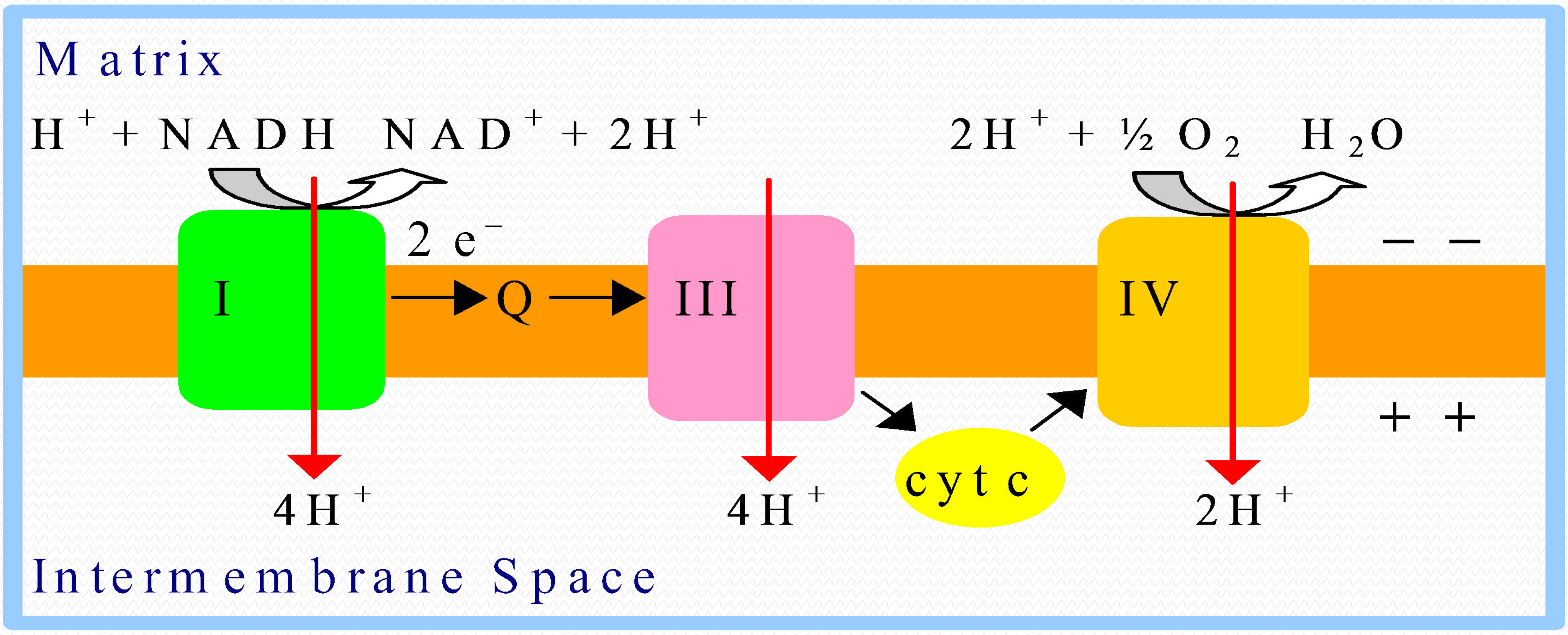
- To make 1 ATP need **30 kJ/mole**

- There needs more than one proton to translocate during ETC process to generate 1 ATP.

- **Ten protons** are pumped out of the matrix during the two electrons flowing **from NADH+H⁺ to O2 (Complex I, III and IV).**

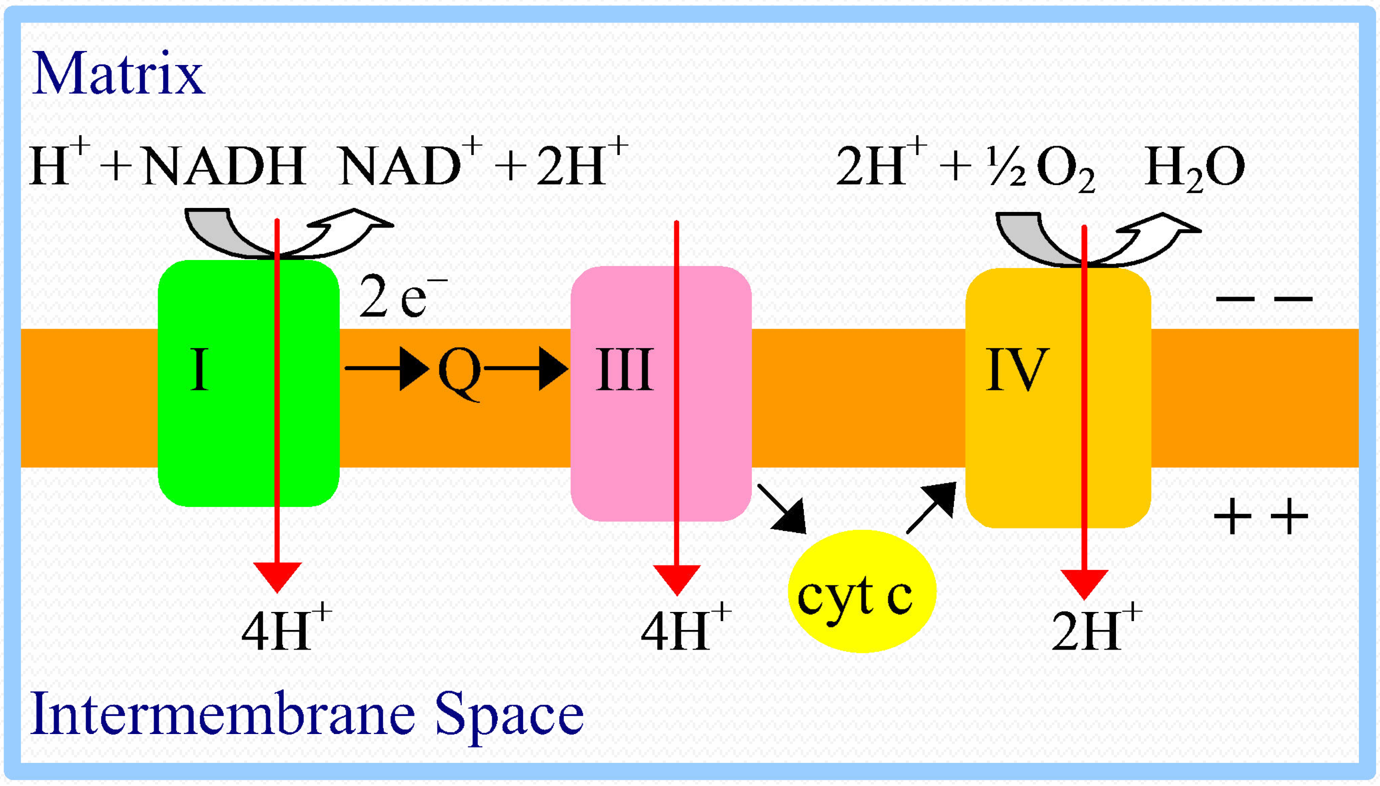
- **Six protons** are pumped out of the matrix during the two electrons flowing from **FADH2 to O2 (Complex III and IV).**

Spontaneous **electron flow** through each of complexes I, III, & IV is **coupled to H^+ ejection from matrix to intermembrane Space**

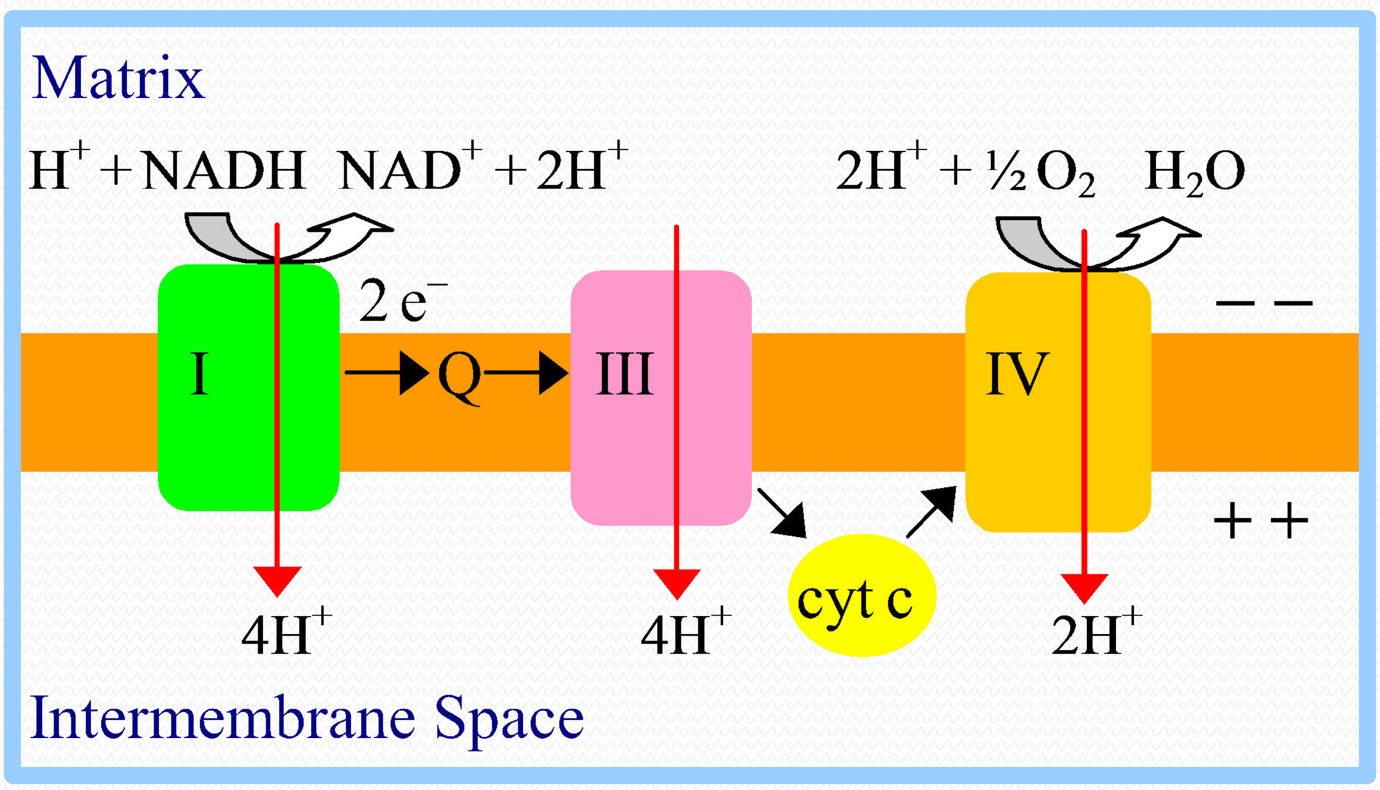


A total of 10 H^+ are ejected from the mitochondrial matrix **per 2 e^-** transferred **from NADH** to oxygen via the respiratory chain.

A total of 6 H^+ are ejected from the mitochondrial matrix **per 2 e^-** transferred **from FADH2** to oxygen via the respiratory chain.

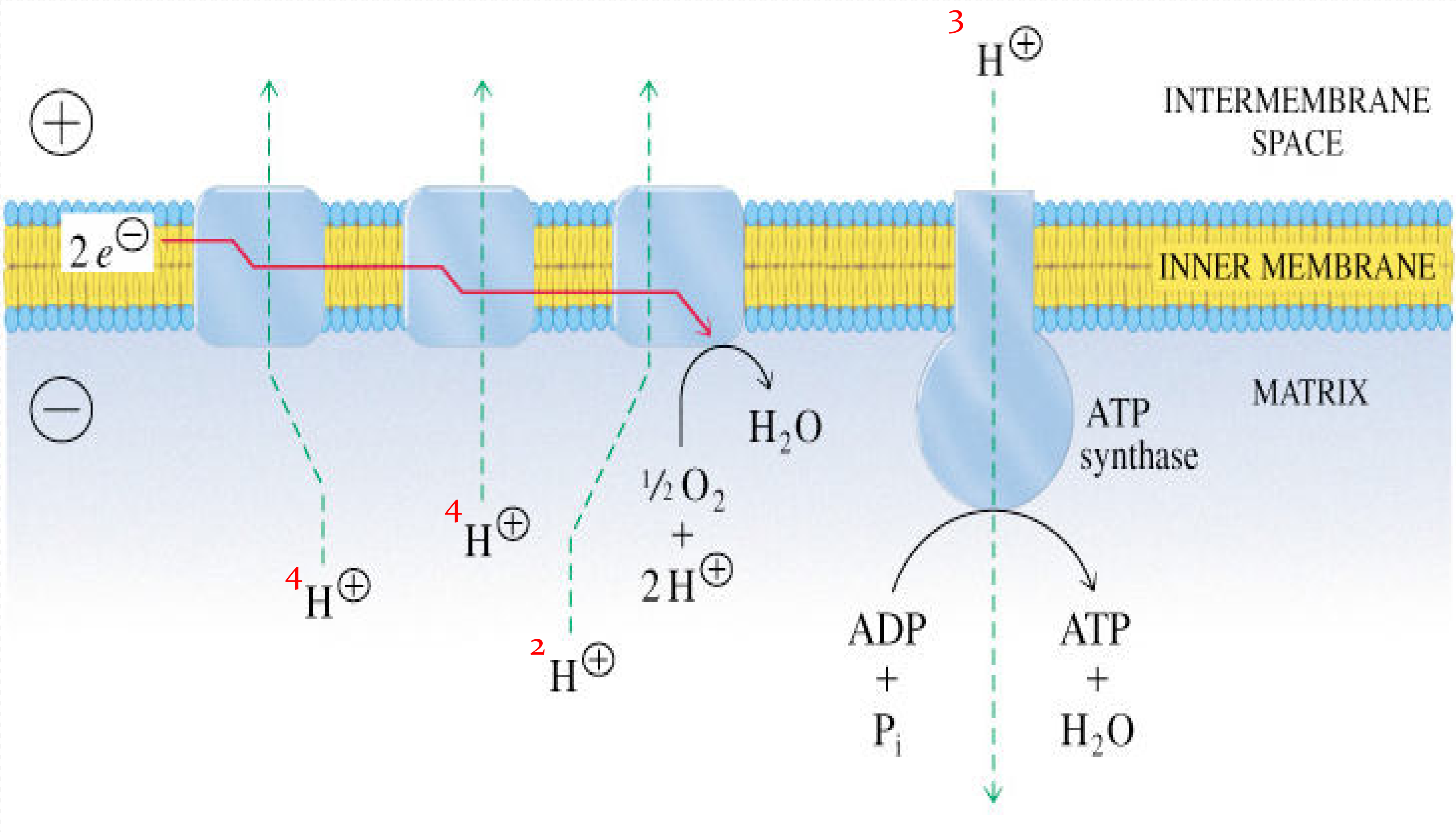


Complex I and **Complex III** transports **$4H^+$** out of the mitochondrial matrix per **$2e^-$** transferred from NADH.



Thus there are **$2H^+$** per $2e^-$ that are effectively transported by **complex IV.**

ATP Yield

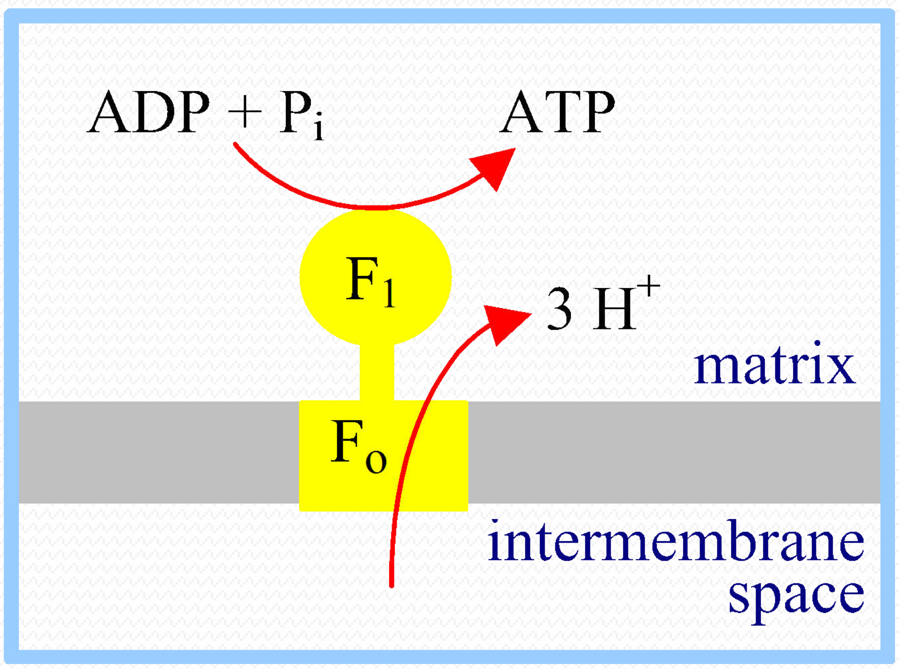


- **10 protons** translocates per **NADH+H⁺**
- **6 protons** translocates per **FADH2**

- **Proton gradient and Proton motive Force created** as electrons transferred to Oxygen forming water

10 H^+ / NADH+H^+

6 H^+ / $FADH_2$



F_1F_o couples ATP synthesis to H^+ transport into the mitochondrial matrix.

Transport of least **3 H^+** per ATP is required.

- **Translocation of 3H^+** required **by ATP Synthase for each ATP produced**
- 1 H^+ needed for transport of Pi.
- **Net: 4 H^+ transported for each ATP synthesized through ATP Synthase.**

P:O Ratio for NADH+H^+

$$10\ H^+ \times \frac{1\ ATP}{4\ H^+} = 2.5\ ATP$$

P.O Ratio for $FADH_2$

$$6\ H^+ \times \frac{1\ ATP}{4\ H^+} = 1.5\ ATP$$

- P:O ratio for NADH: 10 H^+/4H^+ = 2.5 ATP

- P:O ratio for $FADH_2$: 6 H^+/ 4H^+ = 1.5 ATP

ATP Is A Valuable Byproduct Of Oxidative Phosphorylation

- ATP is a **high energy phosphate compound**
- **Biologically important free nucleotide**

ATP has Two High Energy Phosphate Anhydride Bonds

- ATP is energy currency of cell.
- Predominantly generated through Oxidative Phosphorylation.

Sites Of ATP Production In ETC

- **3 sites Of ATP Generation in ETC**
- **Site I/Complex I-**

Electrons transferred from Complex I to CoQ

- **Site II/Complex III-**

Electrons transferred from Cyt b to Cyt c_1.

- **Site III/Complex IV-**

Electrons transferred from Cytochrome aa_3/Complex IV/Cytochrome Oxidase to ½ O_2

Thus ATP Generation
Is Due To Transformation Of
Heat Energy Into Chemical Form Of Bond Energy

Which Satisfy Law Of Thermodynamics
Energy Is never Destructed
Energy Is Transformed From One Form To Another
From One System To Another
One Body To Another

Significance OF ATP

- ATP allows coupling of **thermodynamically unfavorable reactions to favorable reactions**.

