BIOLOGICAL OXIDATION & ELECTRON TRANSPORT CHAIN

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RESPIRATION

Organisms can be classified based on the mechanism to obtain energy-

Autotrophs: are able to produce their own organic molecules through photosynthesis

Heterotrophs: live on organic compounds produced by other organisms **(e.g. Starch/ Glucose)**

All organisms use **cellular respiration** to extract energy from organic molecules.

RESPIRATION

- Respiration – a process by which cells derive energy with a controlled reaction between H^+ and O_2; the end product being water.

- Aerobic organisms are able to capture a far greater proportion of the available free energy of respiratory substrates than anaerobic organisms.

RESPIRATION

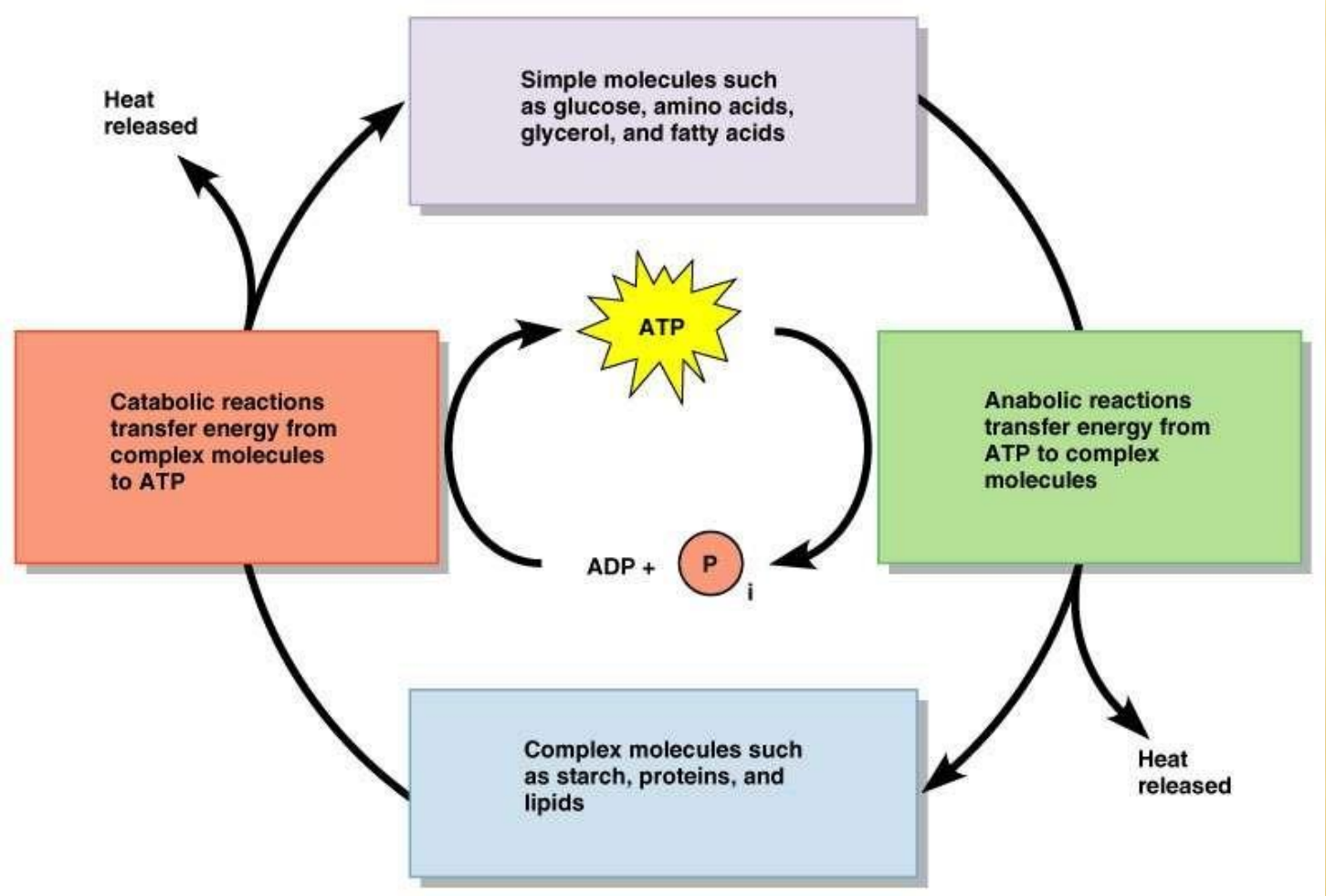
The objective of respiration is to produce ATP.

- Energy is released from oxidation reactions in the form of electrons
- Electrons are shuttled by electron carriers (e.g. NAD^+) to an **electron transport chain**
- Electron energy is converted to ATP in the electron transport chain

METABOLISM

- Metabolism is the sum of the chemical reactions in an organism.
- Catabolism is the energy-releasing processes.
- Anabolism is the energy-using processes.
- Catabolism provides the building blocks and energy for anabolism.