- **Uses of ATP generated in Oxidative Phosphorylation**
  - **Synthetic/Anabolic reactions**
  - **Active transport mechanism.**
  - **Muscular contraction**
  - **Nerve impulse conduction.**

- ATP is continually being hydrolyzed and regenerated
- A person at rest consumes and regenerate 3 ATP/ sec

Staying Alive Energy Wise

- We need 2000 Cal/day or 8,360 kJ of energy per day
- Each ATP gives 30.5 kJ/mole of energy on hydrolysis
- **We need 246 moles of ATP**
- Body has less than 0.1 moles of ATP at any one time
- We need to make 245.9 moles of ATP
- Each mole of Glucose yields 38 ATPs or 1160 kJ
- We need **7.2 moles of Glucose** (1.3 kg or 2.86 pounds)
- Each mole of Stearic acid yields 147 ATPs or 4,484 kJ
- We need **1.86 moles of stearic acid** (0.48 kg or 1.0 pound of fat)

Remember

- **CoQ accepts** electrons and Protons by **complexes I and II**
- **Acceptance of Protons and Electrons from Complex II by CoQ does not generate ATP**

E.T.C is a Mode For Free Radical Generation

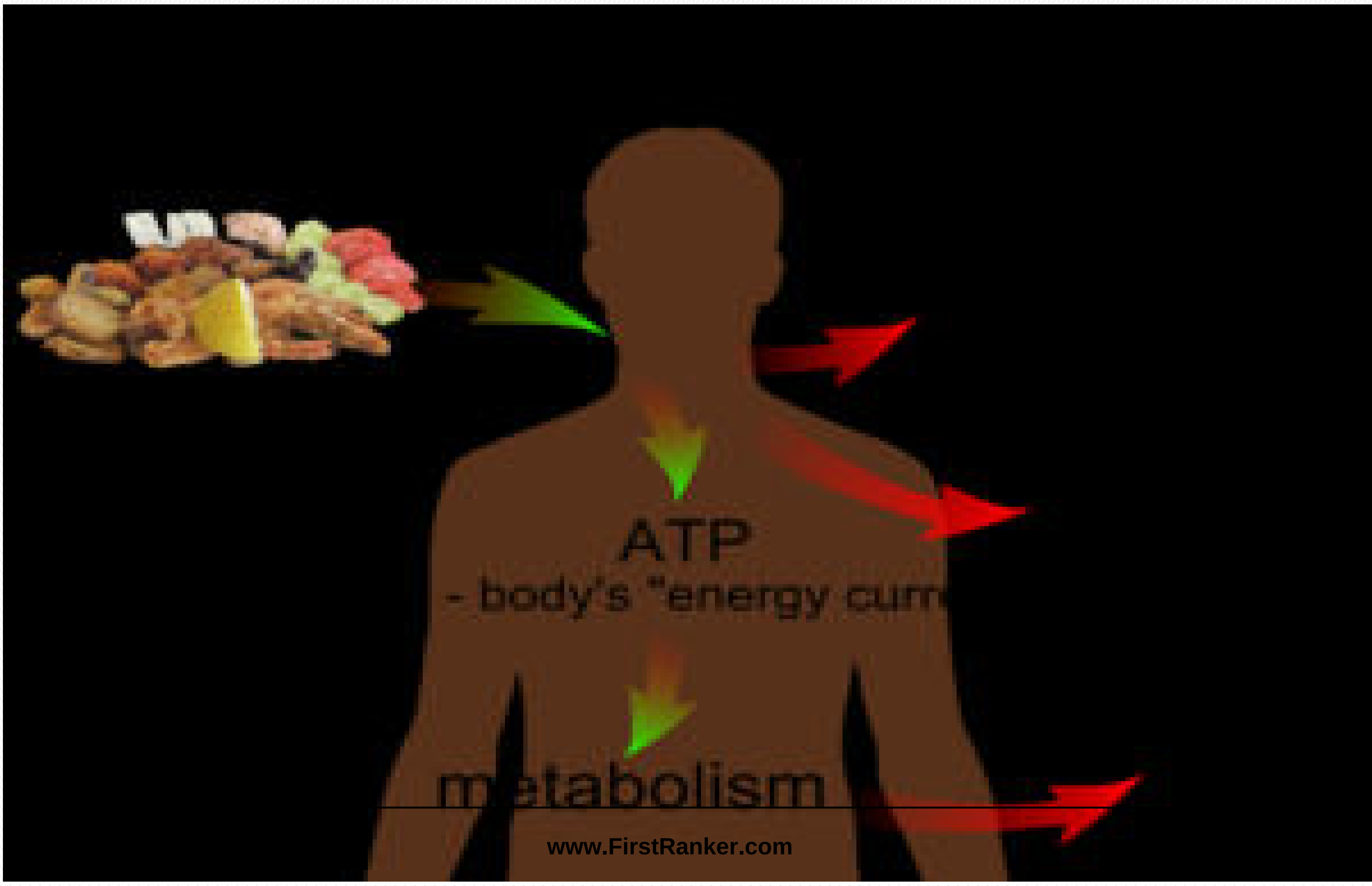
- During E.T.C operation there occurs leakage of small amounts of electrons

- Which are transferred directly to oxygen to form super oxide ion (Free radicals/ROS)

What is a Free Radical ?

- Any chemical species with one of more unpaired electrons
- Unstable/Highly Reactive to get stabilized
- Powerful Oxidant
- Short half life (nanoseconds)
- Can exist freely in environment

ETC



Factors For Universal Metabolism

- Nutrition
- Environment
- Life Style Habits

- **Factors Associated To ETC**

- **Metabolites- Carbs ,Proteins , Lipids**
- **Vitamins , Minerals and Antioxidants**
- **Oxygen Concentration**
- **Respiration Process**
- **Hemoglobin Structure and Function**
- **Mitochondrial DNA**

REGULATORS OF OXIDATIVE PHOSPHORYLATION

Important Direct Substrates

Regulators Of Oxidative Phosphorylation and ATP Generation

- NADH/$FADH_2$
- O_2
- ADP and pi

Indirect Substances Involved

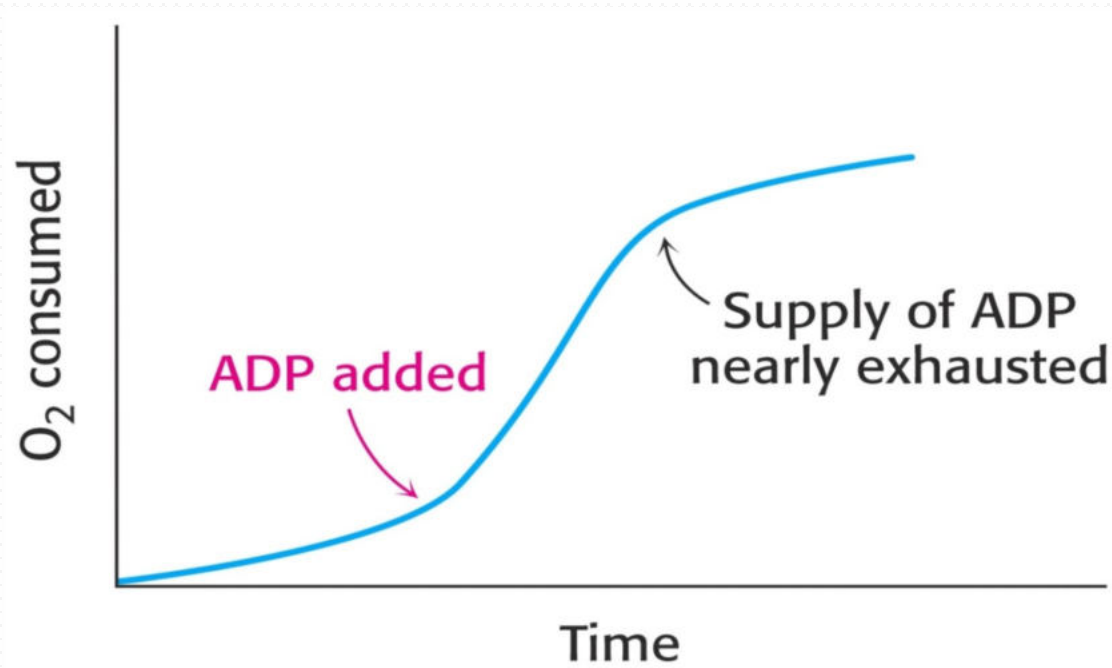
- Glucose
- Fatty acids
- Insulin
- Amino acids and Proteins
- Iron
- Vitamin C
- Vitamin B Complex members- Niacin, Riboflavin

ATP/ADP Ratio Regulates Mechanism Of Oxidative Phosphorylation

Respiratory Control

The most important factor in determining the rate of oxidative phosphorylation is the level of ADP.

The regulation of the rate of oxidative phosphorylation by the ADP level is called respiratory control



- ADP and pi is required for ETC process.

- **Intramitochondrial ratio ATP/ADP is a control mechanism**

- At high ATP/ADP ratio
- **ATP acts as an allosteric inhibitor** for **Complex IV** (Cytochrome Oxidase)
- Inhibition is reversed by increasing ADP levels.

- **ADP levels** reflect rate of **ATP consumption and energy state of the cell.**

- **At low ADP levels – Low oxidative phosphorylation**

- Electron transport is tightly coupled to phosphorylation.
- ATP cannot be synthesized by oxidative phosphorylation unless there is heat energy released from electron transport.
- Electrons do not flow through the electron-transport chain to O_2 unless ADP is phosphorylated to ATP.

Inhibitors OF ETC Complexes

OR

Inhibitors Of Oxidative Phosphorylation

ETC Complexes Inhibitors
OR
Interruptors of Oxidative Phosphorylation Mechanism

- **Enemies/Distractors of ETC components who stop its normal** operation.
- **Block ETC operation** and stop **ATP generation.**

ETC Complexes Inhibitors

- Chemical compounds **having affinity for ETC components/complexes**
- Chemically **interact with ETC complexes, bind** and **inactivate them**
- **Affects** normal **functional operation of ETC**
- **Low/No ATP production**
- **Cessation of cellular activities**

- **Complex I/Site I - E.T.C Inhibitors**

  - ❖ **Amobarbital /Amytal**
  - ❖ **Rotenone (Fish/Rat Poison)**
  - ❖ **Mercurials**
  - ❖ **Piercidin –A**

  (Volatile Anesthetics)

  - ❖ **Halothane (Malignant Hyperthermia)**
  - ❖ **Fluothane**
  - ❖ **Isoflurane**
  - ❖ **Sevoflurane**

- **Complex III/ Site II -E.T.C Inhibitors**

  - ❖ **British Anti Lewisite (BAL)**
  - ❖ **Antimycin –A**
  - ❖ **Dimercaprol**

- **Complex IV/Site III /Cytochrome Oxidase Inhibitors :**
  - Cyanide
  - Carbon Monoxide
  - H_2S
  - Azide

- Cyanide is most potent inhibitor of ETC
- It binds to Fe^{3+} of cytochrome oxidase blocking mitochondrial respiration leading to cell death.
- Cyanide poisoning causes death due to tissue asphyxia (mostly of CNS)

Complex V Inhibitors

ATP Synthase Inhibitors

- **Oligomycin**

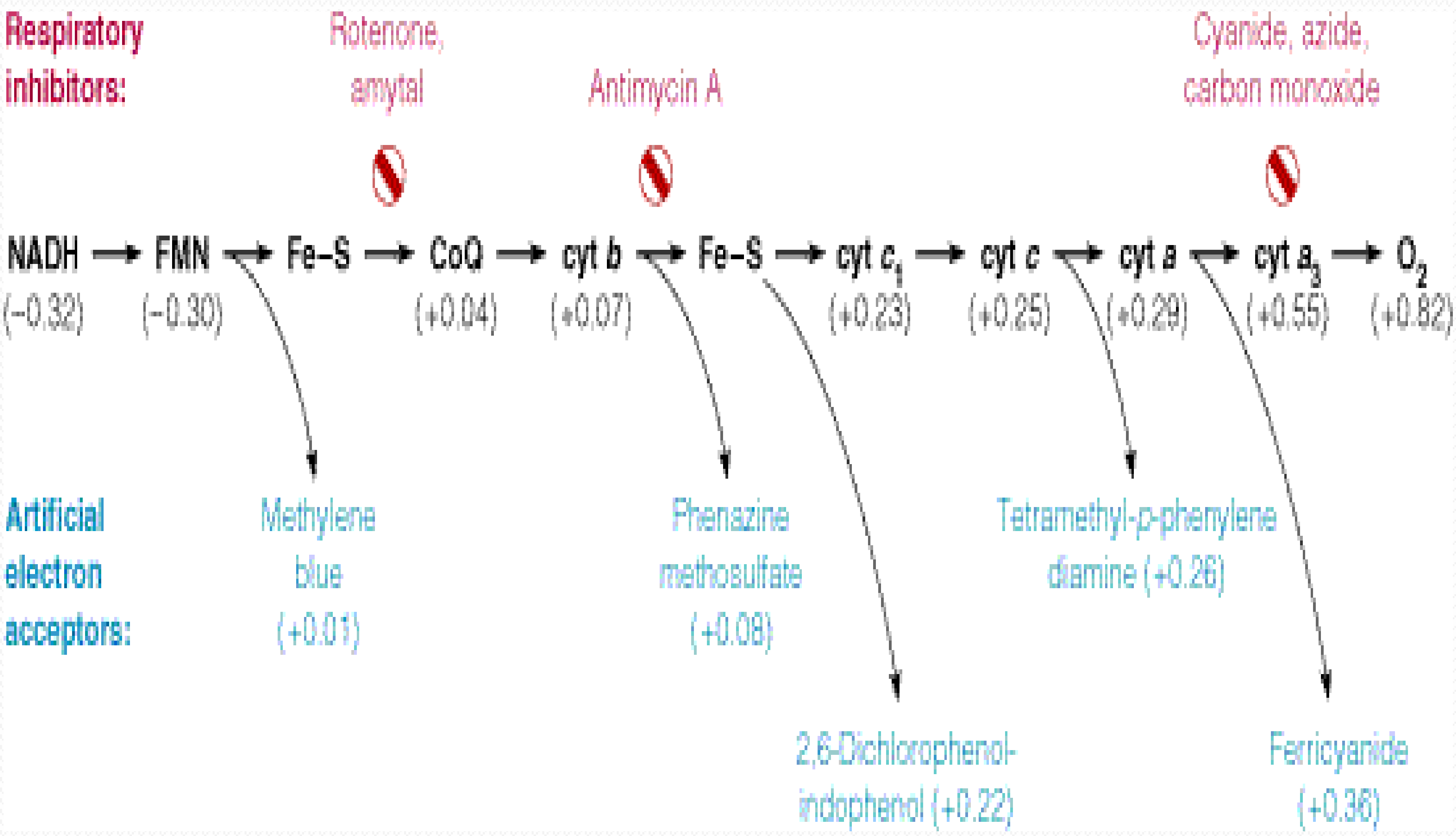
- **Fo particle** of ATP Synthase serve as **proton channel**
- An antibiotic Oligomycin binds with **Fo particle of ATP Synthase**
- **Do not translocate Protons through it.**
- **Inhibits activation of ATP Synthase phosphorylation of ADP to ATP.**

- **Atractyloside**

- A **Glycoside prevents translocation of ADP** across mitochondrial membrane.
- Make it **unavailable for phosphorylation reaction**

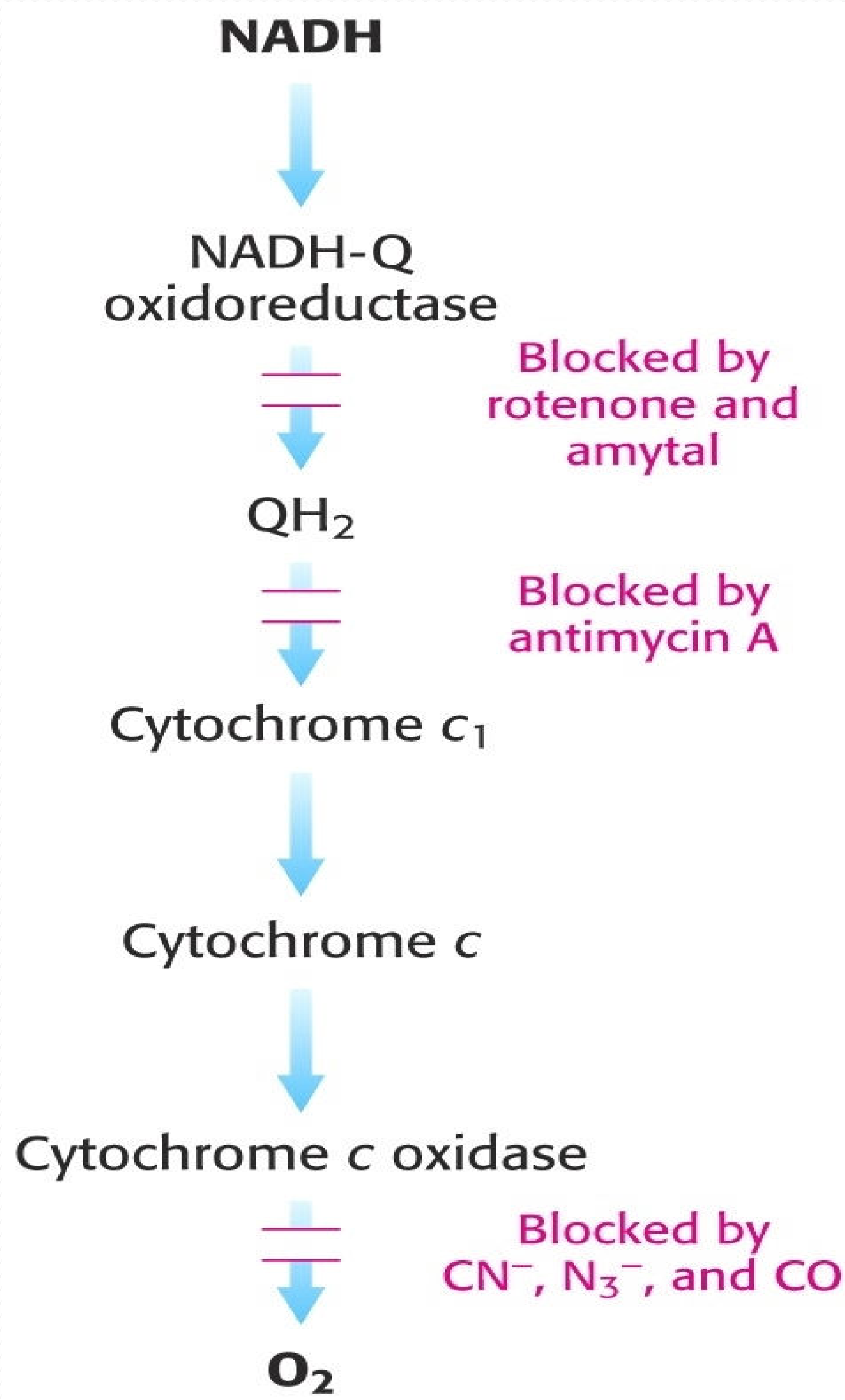
- **Bongregate**
- Pseudomonas toxin has inhibitory action **similar like Atractyloside.**

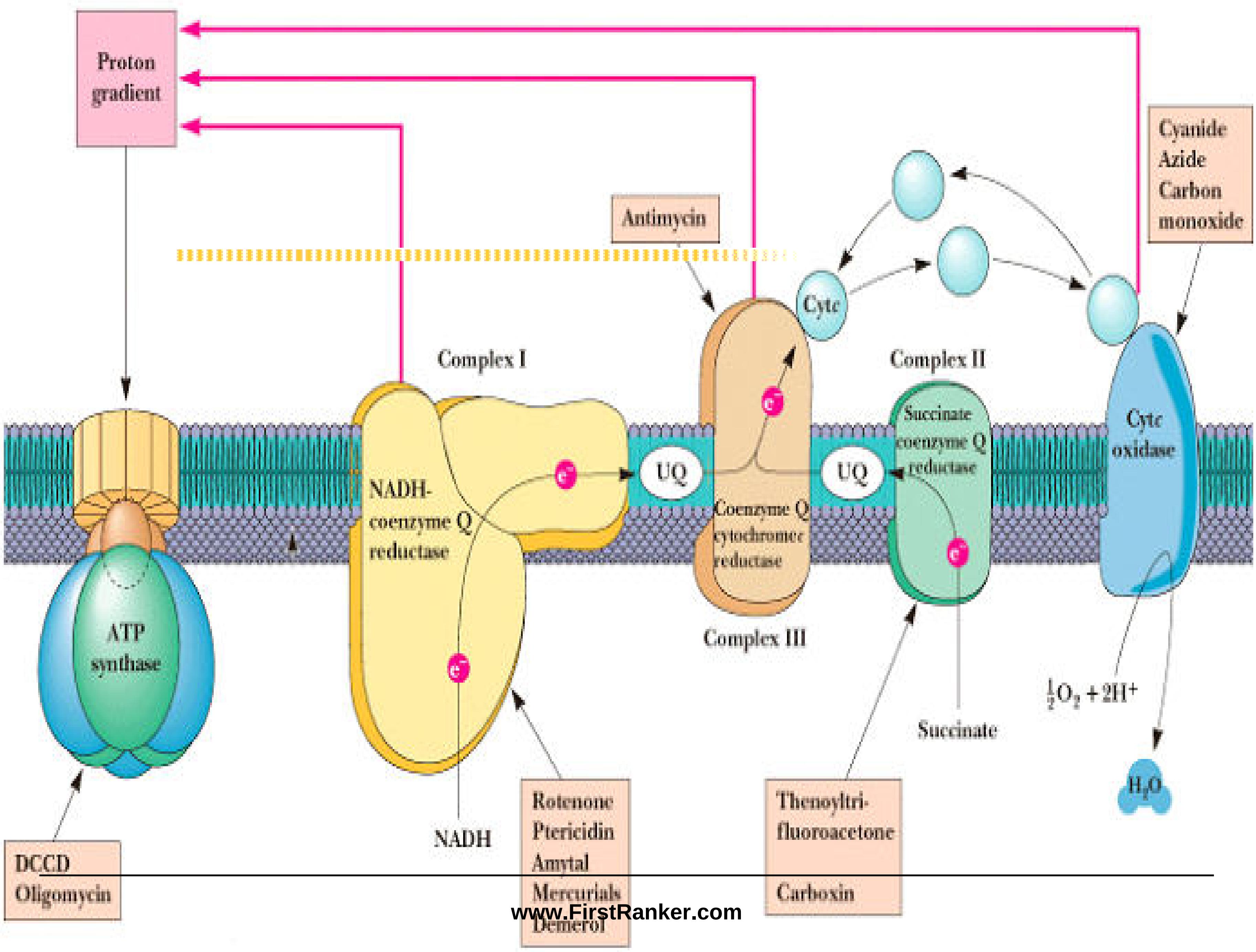
Artificial Electron Acceptors/ Distractors Of ETC



- These chemicals arrest respiration by inhibition of ETC complexes

Specific inhibitors of Electron Transport Chain and ATP-Synthase





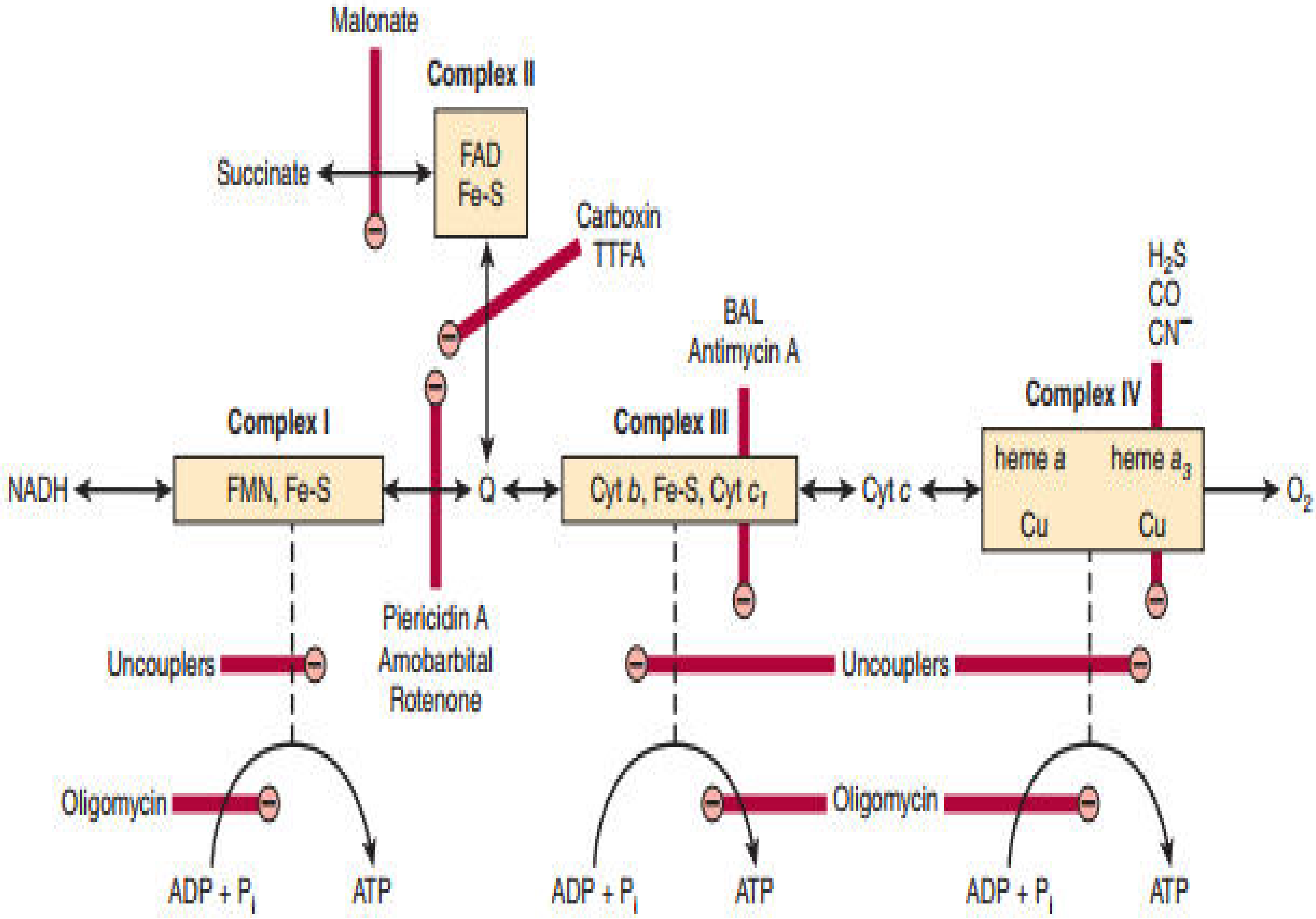


FIGURE 13–9 **Sites of inhibition (⊖) of the respiratory chain by specific drugs, chemicals, and antibiotics.** (BAL, dimercaprol; TTFA, an Fe-chelating agent. Other abbreviations as in Figure 13–5.)

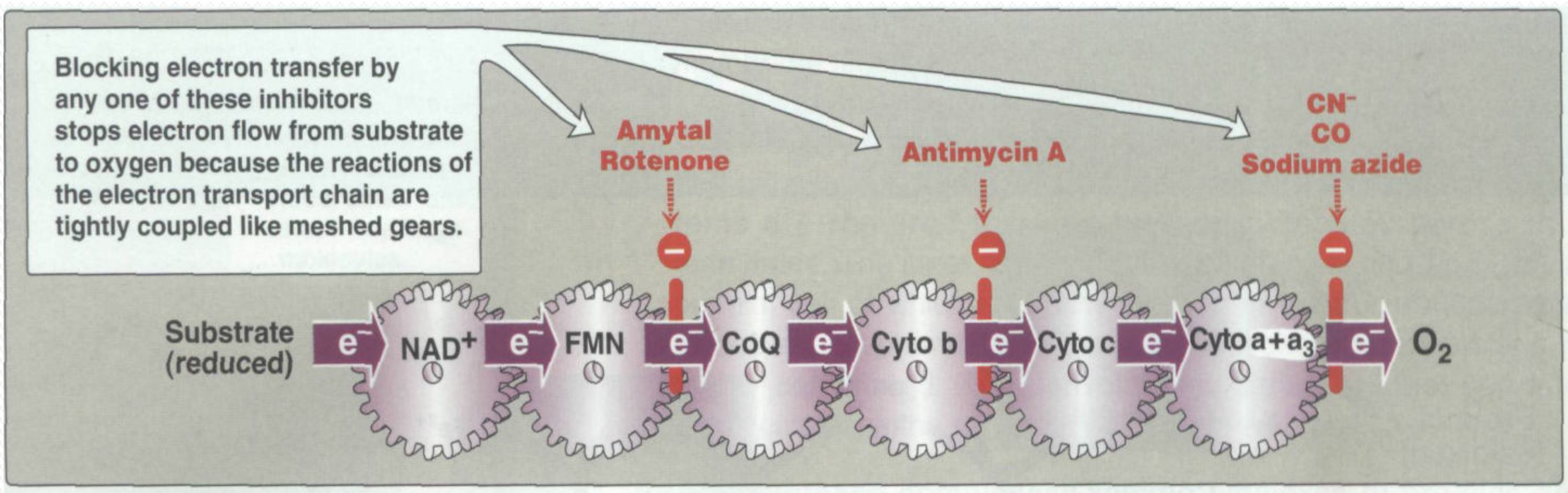
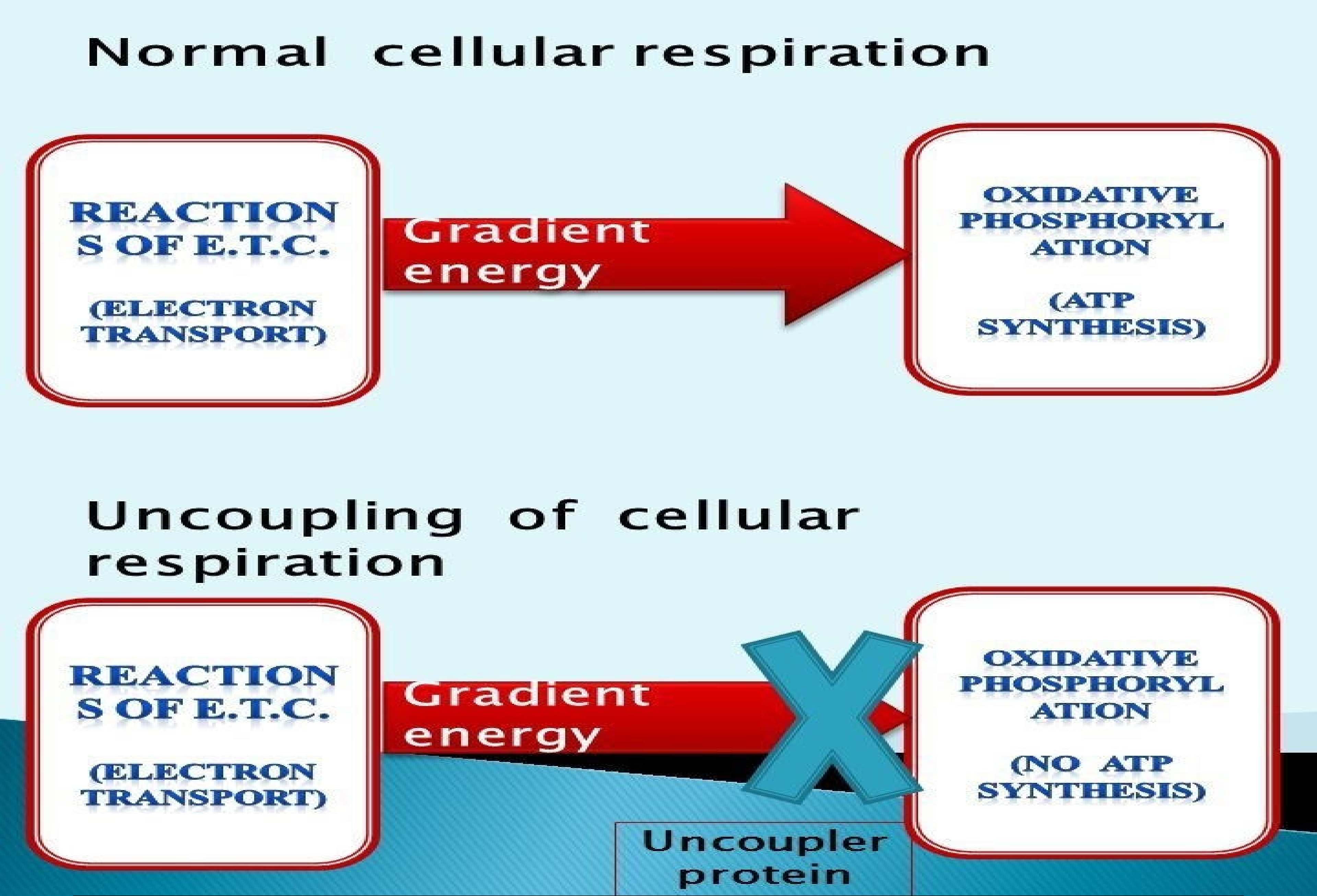


Figure 6.10
Site-specific inhibitors of electron transport shown using a mechanical model for the coupling of oxidation-reduction reactions. [Note: Figure illustrates normal direction of electron flow.]

Uncouplers Of Oxidative Phosphorylation

What are Uncouplers?

- Uncouplers are **chemical agents**
- Uncouplers are mostly **lipid soluble aromatic weak acids**
- They **Uncouple/Delink two tightly coupled natural processes**
  - **E.T.C** (Oxidation) **uncoupled from Phosphorylation (ATP generation)**
- **They just carry out Oxidation without Phosphorylation**



Uncouplers break the connection between Electron Transport Chain and Phosphorylation

Electron transport is a motor

Phosphorylation is the transmission

Uncouplers put the car in NEUTRAL

Uncouplers Action Illustrates As Total Solar Eclipse

- **Uncouplers just bring oxidation (E.T.C/Sun Rise) without phosphorylation**(Interrupted Sun Light)

- **Uncoupler (Moon In between) inhibits generation of ATP (Dark/No Day)**

Types Of Uncouplers

Physiological Uncouplers

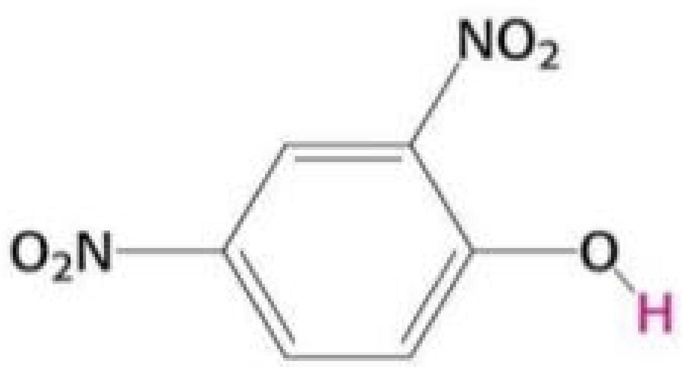
- **Thermogenin /Uncoupling Protein-1**
- **Excess of Thyroxine**
- **Long Chain Fatty acids**
- **Unconjugated Hyperbilirubinemia**

Chemical Uncouplers

- 2,4 Di Nitro Phenol
- Di Nitro Cresol
- Dicumarol
- Aspirin
- *p*-Triflouromethoxy Carbonyl Cyanide Phenylhydrazone (FCCP)
- Valinomycin
- Pentachlorophenol
- Snake Venom-Phospholipases

2,4-dinitrophenol (DNP)

- A small lipophilic molecule
- A protein carrier
- Can easily diffuse through the IMM
- Also used as drug to lose weight
- Due to many side effects FOOD & DRUG ADMINISTRATION has banned this drug

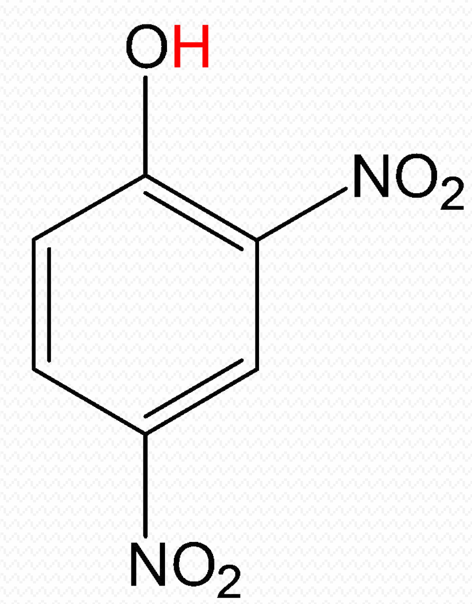


2,4-Dinitrophenol (DNP)

Mode Of Action Of Uncouplers

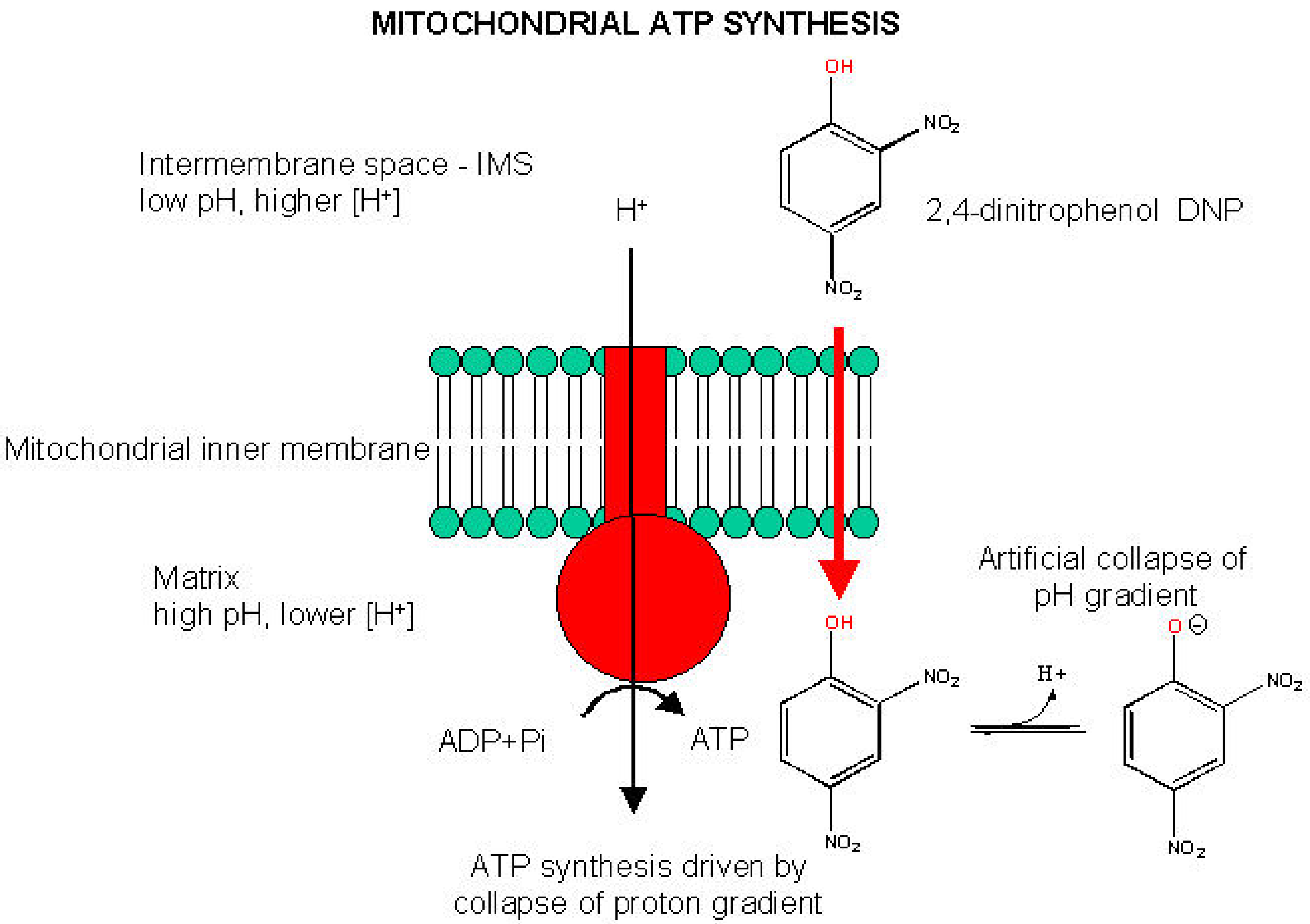
- Certain Uncouplers are **ionophores, lipophilic substances.**
- They **carry protons from intermembrane space across mitochondrial membrane to matrix**
- From **site other than specific site**. (i.e not through Fo and F1 particles of ATP Synthase).