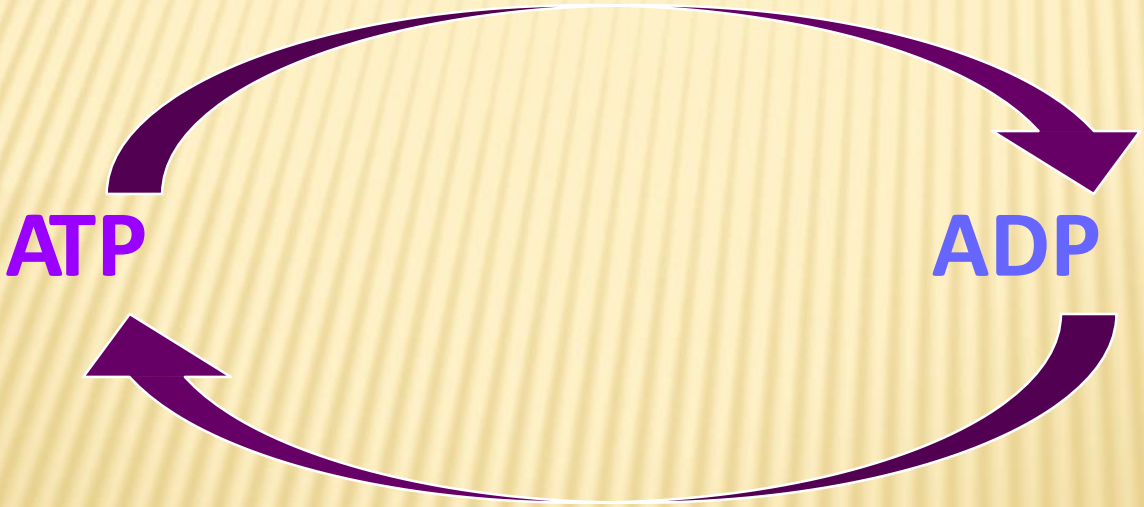
METABOLISM


Heat released
Simple molecules such as glucose, amino acids, glycerol, and fatty acids
ATP
Catabolic reactions transfer energy from complex molecules to ATP
Anabolic reactions transfer energy from ATP to complex molecules
ADP +
P
i
Complex molecules such as starch, proteins, and lipids
Heat released

ATP COUPLES ENERGY BETWEEN CATABOLISM AND ANABOLISM

Energy available for work & chemical synthesis (e.g. movement, signal amplification, etc.)