- Certain **Uncouplers changes permeability** of mitochondrial membrane to protons.
- **Translocate protons easily through mitochondrial membrane.**



2,4-dinitrophenol

2,4 DNP dissolve in membrane and function as **carriers for H^+**.



A- Uncouplers

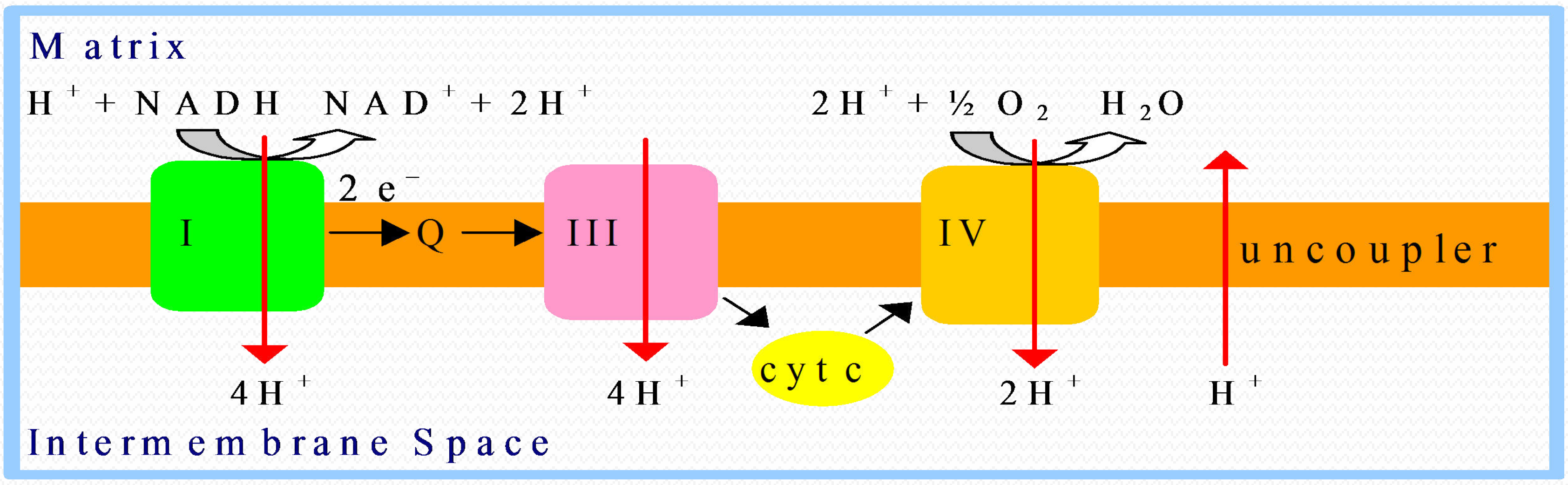
These compounds **abolished the coupling between oxidation and phosphorylation** through increasing the permeability of the IMM

↓

Failure of formation of the electrochemical gradient

↓

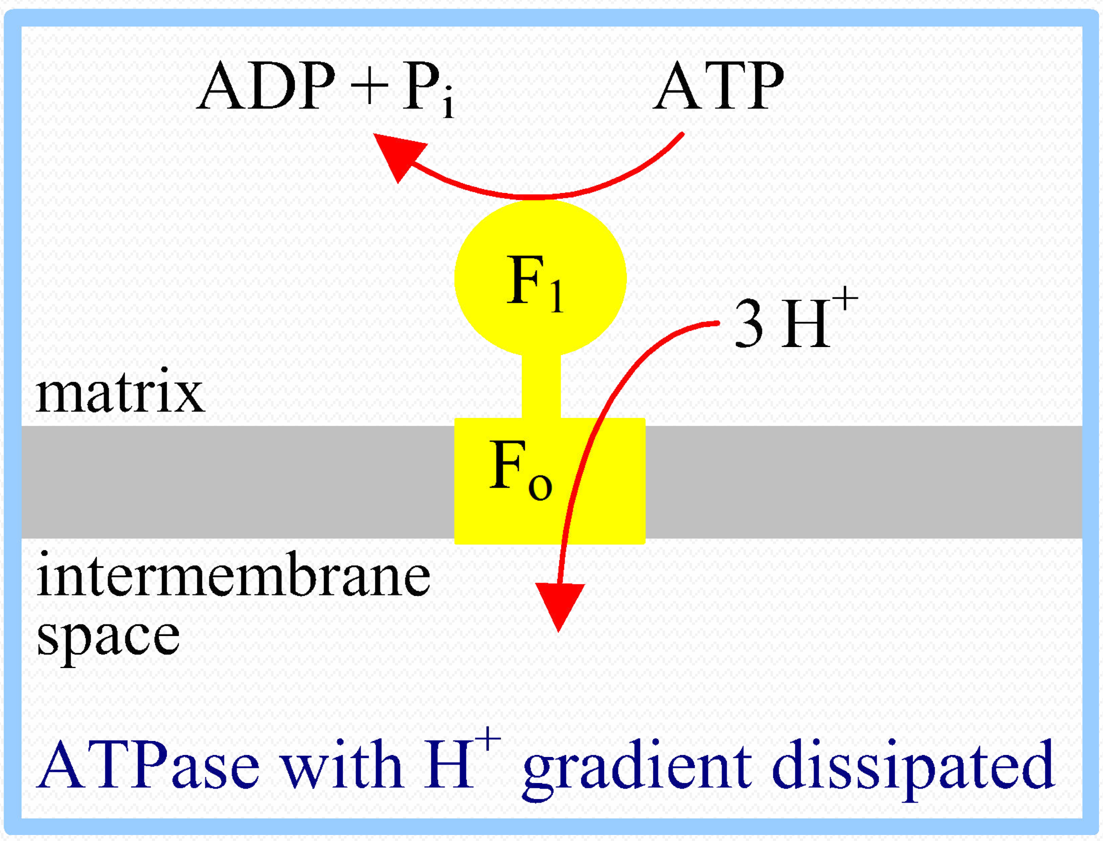
ATP formation stops while oxidation proceeds.



Uncouplers block oxidative phosphorylation by **dissipating H^+ electrochemical gradient**.

Protons pumped out **leak back into mitochondrial matrix,**

preventing development of proton gradient and proton motive force.



ATP Synthase reaction **runs backward** in presence of an **uncoupler**.

- Hydrolysis of ATP is spontaneous.

- Thus Uncouplers by their action **deplete proton gradient of intermembrane space during ETC operation.**

Uncouplers Dissipate More Heat

- **Uncouplers Do not** allow to **develop required proton gradient** and
- Do not form **proton motive force** in the **intermembrane space** of mitochondria
- **No translocation of Protons through ATP Synthase**
- Causes **no stimulation or activation of ATP Synthase**
- **No catalysis of Phosphorylation** of ADP with pi to generate ATP

- **During uncoupling phenomena**

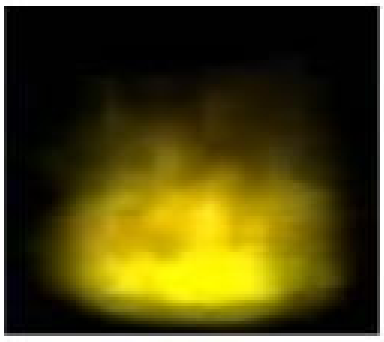
- **Free energy released as Heat energy** more than 7.3 Kcal is not conserved for Phosphorylation reaction dissipiated as it is in form of heat

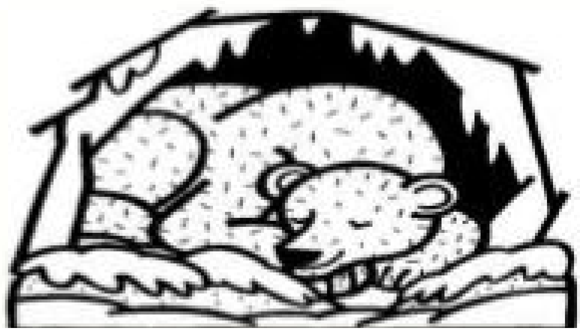
- A very high heat energy released then causes **swelling of Mitochondria** and exhibit malignant hyperthermia.

Physiological Uncoupling
By
Uncoupling Protein (UCP-1)

Thermogenine (UCP1):

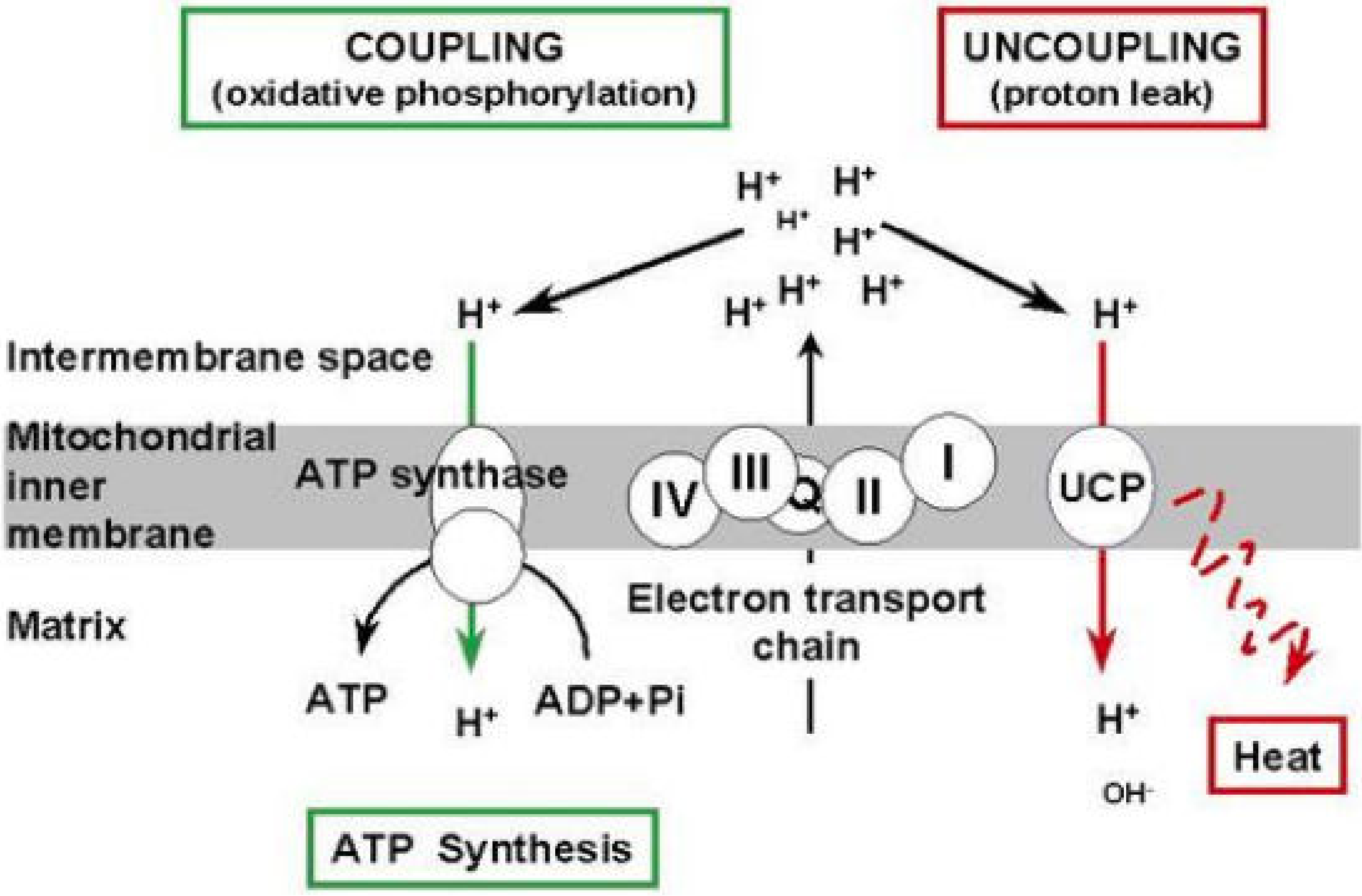
- It is considered as a physiological uncoupling protein.
- It is present in the brown adipose tissue of newly born, some people and hibernating animals.
- It allows protons to pass the mitochondrial matrix without passing F0-F1 complex.
- No ATP is formed and energy is released in the form of heat.



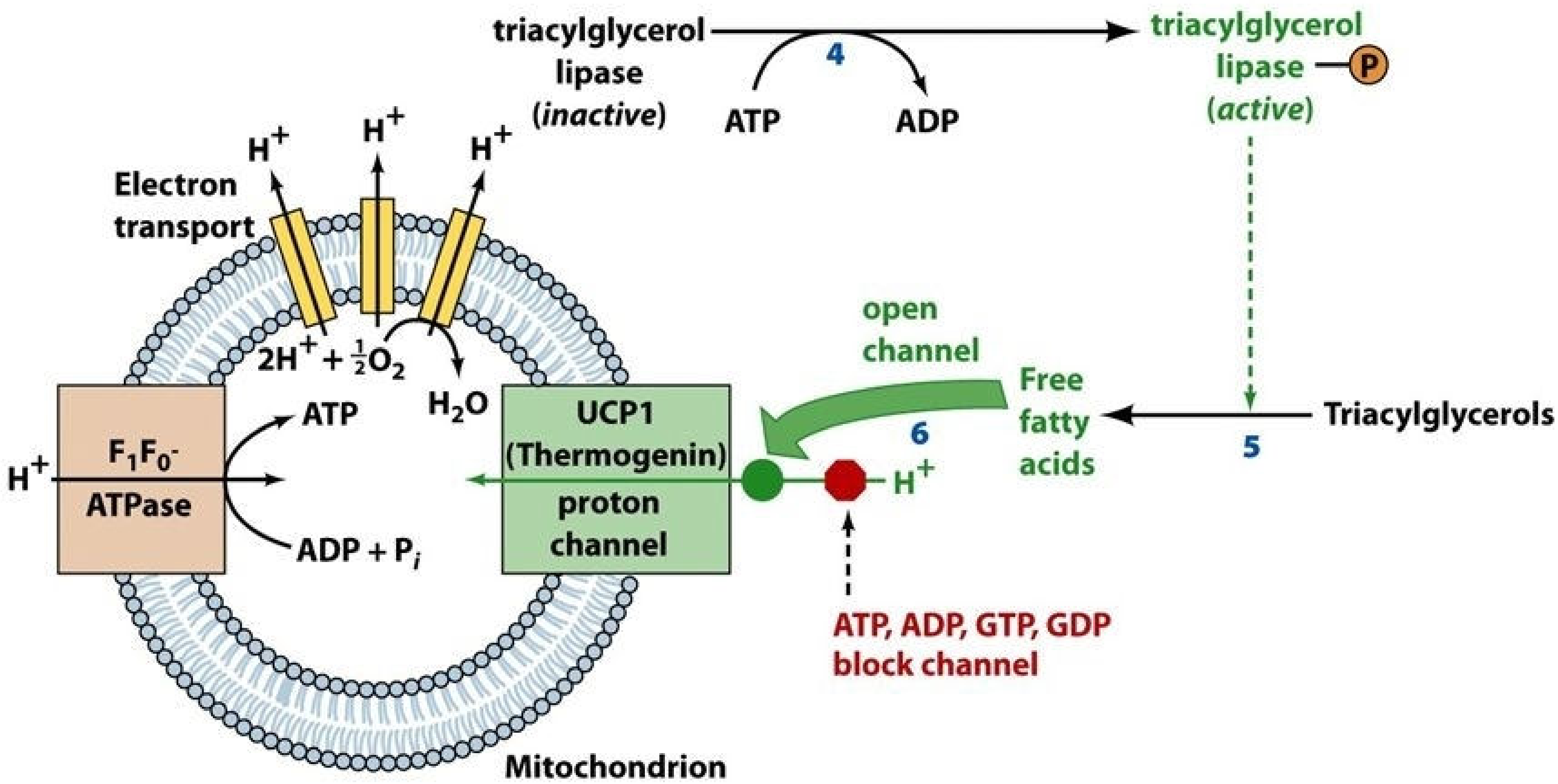


How UCP works

Collins Figure 1

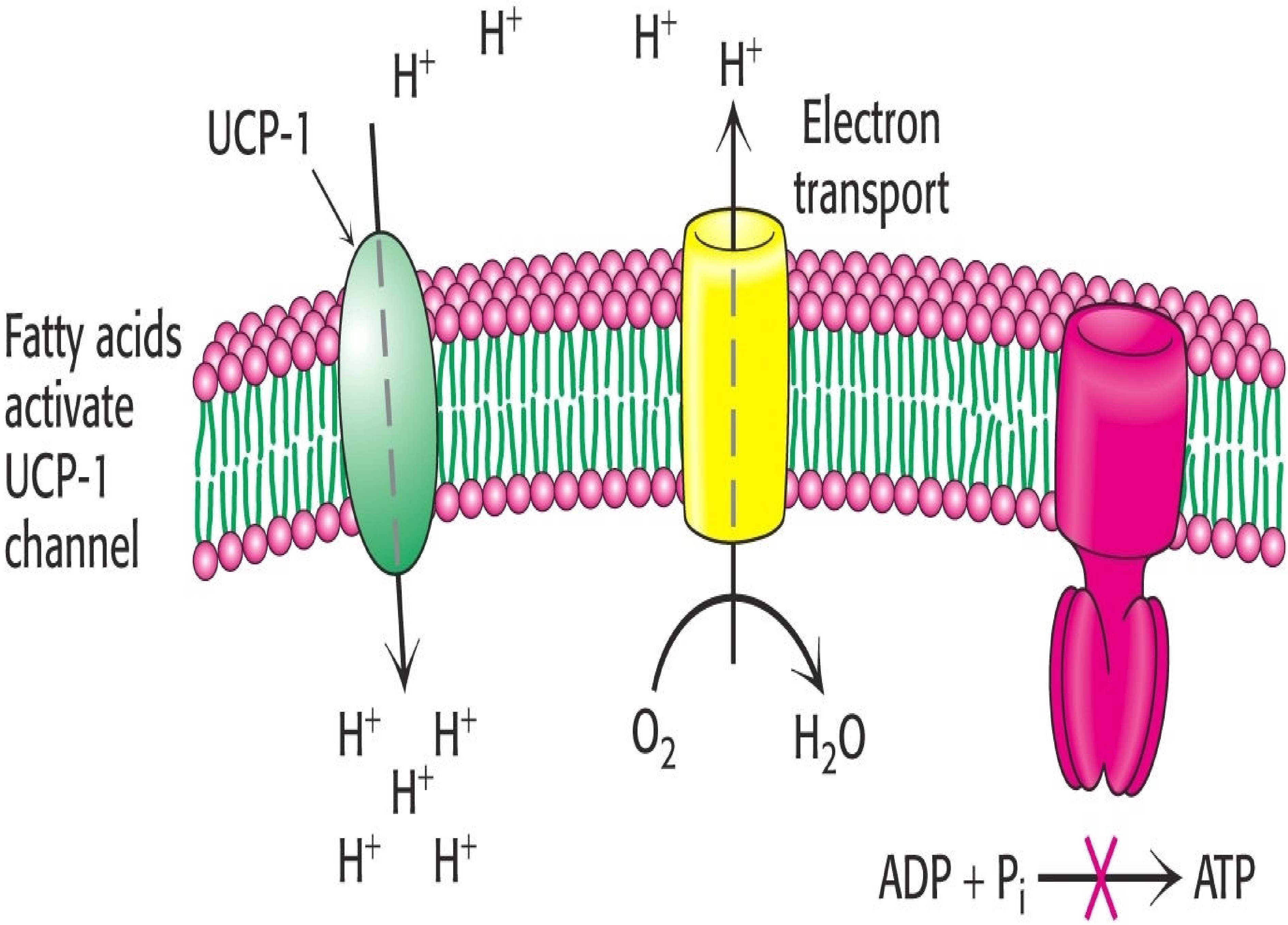


Mechanism of Hormonally-Induced Uncoupling of Oxidative Phosphorylation in Brown Adipose Tissue



- An **Uncoupling Protein** (**UCP-1**)/ **Thermogenin** is produced in **brown adipose tissue** of **newborn mammals** and **hibernating mammals**.