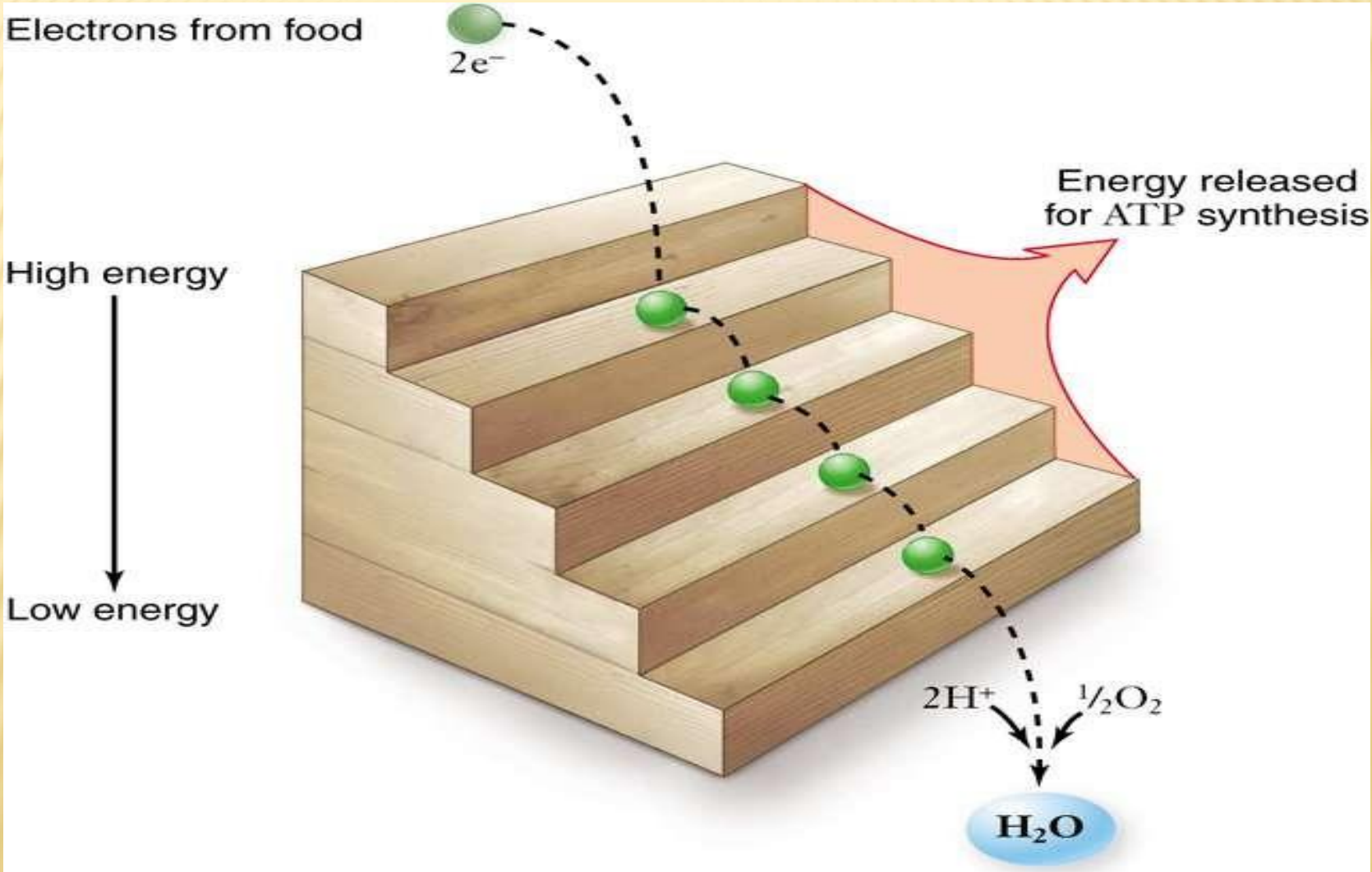
← anabolism



Energy from food (fuel molecules) or from photosynthesis

← catabolism

BIOLOGICAL OXIDATION

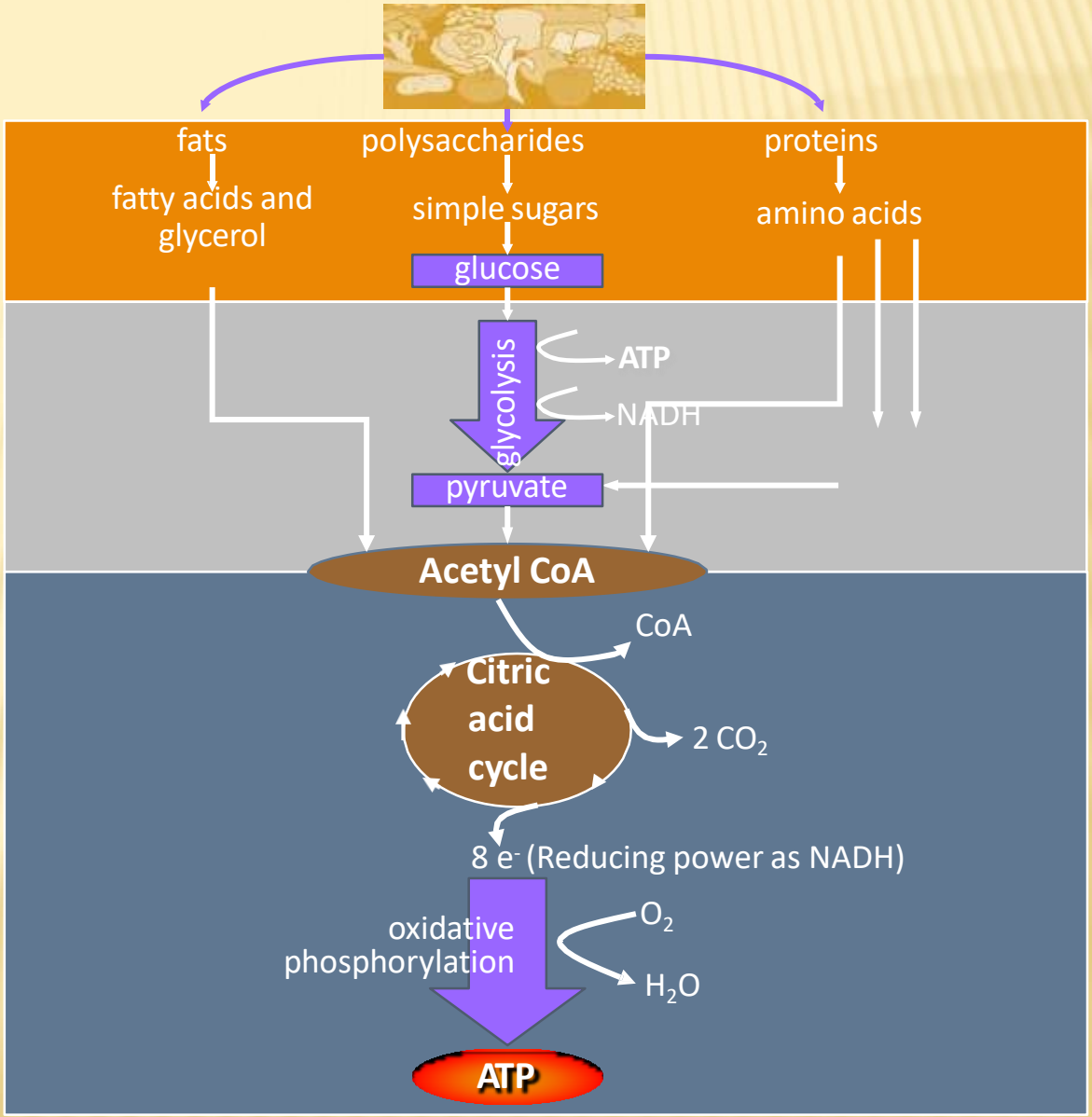


CELLULAR METABOLISM

Part 1:
Breakdown of large macromolecules to simple subunits

Part 2:
Breakdown of simple subunits to acetyl CoA accompanied by production of limited amounts of ATP and NADH

Part 3:
Complete oxidation of acetyl CoA to H_2O and CO_2 accompanied by production of large amounts of NADH and ATP in mitochondrion



ATP IS THE PRINCIPAL CARRIER OF CHEMICAL ENERGY IN THE CELL!

High Energy compound.

$\Delta G^{o'} = -7.3$ kcal/mol

- Major activities promoted by ATP:
- -locomotion
- -membrane transport
- -signal transduction
- -keeping materials in the cell
- -nucleotide synthesis

BIOLOGICAL OXIDATION

- Involves the transfer of electrons
- oxidation being termed for the removal of electrons & reduction for gain of electrons
- Oxidation is always accompanied by reduction of an e^- acceptor