- This UCP-1 protein of an inner mitochondrial membrane functions as a **H^+carrier**.



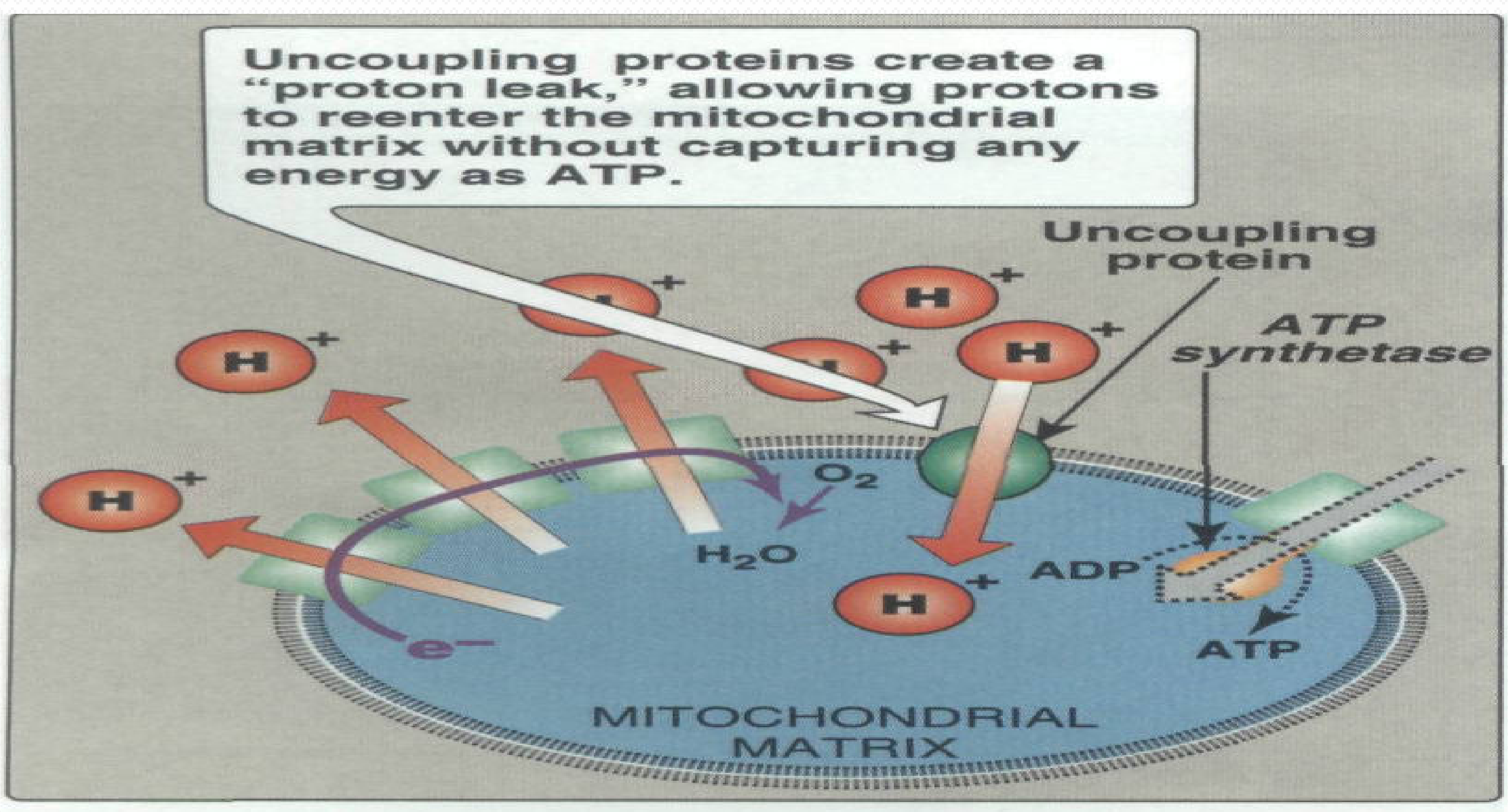


Figure 6.14
Transport of H^+ across mitochondrial membrane by 2,4-dinitrophenol.

- Uncoupling by UCP-1 **protein blocks development of a H^+ electrochemical gradient**, thereby stimulating respiration.
- Free energy of ETC is dissipated as heat.

- Uncoupling of ETC and phosphorylation occurs in animals as a means to produce heat
- **Non shivering thermogenesis**
- **Occurs in brown adipose tissues** (rich in mitochondria)

Significance Of Physiological Uncouplers

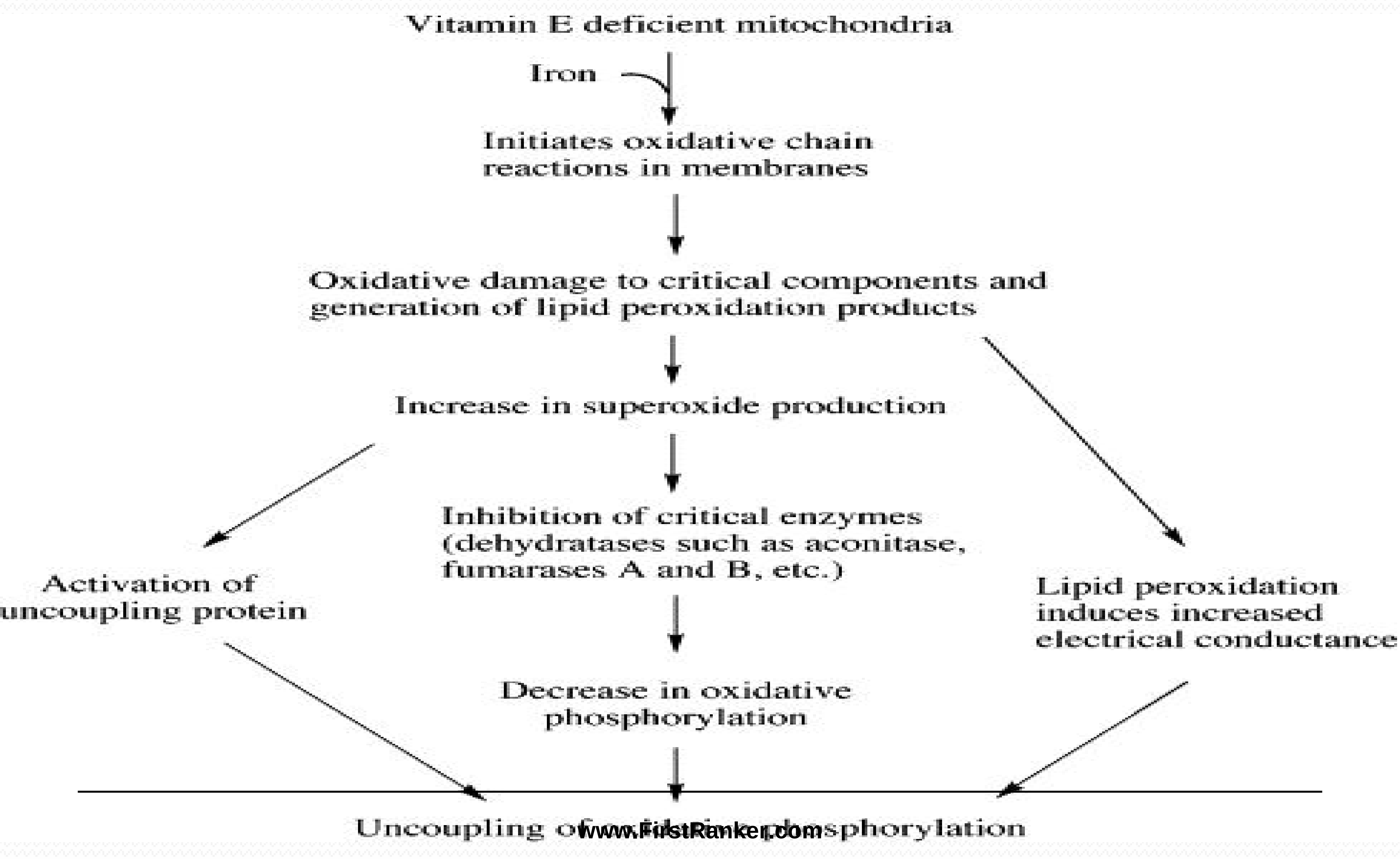
- In **extreme cold conditions** and in hibernating animals
- Physiological Uncouplers bring uncoupling phenomena
- The heat liberated inside body helps to **restore and maintain body temperature.**

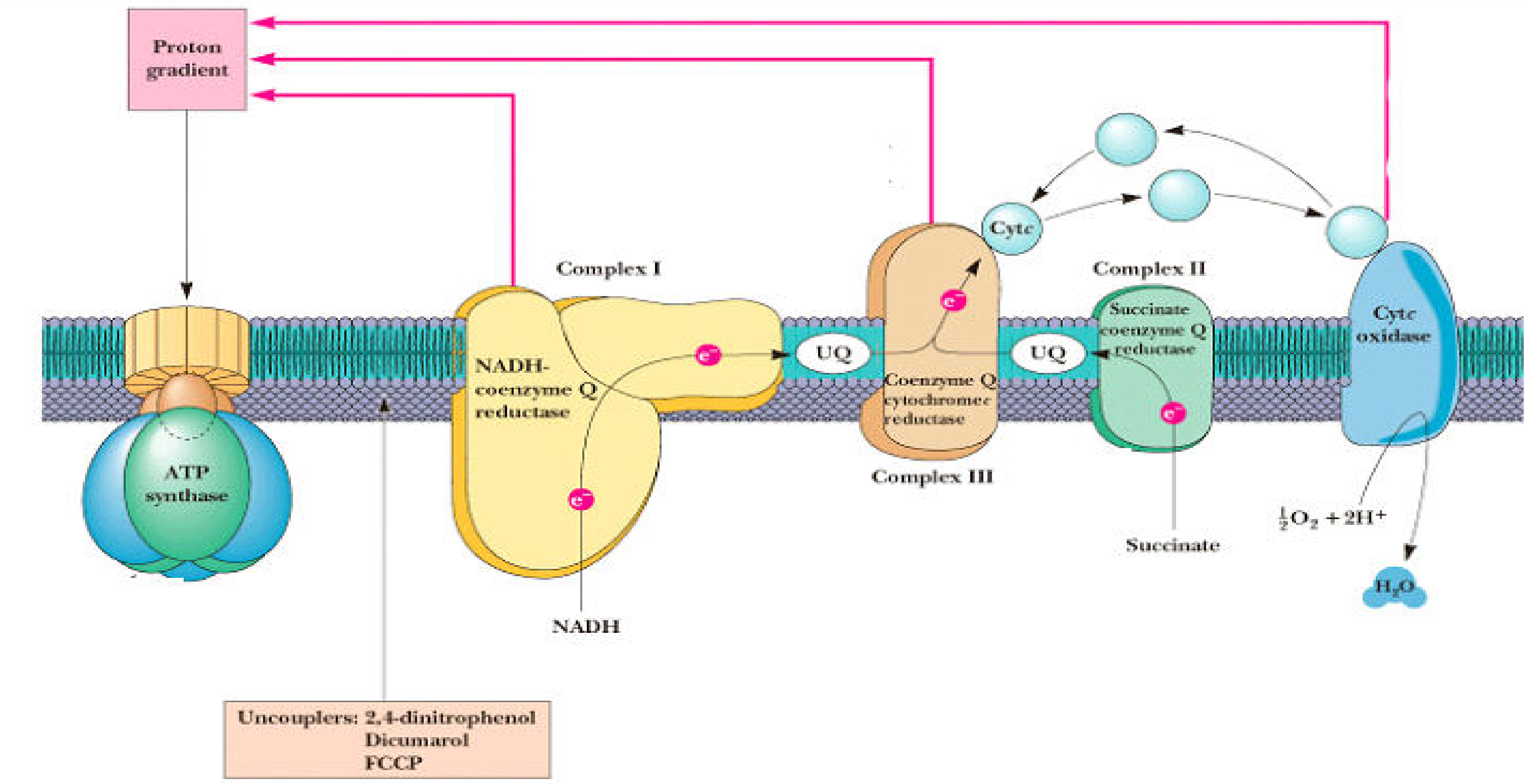
Brown adipose (fat) cells contain natural Uncouplers to warm animals cold adaptation and hibernation.

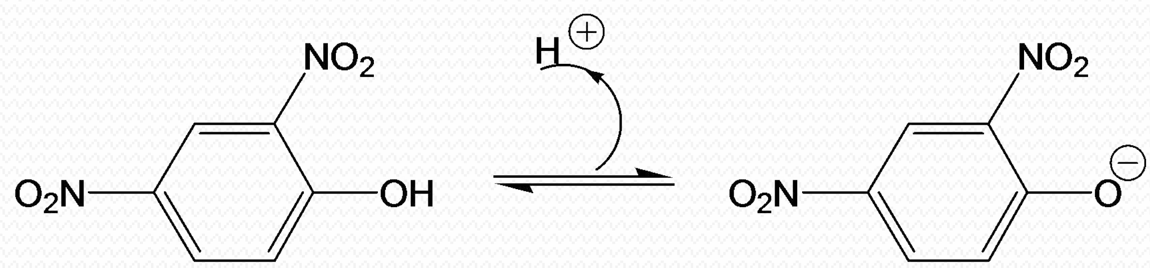
As per the Required condition Of Body

- This "**non-shivering thermogenesis**" **is costly in terms of respiratory energy**
- **Heat energy unavailable for** ATP synthesis
- But provides **valuable warming to an organism.**

Effect Of Poor Antioxidant Activity







ETC Inhibitors and Uncouplers

Table 1. Inhibitors of Respiration and Oxidative Phosphorylation

Site-Specific	Target Complex
Carbon monoxide	IV
Cyanide	IV
Sodium Azide	IV
Rotenone	I
Antimycin A	III
Amytal	I
Phosphorylation	
Oligomycin	F_o
Uncouplers	
2,4-Dinitrophenol (DNP)	Proton gradient
Trifluorocarbonylcyanide Phenylhydrazone (FCCP)	Proton gradient

Any compound that stops electron transport will stop respiration…this means you stop breathing

Electron transport can be stopped by inhibiting ATP synthesis

An uncoupler breaks the connection between ATP synthesis and electron transport

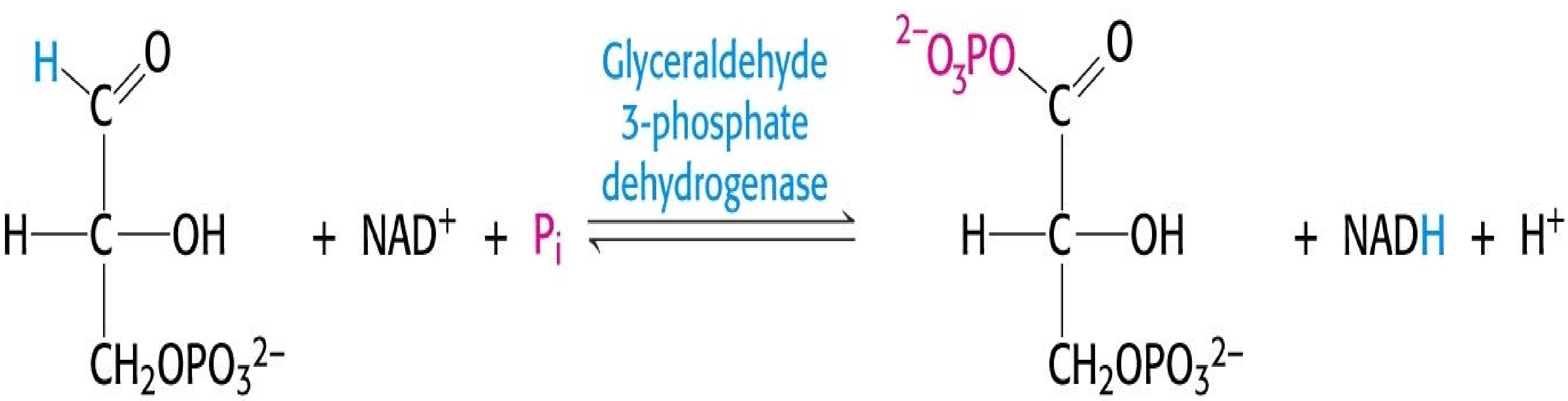
Shuttle Systems

Shuttling Reducing Equivalents OF $NADH+H^+$ from Cytosol into the Mitochondrion

Shuttle

- A **vehicle or aircraft** that **travels regularly between two places**
- **Biochemical shuttle** is a **biochemical system for translocating Protons and electrons produced during Glycolysis**
- **Across a semipermeable inner membrane of mitochondrion**
- For oxidative phosphorylation mechanism

NADH+H⁺ is generated in the **cytosol** during **Glycolysis**



Glyceraldehyde 3-phosphate (GAP)

1,3-Bisphosphoglycerate (1,3-BPG)

- **Cytosolic NADH+H^+ itself** is **not carried** across the mitochondrial membrane.

- **Instead its Protons and Electrons** of **NADH+H^+ are carried through shuttle systems**.

- **Since NAD$^+$ and NADH +H^+ are impermeable to** an inner **mitochondrial membrane**

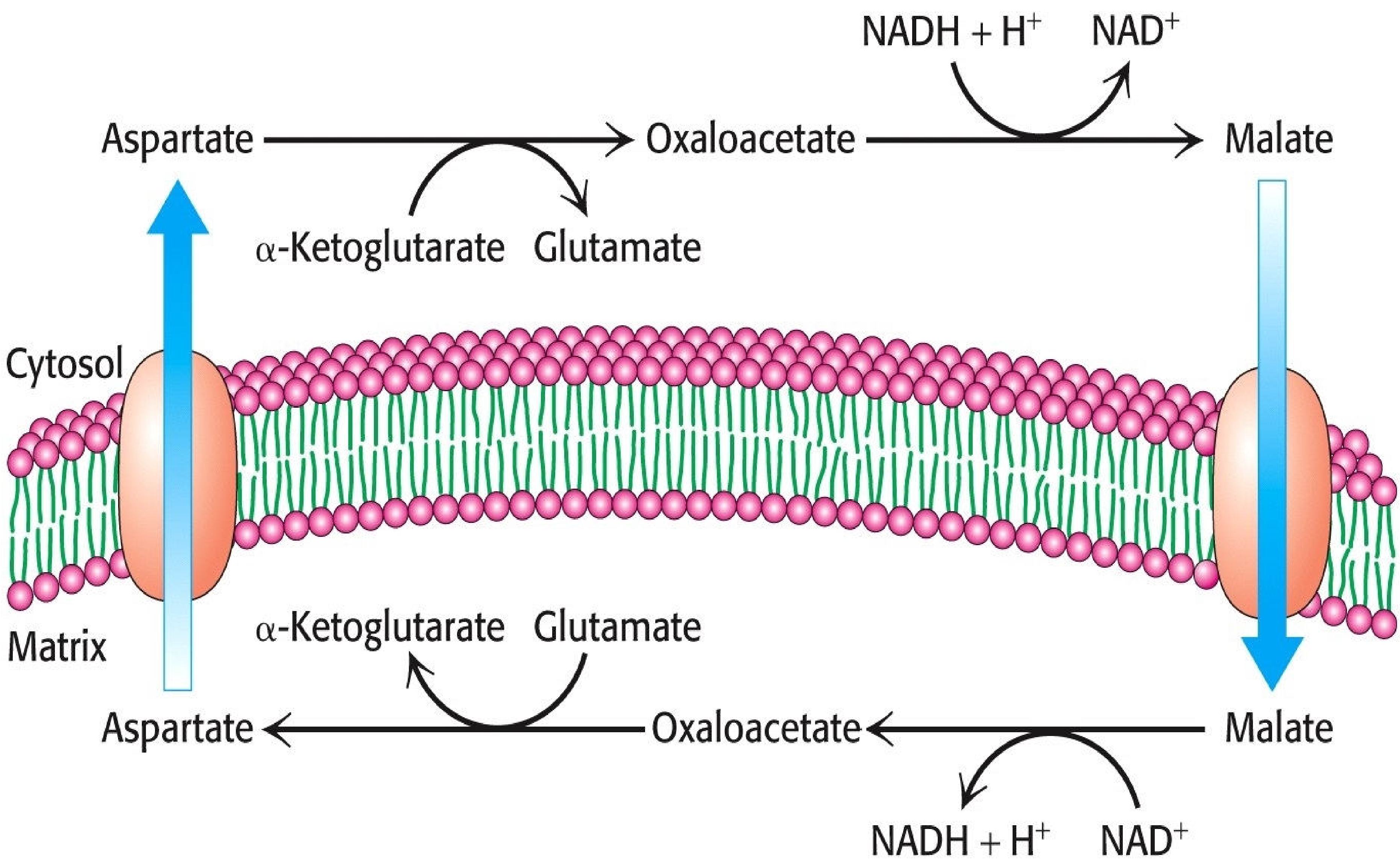
- This reducing equivalents **must be shuttled** into mitochondrial matrix before they can enter the ETC.

Cytosolic NADH+H$^+$ Enter Mitochondria via 2 Shuttle Systems

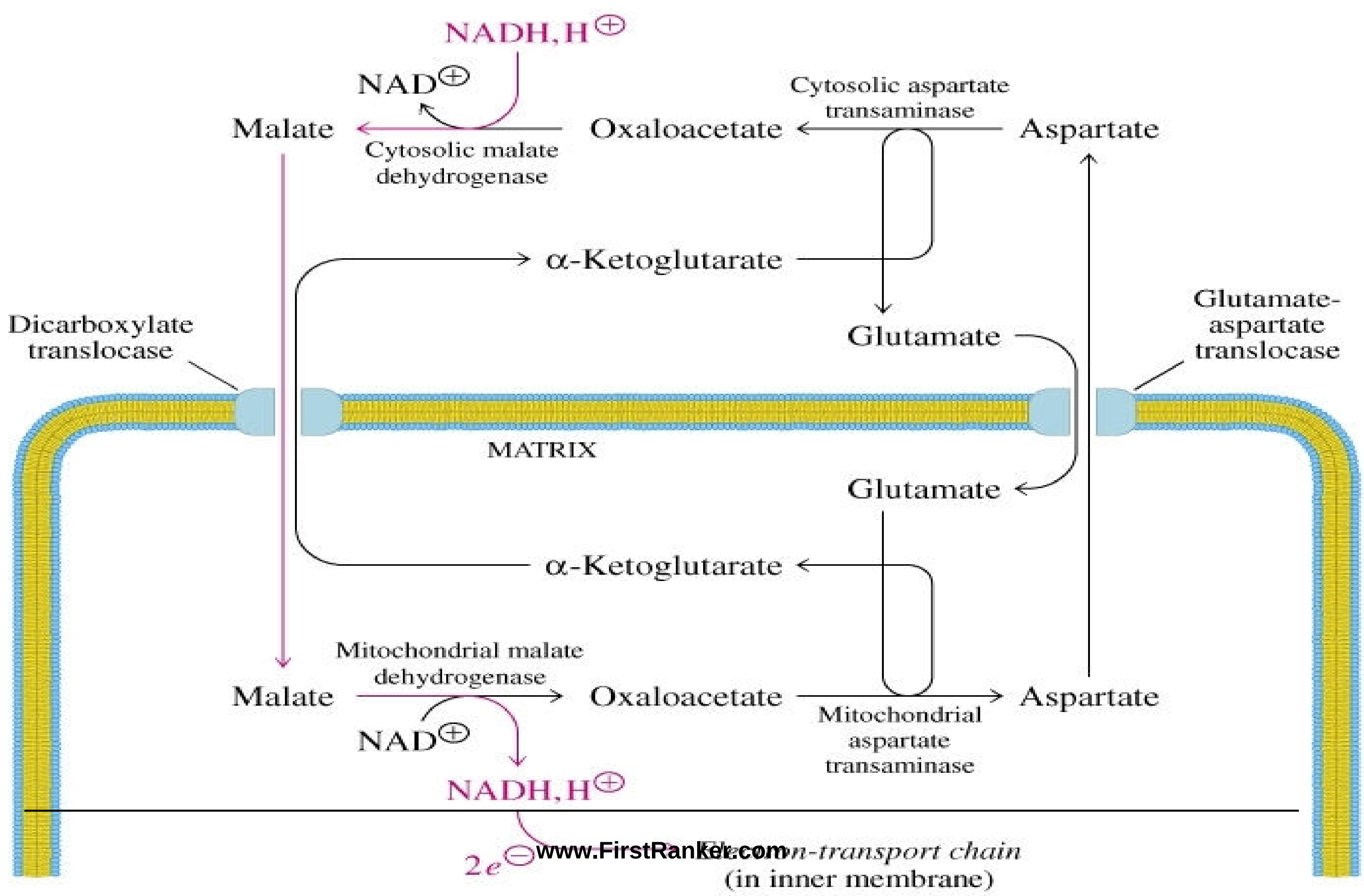
- **Two shuttles Involved:**

- Malate-Aspartate Shuttle
- Glycerol 3-phosphate Shuttle

Malate-Aspartate Shuttle



Malate/Aspartate Shuttle System

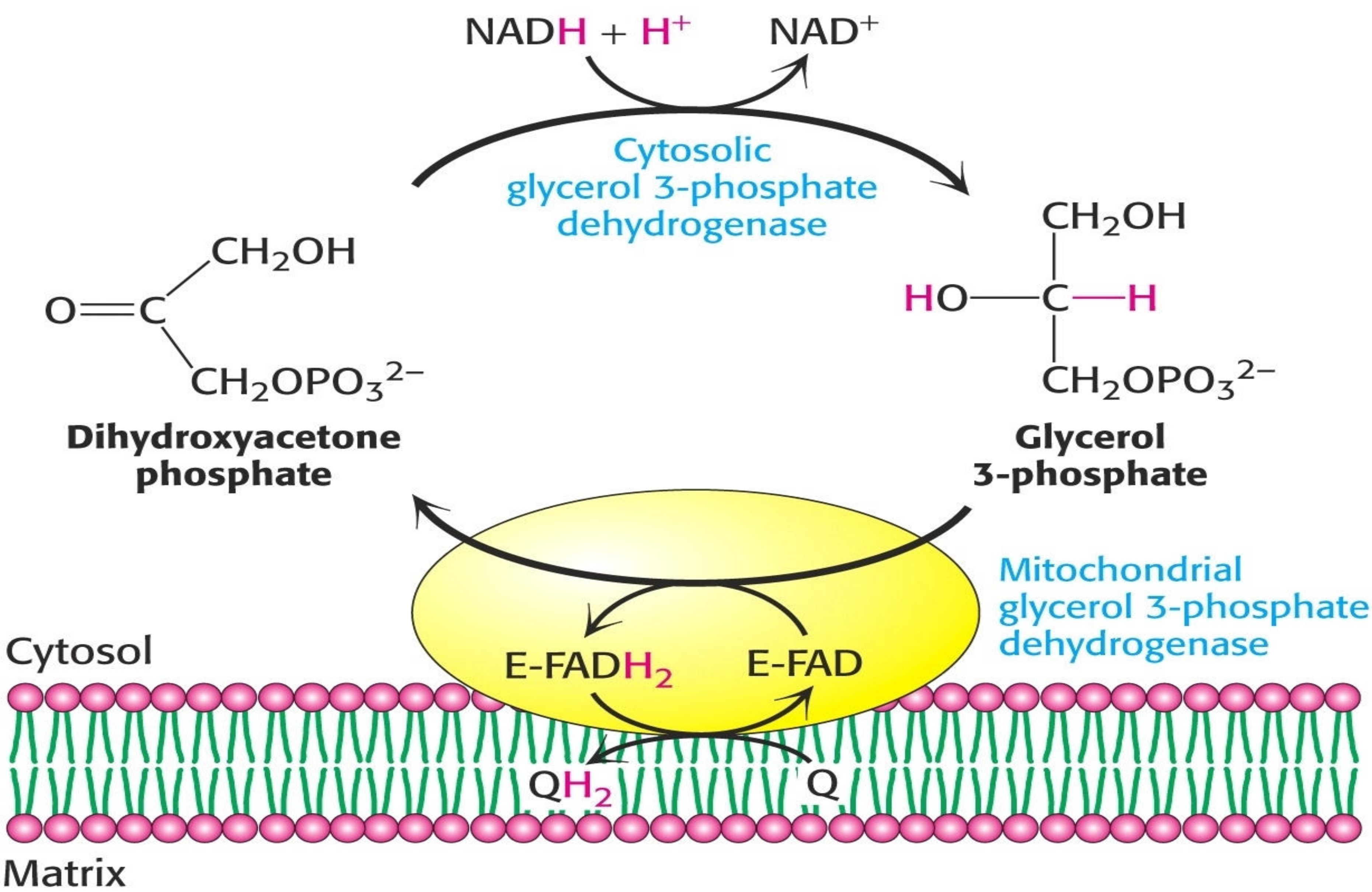


- **Malate Aspartate Shuttle**

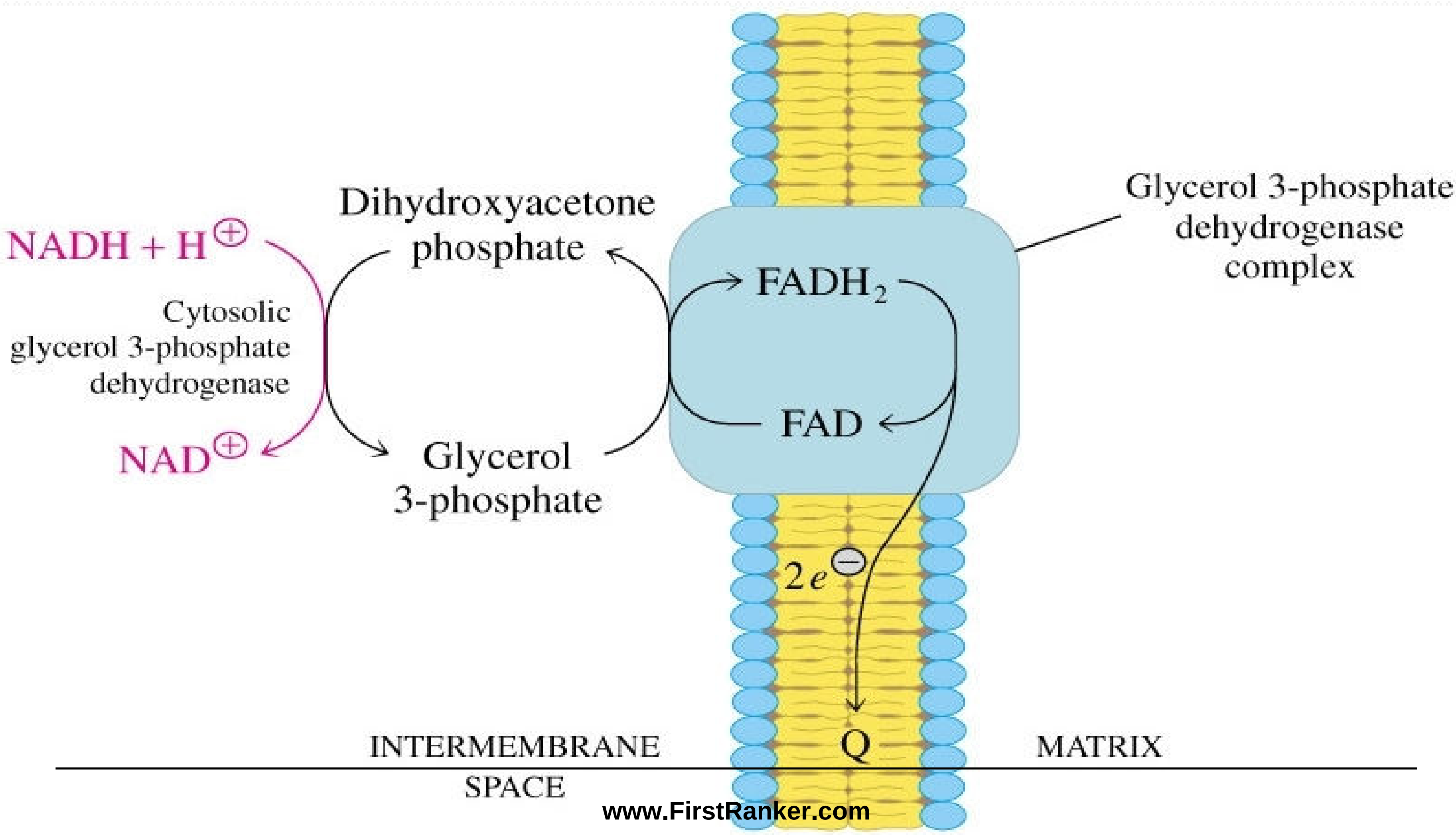
- Active in **Heart and Liver.**
- 2.5 molecules of ATP are produced

Glycerol-3-Phosphate Shuttle

Glycerol-3-Phosphate Shuttle



Glycerol 3 Phosphate Shuttle



Glycerol Phosphate Shuttle

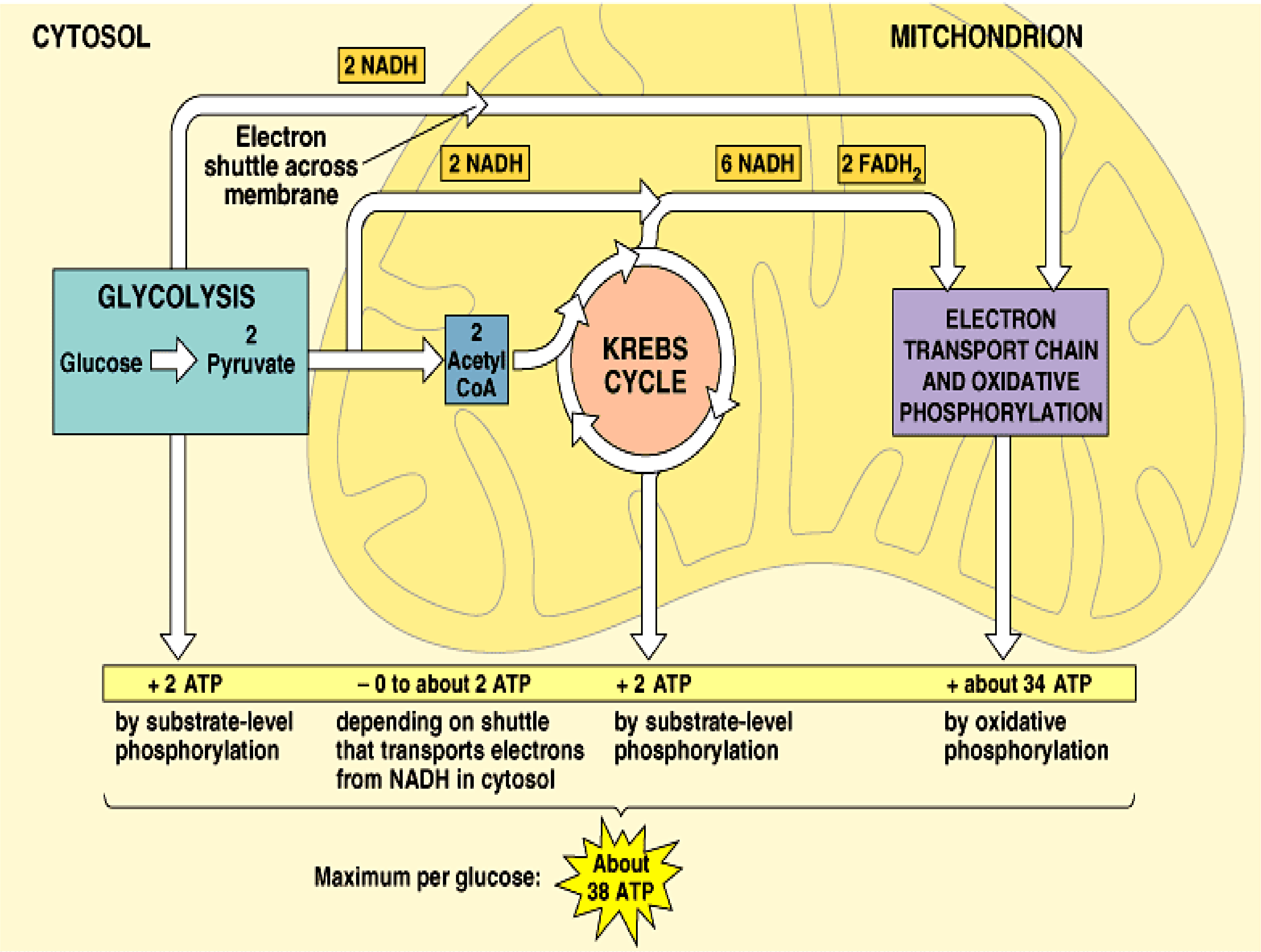
- Active in **Skeletal muscles** and **Brain**
- $FADH_2$ formed in this enter the electron-transport chain **through CoQ**
- Generates only **1.5 molecules of ATP**

Summary of Shuttle Systems

- **Total ATPs Generated / 1 Glucose Oxidation**

- **Heart and Liver** 32.0 ATP
  - **Uses Malate Aspartate Shuttle**

- **Muscle and Brain** 30.0 ATP
  - **Uses Glycerol phosphate Shuttle**



Factors Affecting Oxidative Phosphorylation Mechanism

- **Oxygen** supply to cells
- **Hemoglobin** structure and function
- **Respiratory system** and its function
- **Mitochondrial structure** and ETC components.