ENZYMES INVOLVED IN OXIDATION AND REDUCTION REACTIONS

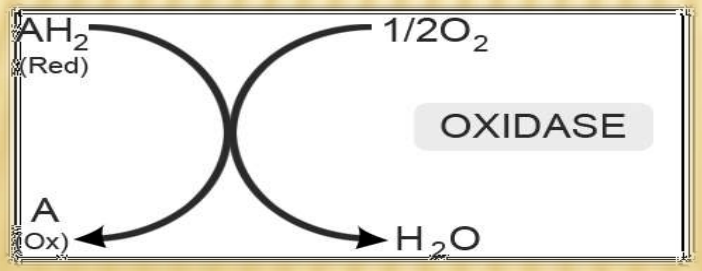
- Are called as Oxidoreductases which include : oxidases, dehydrogenases, hydroperoxidases and oxygenases.
- **Oxidases** use oxygen as an electron acceptor
- **Dehydrogenases** can't use O_2 as an electron acceptor
- **Hydroperoxidases** use H_2O_2 as a substrate
- **Oxygenases** catalyze the direct transfer of O_2 into the substrate
- Oxidases & dehydrogenases are involved in respiration; hydroperoxidases neutralize free radicals & oxygenases are involved in biotransformation reactions.

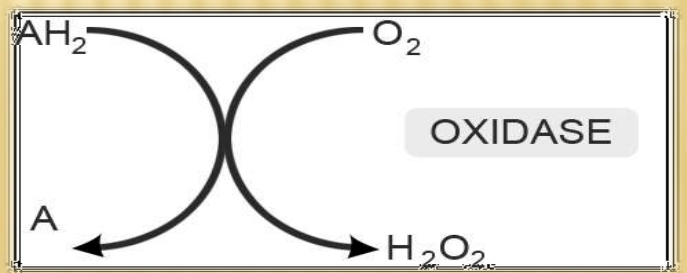
OXIDASES

- Catalyze the removal of hydrogen from a substrate with the involvement of oxygen as a H – acceptor, forming water or hydrogen peroxide.

- Exist in two different forms :
  - Some of them are copper containing such as, Cytochrome oxidase , the terminal component of ETC which transfer the e^- finally to O_2.

  - Other are flavoproteins such as , L – amino acid oxidase (FMN linked) and Xanthine oxidase (FAD linked)