- **Presence of Nutrients**
- **Enzyme** function **and Coenzymes** availability
- Adequate amount of ADP and pi.
- Presence of ETC inhibitors

Pathological Conditions Affecting Oxidation Phosphorylation Mechanism Which Lower Down ATP Production

1. Hypoxia
2. Anemia
3. Ischemia
4. Hemoglobinopathies
5. Emphysema
6. Respiratory Distress Syndrome
7. Asthma
8. Prolonged Starvation
9. Malnutrition
10. Diabetes mellitus
11. ETC inhibition by chemicals/drugs
12. Inherited Disorders of Mitochondria

Inherited /Genetic Disorders

Related To Mitochondrial Oxidative Phosphorylation Mechanism

Mitochondrial DNA

- **Mitochondrial genes encode for ETC complexes**
  - **Complex I**
  - **Complex III**
  - **Complex IV**
  - **Complex V**

- **Mutations in any one or more genes** of mitochondrial DNA controlling mechanism of Oxidative phosphorylation **lead to its inherited disorders**

1. MELAS

- An inherited disorder caused due to **defect of complex I or IV of E.T.C**
- Associated with
  - **Mitochondrial Myopathy**
  - **Encephalopathy**
  - **Lactate accumulation**
  - **Acidosis**
  - **Stroke**

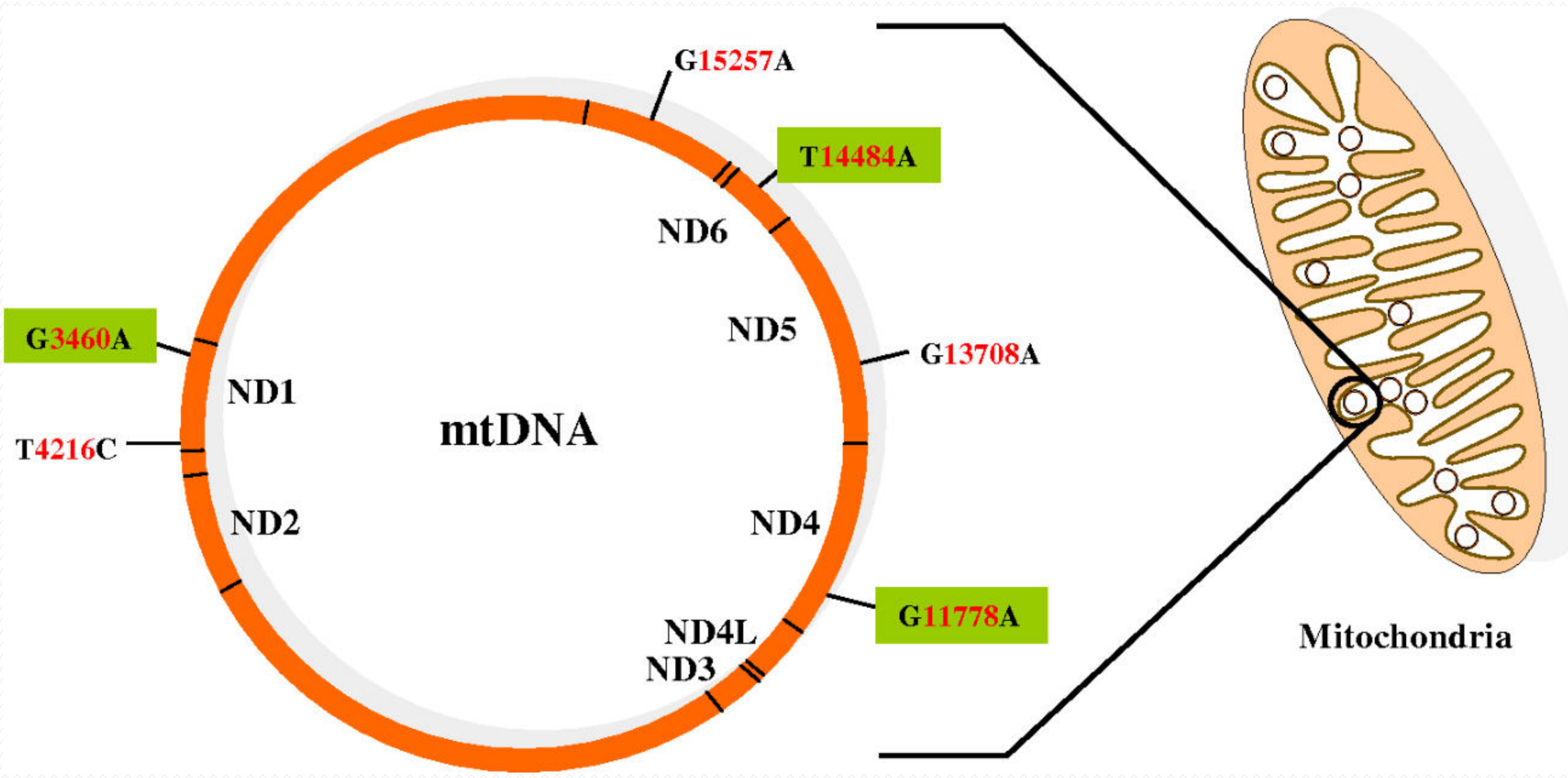
2. Fatal Infantile Mitochondrial Myopathy

- **Defect in E.T.C components located in mitochondria**
- **Cytochrome c Oxidase defect**
- Associated with renal dysfunction.
- Mostly fatal in early age

3. Leber's Hereditary Optic Neuropathy (LHON)

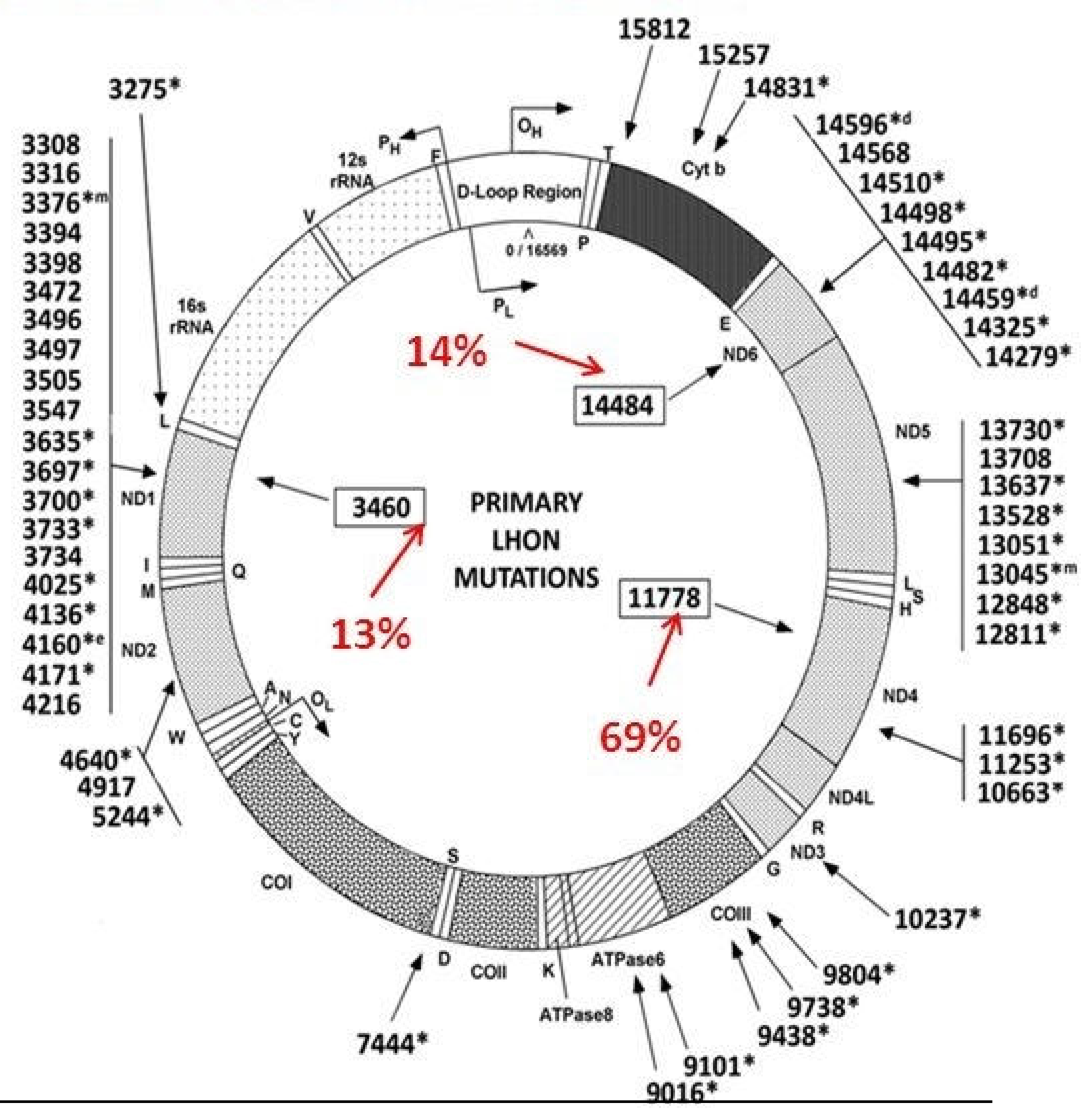
- Caused due to **mutations in mitochondrial DNA**
- Affects oxidative phosphorylation mechanism
- Loss of **bilateral vision due to neuroretinal degeneration**.

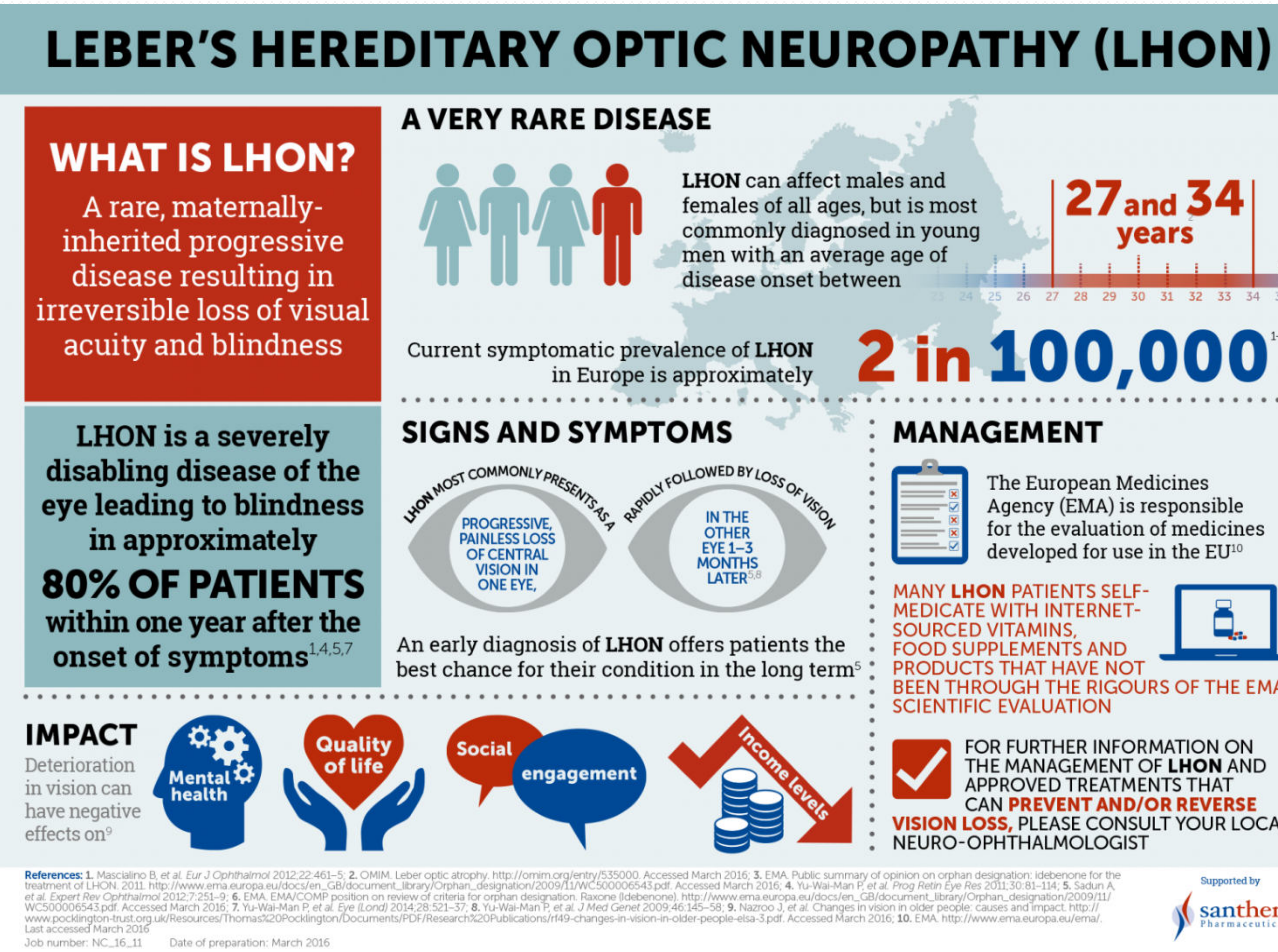
Mutant Genes Of LHON



primary LHON mutations

Three point mutations in mtDNA, known as the account for about 90% of cases of LHON worldwide.





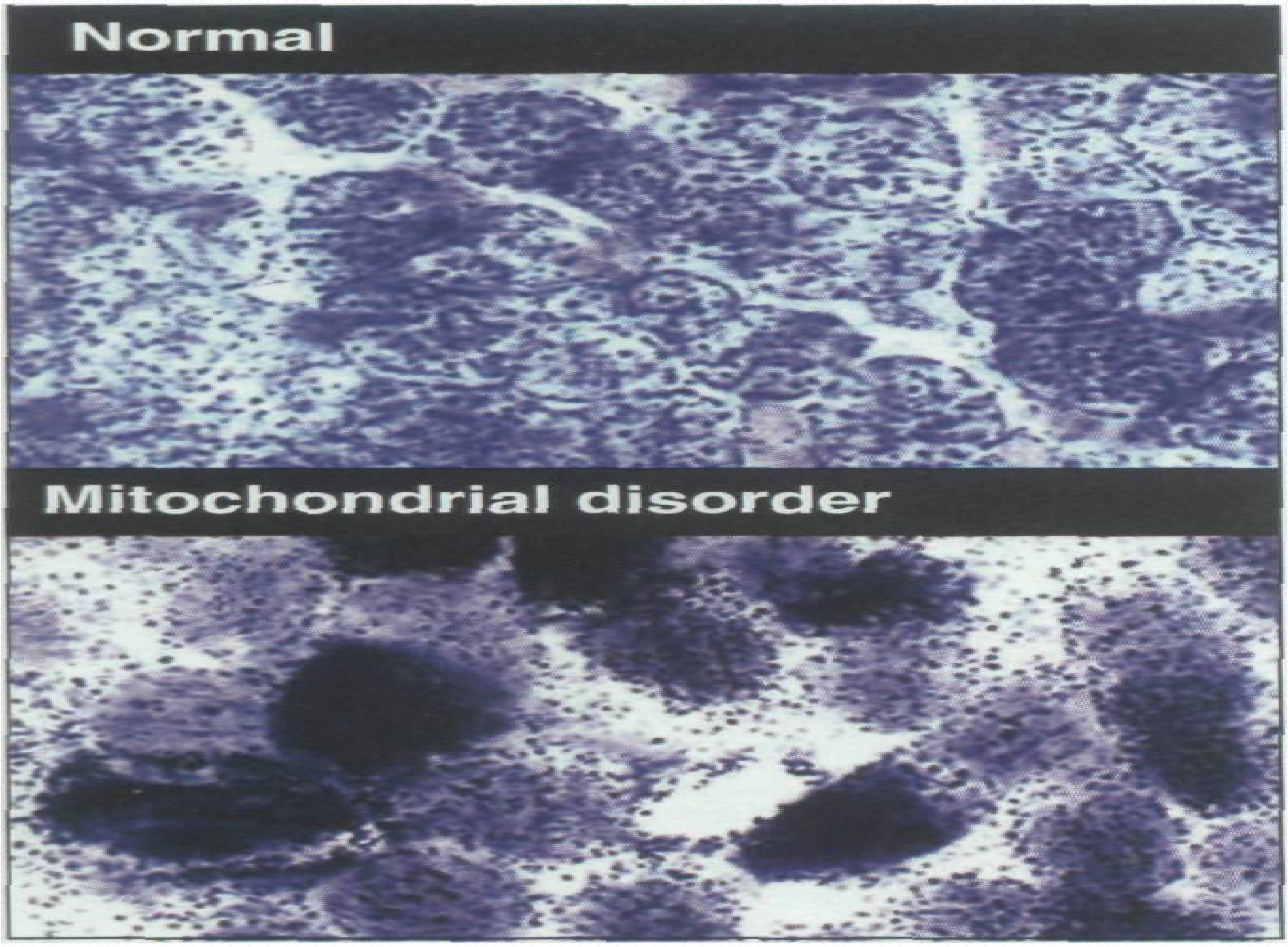
Maternally transmition of mutant mtDNA

- LHON is caused by a mtDNA point mutation, it is inherited maternally, and therefore affected or carrier *men* cannot transmit their mutation to their children.

- Affected or carrier *women*, on the other hand, transmit their LHON mutation to all of their children.

- Children of **homoplasmic** women are at the highest risk for LHON disease expression, as they are homoplasmic themselves.