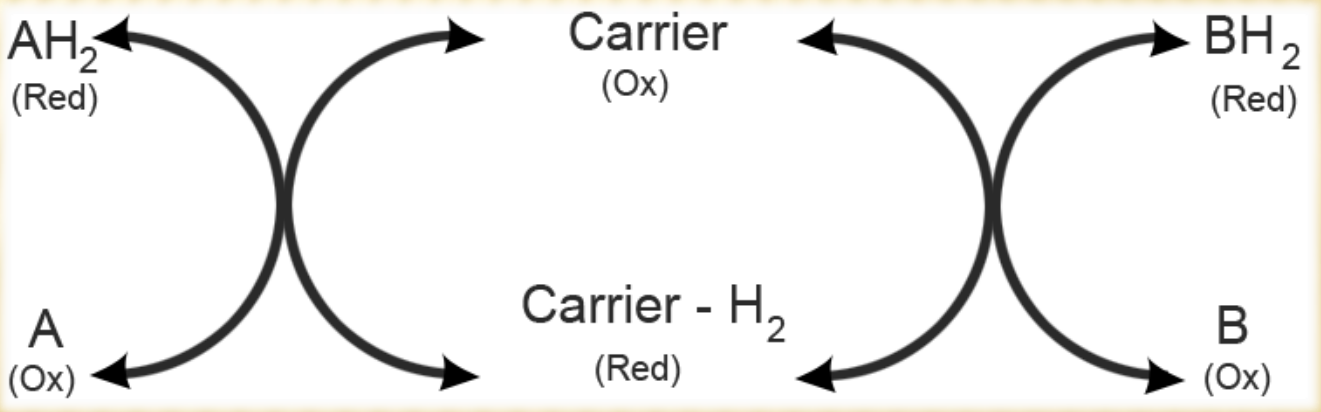
DEHYDROGENASES

Perform 2 main functions:

- Transfer hydrogen from one substrate to another in a coupled Oxidation /Reduction reaction
- As components of Electron transport chain such as cytochromes

Dehydrogenases use coenzymes – nicotinamides & riboflavin - as hydrogen carriers

- Nicotinamides can be in the form of NAD^+ or $NADP^+$
- Riboflavin can be – FMN or FAD same as oxidases



HYDROPEROXIDASES

- Includes 2 sets of enzymes : catalases and peroxidases
- Peroxidases reduce H_2O_2 at the expense of several other substances

$$H_2O_2 + AH_2 \longrightarrow 2H_2O + A$$

- Catalases uses H_2O_2 as electron acceptor & electron donor

$$2H_2O_2 \longrightarrow 2H_2O$$

Peroxisomes are rich in oxidases and catalases

OXYGENASES

Catalyze the incorporation of O_2 into substrates in 2 steps

- Oxygen is bound to the active site of the enzyme
- Bound O_2 is reduced or transferred to the substrate

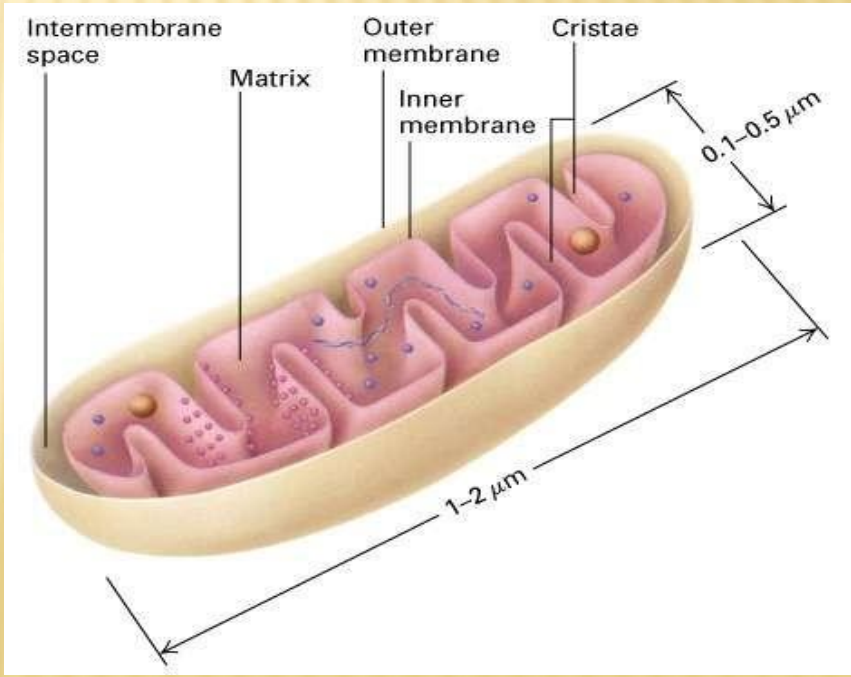
Consist of two sets of enzymes

1. Dioxygenases : incorporate both atoms of oxygen into the substrate ; $A + O_2 \longrightarrow AO_2$
2. Monooxygenases : incorporates one atom of oxygen into the substrate & the other is reduced to water

$$A-H + O_2 + ZH_2 \longrightarrow A-OH + H_2O + Z$$

MITOCHONDRION

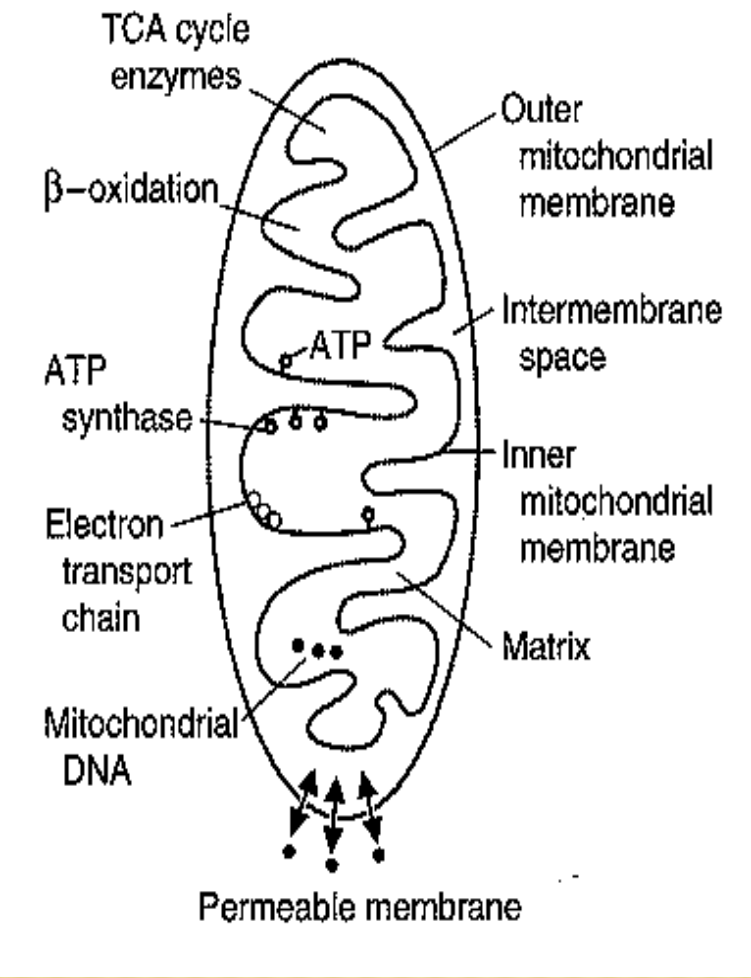
- Mitochondria, have been termed the "powerhouses" of the cell since the final energy release takes place in the mitochondria only.
- Mitochondria have an outer membrane that is permeable to most metabolites, an inner membrane that is selectively permeable, enclosing a matrix within .

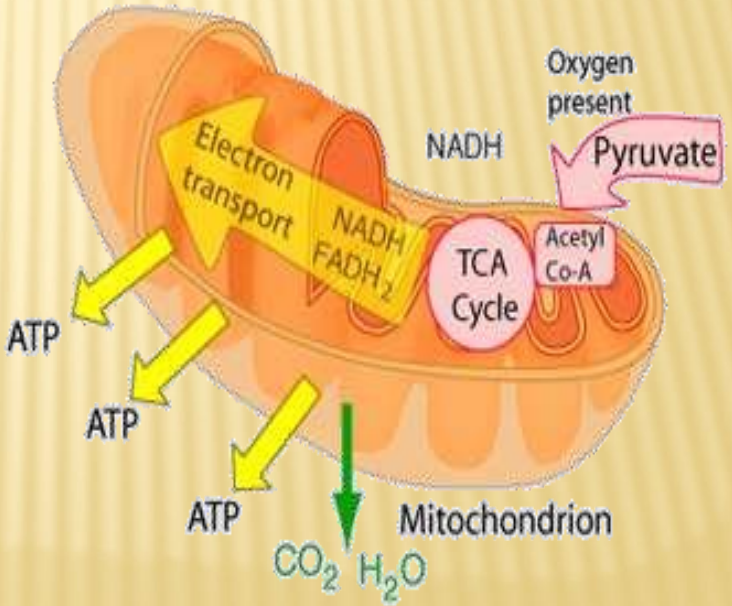


MITOCHONDRION

- The outer membrane is characterized by the presence of various enzymes, including acyl-CoA synthetase and glycerol phosphate dehydrogenase.
- Adenylyl kinase and creatine kinase are found in the intermembrane space.
- The phospholipid cardiolipin is concentrated in the inner membrane together with the enzymes of the respiratory chain, ATP synthase and various membrane transporters.
- The matrix encloses the enzymes of TCA cycle, beta oxidation and pyruvate dehydrogenase complex.