- Children of **heteroplasmic** women, however, receive a variable and unpredictable amount of mutant mtDNA from their mothers, not determined by the extent of maternal heteroplasmy, **and may have insufficient mutant mtDNA to reach the threshold for disease expression**



4. Mitochondrial DNA Deletion Syndrome

Mitochondrial DNA Deletion Syndrome

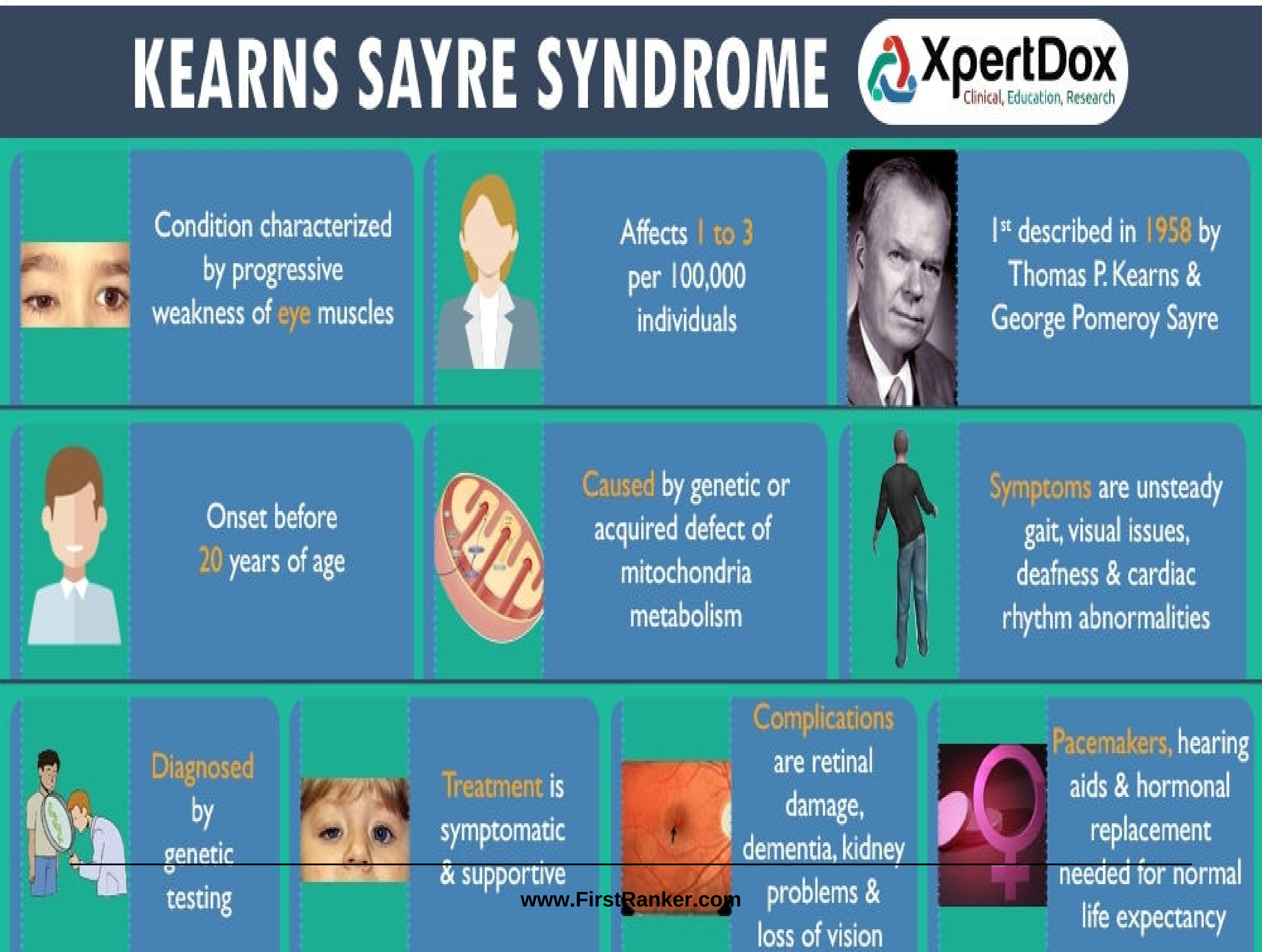
- **Kearns Sayre syndrome**

Ophthalmoplegia (inability to move eyes)
Ptosis (droopy eyes
Onset second decade
muscle

- **Pearson syndrome**

Sideroblastic anemia with pancytopenia
Exocrine pancreatic insufficiency
Onset: early infancy
Blood

- Multisystemic disease
- PEO
- Mitochondrial myopathy

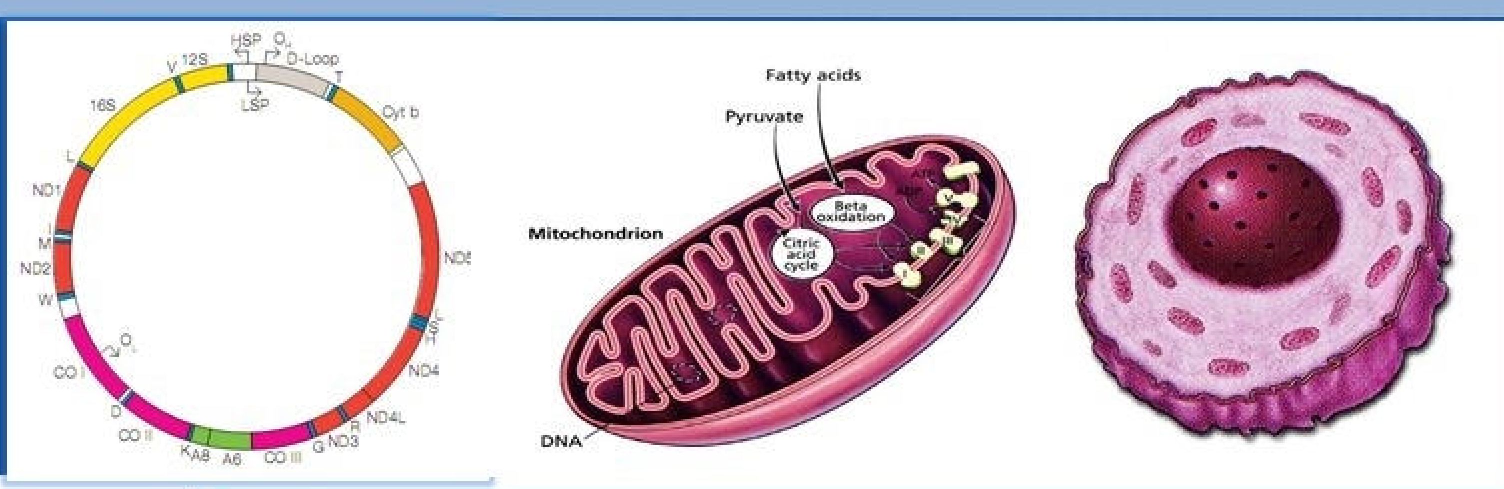


Kearns-Sayre syndrome (KSS)

- Cause
  - by a 5,000 base deletion in the mitochondrial DNA
  - Heteroplasmic, not maternally inherited (sporadic)
  - start before the age of 20
- Symptoms
  - vision loss, dysphagia, proximal weakness, hearing loss, cerebellar ataxia and cardiac conduction defects
- Diagnosis and Treatments
  - molecular diagnosis
  - no cures, only palliative medications

Pearson Syndrome

- The majority of cases are **sporadic**, although more rarely familial cases do occur, which can be **maternally or autosomally inherited.**
- It can result from either **deletion** or combined **duplication/deletion mutations**.



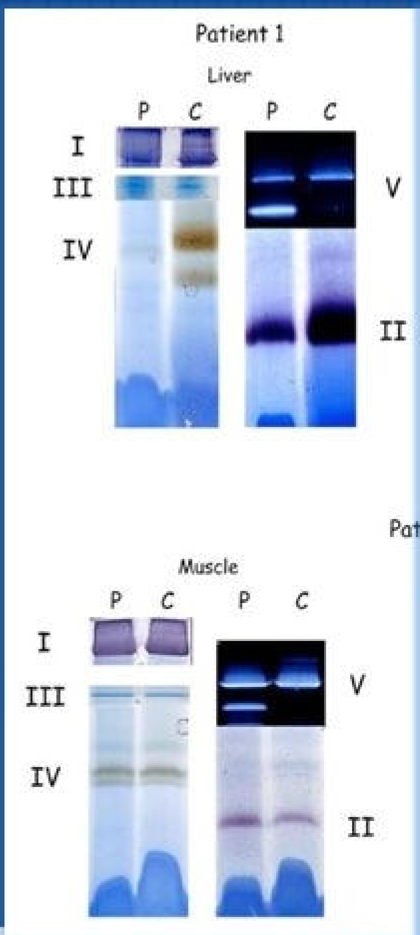
Pearson Syndrome

- It is characterised by a **sideroblastic anaemia** with vacuolisation of marrow precursors, accompanied by **neutropenia**, **thrombocytopenia**, **exocrine pancreatic dysfunction** and **abnormal liver function**, but **neurological symptoms**.

Pearson Syndrome

- Neonates may be well at birth, but some 40% of patients present in the first year with **persistent hypoplastic anemia**, other **cytopenias**, **low birth weight**, **microcephaly**, and **multiple organ system involvement (GI, neuromuscular, and metabolic).**
- **Hydrops fetalis** has also been reported. Anemic newborns may need transfusion.

Pearson Syndrome



- It is diagnosed by the presence of a single large scale rearrangement of mtDNA, as observed in Southern blot hybridization analysis of blood DNA.
- Southern blot or long-range PCR for the detection of mtDNA rearrangement is recommended.

5. Luft's Disease

- Luft's Disease is a **mitochondrial disease**
- **First patient** who was diagnosed with this disease was a **30 year old Swedish woman by Dr Rolf Luft**
- Caused by **abnormal mitochondria**

Biochemical Cause

Mitochondria Respire Wildly

- **Respiratory control is lost**
- Partial Uncoupling is caused by an **abnormality in mitochondrial membrane**
- Electron transport is only **loosely coupled to ATP production**
- **Oxidation process proceed independent of ADP phosphorylation to generate ATP**
- An extra energy **evolves in form of heat**
- **This elevates body temperature up to 38.4 °C** which **raises BMR**

Luft's Disease Is Characterized By

- Abnormal **excessive production of heat**
- Characterized by **hypermetabolism and abnormal transpiration.**
- Patient experiences **excessive sweating during winter**
- Make them to **change their clothes 10 times a day.**

- Onset is in **childhood**
- **Thyroid function is normal**
- Since there is less ATP production and an extra energy is lost in the form of heat
- Metabolic processes are stimulated

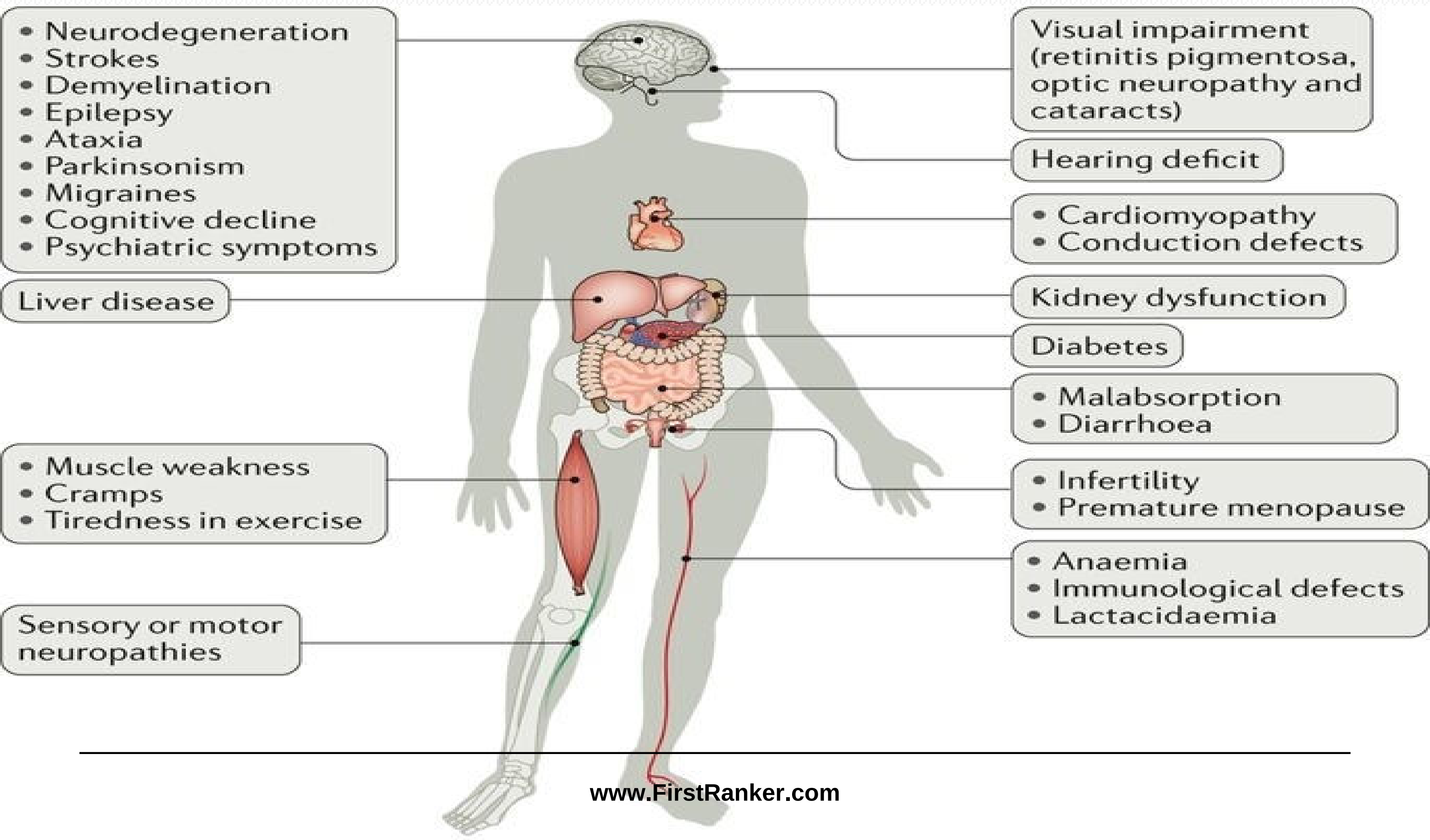
Luft's Disease
Non Thyroidal Hypermetabolism

- Due to **high BMR and low ATP production**
- **High caloric intake**
- There is **failure to put on weight despite a good diet**
- There is **progressive weight loss despite increased food intake**
- **Excessive perspiration**
- **Excessive thirst** indicate a state of severe hyper metabolism of non thyroid origin (**since thyroid hormones -T3 and T4 are normal**)

Manifestations of Luft's Disease

- Heat intolerance
- Profuse perspiration
- Polydipsia without polyuria
- Severe hyper metabolism
- Polyphagia
- Muscular wasting and weaken
- Absent deep reflexes, and Resting tachycardia.

Multiorgan Dysfunction Risk In Luft's Disease



Case Study

- An elderly couple was brought by ambulance to an emergency department after their daughter noticed that they were both **acting "strangely."** The couple had been in good health prior to the weekend. Their daughter had gone out to spend the week-end with her friends. The couple had been snowed in at their house until the snowplows cleared the roads. They had plenty of food and were kept warm by a furnace and blankets. On reaching home after two days, their daughter noticed that they both were complaining of **bad headaches, confusion, fatigue, and some nausea**. On arrival to an emergency department, both patients were afebrile with normal vital signs and O_2 saturation of 99 percent on 2 L of O_2 by nasal cannula. Their lips appeared to be very red. Both patients were slightly confused but otherwise oriented. The physical examinations were within normal limits.
Carboxyhemoglobin levels were drawn and were elevated.
What is most likely cause of these patients' symptoms?

Case 1 – Kearns-Sayre Syndrome

- 16 year-old boy
- CC: Ataxia and droopy eyelids.
- HPI:
  - His ptosis started at age 5 and his parents note that he turns his head more then usual when trying to look around. He also has noted that his balance is off and he occasionally drops objects.
- PMH:
  - short stature
  - diabetes
  - complete heart block
- Exam:
  - Bilateral ptosis and restricted bilateral horizontal eye movements. His fundoscopic exam reveals pigmentary retinopathy. His has 4/5 strength in his proximal arms and legs and is unable to tandem walk.
- Workup:
  - Serum lactate is slightly elevated. CSF shows elevated protein and lactate. Muscle biopsy shows ragged red fibers.
- Genetics: mtDNA deletions, usually sporadic

Questions

- **Long Essays.**
- Q.1 Define Biological oxidation. Enumerate and Describe various enzymes carrying out biological oxidation reactions with suitable examples.

- Q.2 Describe Respiratory chain and Give its significance.

OR

- Explain the Electron. Transport chain (E.T.C.) and its significance.

OR

- How the reduced equivalents generated in anaerobic dehydrogenase reactions are reoxidized.

- Q.3 What is oxidative phosphorylation? Explain the mechanism with respect to various theories and hypothesis.

- **Short Notes**
- Cytochromes
- Inhibitors of E.T.C
- Shuttle systems and its significance
- Inhibitors and Uncouplers of oxidative phosphorylation

- Complexes of E.T.C.
- Redox potential and free energy changes.
- Inherited Disorders related to E.T.C. abnormality.
- ATP – Mode of its formation and it's role in the Body.

Short Answer Questions

- Give the sites for ATP generation of in E.T.C.
- Enumerate the High energy compounds of our body
- Substrate level phosphorylation and it's importance.

- Enumerate the Enzymes catalyzing Biological oxidation reactions. Write the class to which these enzymes classified.

- Inherited Disorders of Mitochondrial Dysfunction

- Define P.O ratio. What is the P:O ratio for reduced NADH+H^+ & $FADH_2$ respectively.
- List the components of E.T.C. and their location.
- Redox couple & Redox potential.

- FlavoProteins
- Product of Aerobic and Anaerobic dehydrogenation reactions.
- Write enzymes catalyzing Aerobic and Anaerobic dehydrogenation reaction's during metabolism.

THANK YOU

- Laboratory data showed lactic acidosis,
- Proteinuria
- Glycosuria and
- Generalized aminoaciduria
- Muscle biopsy showed large clumps of granules positive with oxidative enzyme stains and increased lipid droplets. Ultrastructural studies showed large aggregates of mitochondria, many of which were greatly enlarged and contained disoriented or concentric whorls of cristae and paracrystalline inclusions.

- **A 1-month-old boy** was admitted because of failure to thrive. He was floppy and had bilateral ptosis, diminished reflexes, and poor suck. He had **aspiration pneumonia**, developed seizures, and died at age 3 1/2 months.
- He was an only child, and family history was negative.
- **Cytochrome c oxidase was absent in fresh frozen sections by histochemical staining.**
- By biochemical assay, cytochrome c oxidase (cytochrome aa3) was 6% of normal in muscle biopsy and undetectable in autopsy muscle; spectra and content of cytochromes showed lack of cytochrome aa3, decreased cytochrome b and normal cytochrome cc1.
- In kidney, cytochrome-c-oxidase activity was 38% of normal and spectra showed decreased cytochromes aa3 and b.
- The association of fatal infantile mitochondrial myopathy, **lactic acidosis** and renal dysfunction was previously reported by Van Biervliet et al and appears to be a distinct nosologic entity, one of the few biochemically defined mitochondrial myopathies.

- A case of cytochrome c oxidase deficiency primarily affecting skeletal muscle is described. The child was admitted at 4 weeks due to failure to thrive and examination at that time revealed weakness and hypotonia. His condition deteriorated until at 11 weeks respiratory arrest necessitated artificial ventilation and death occurred at 14 weeks. Biochemical investigation showed lactic acidemia and generalized aminoaciduria. Histochemical examination of muscle obtained at biopsy showed strong reactions for some oxidative enzymes, but by contrast cytochrome c oxidase could not be detected. Cytochrome c oxidase activity was less than 5% of control values in an extract of fresh muscle. The reduced-minus oxidized absorption spectra of muscle mitochondrial fractions prepared from post-mortem tissue showed an absence of cytochrome aa3 and a partial deficiency of cytochrome b. Ultra -structural examination showed abnormal mitochondria with loss of cristae and an abnormal granular matrix. The family history suggests autosomal recessive inheritance.