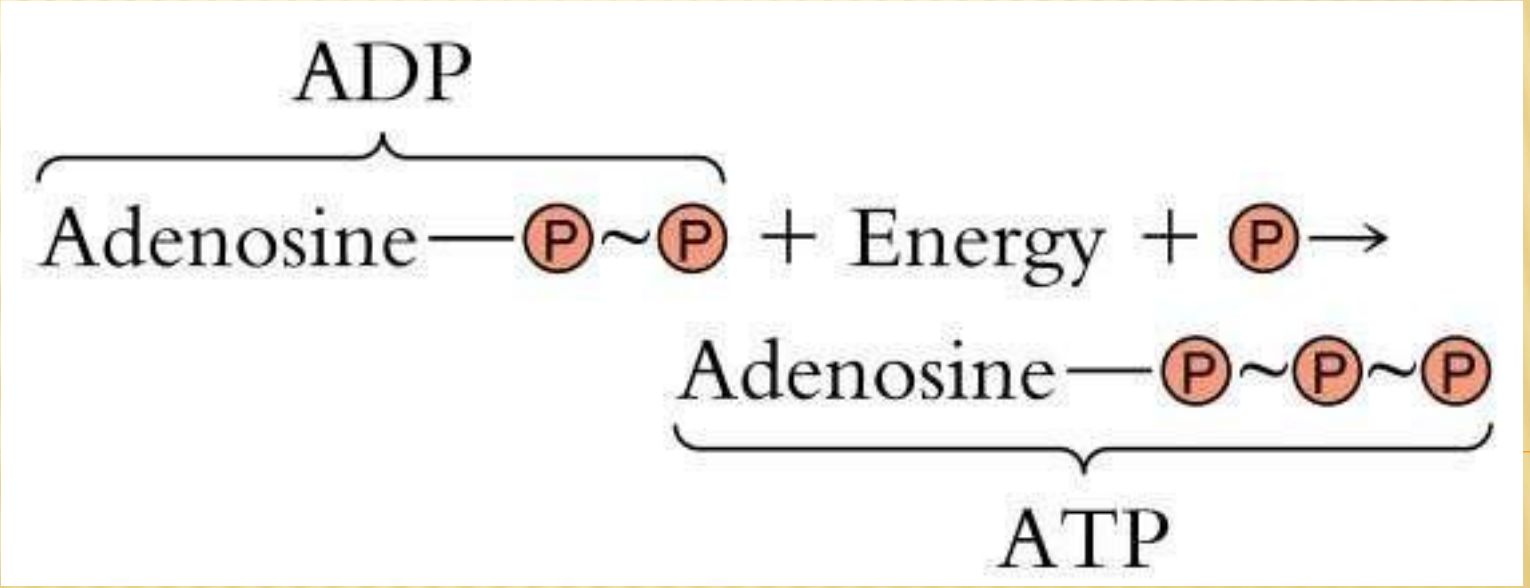
MITOCHONDRION





THE GENERATION OF ATP

ATP is generated by the phosphorylation of ADP



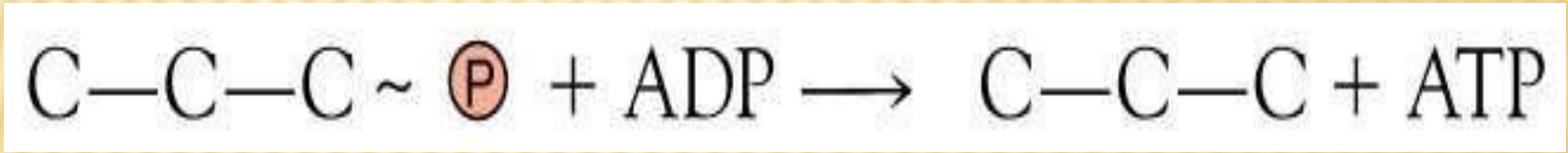
THE GENERATION OF ATP

ATP can be generated either by-

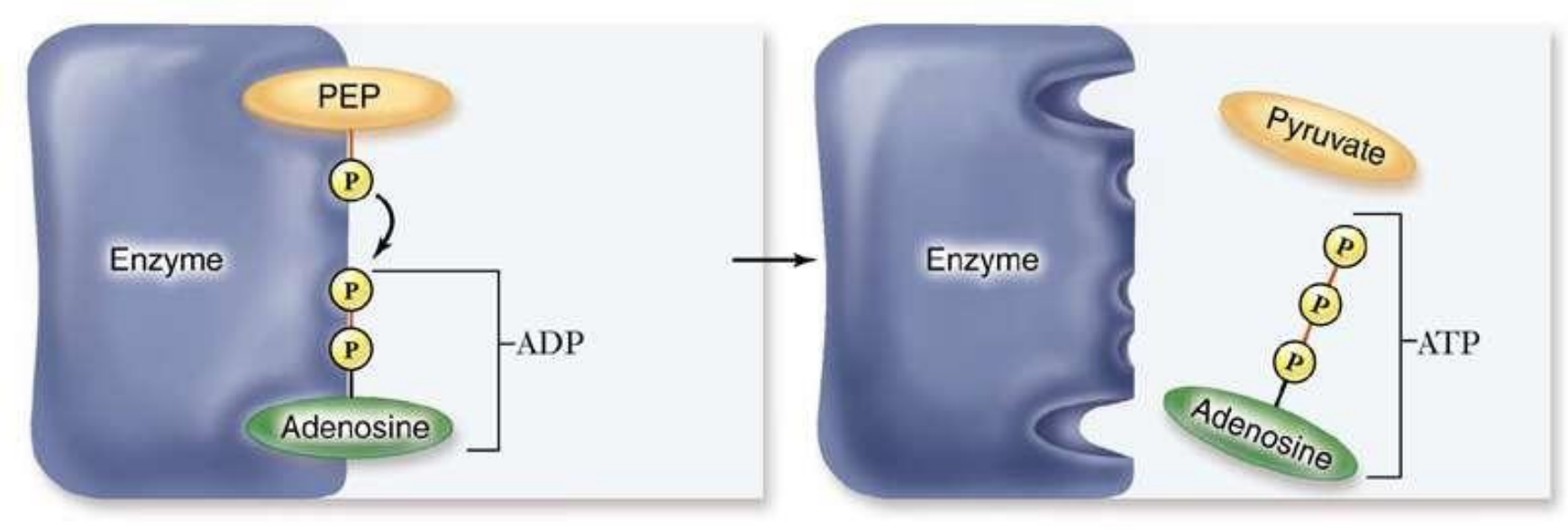
- Substrate level phosphorylation or
- By Oxidative phosphorylation

SUBSTRATE-LEVEL PHOSPHORYLATION

- Substrate-level phosphorylation is the transfer of a high-energy PO_4^- to ADP at the expense of the energy of the substrate without involving the electron transport chain.
- The substrate has higher energy level than the product, the surplus energy is used up for ATP formation.



SUBSTRATE LEVEL PHOSPHORYLATION



Substrate-level phosphorylation – transferring a phosphate directly to ADP from another molecule

OXIDATIVE PHOSPHORYLATION

Oxidative phosphorylation is the process by which the energy stored in NADH and $FADH_2$ is used to produce ATP.

A. Oxidation step**:** electron transport chain

$$NADH + H^+ + O_2 \longrightarrow NAD^+ + H_2O$$

$$FADH_2 + O_2 \longrightarrow FAD + H_2O$$

B. Phosphorylation step

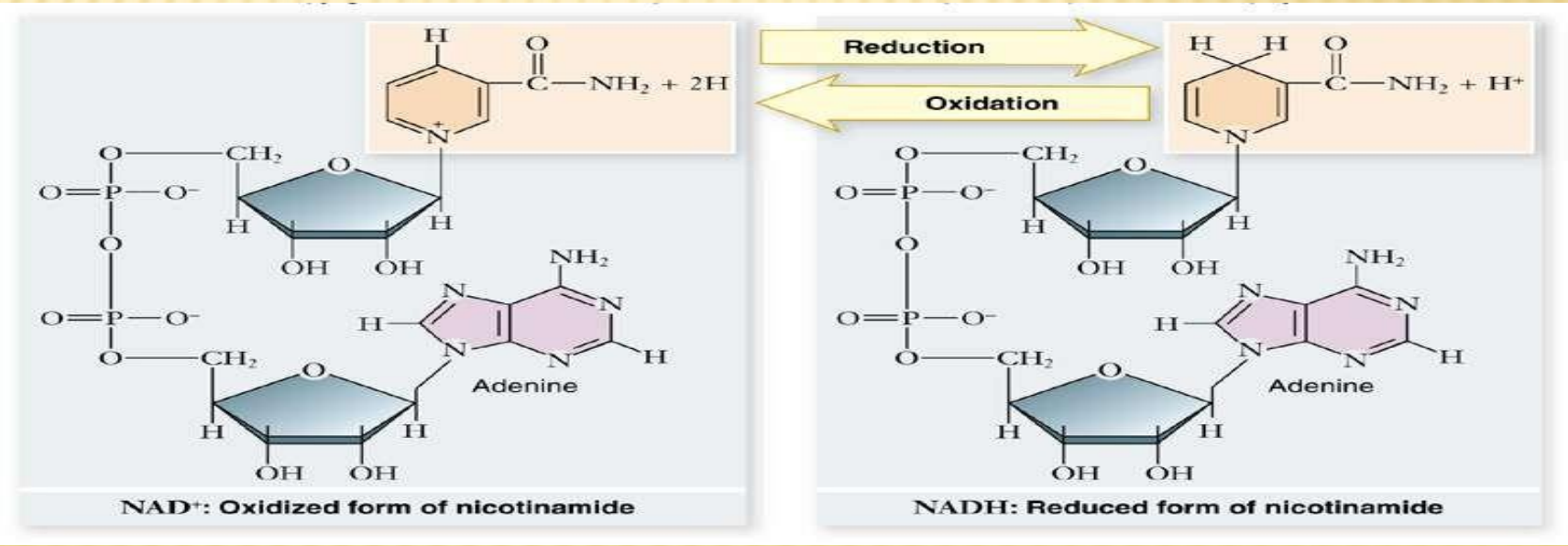
$$ADP + P_i \longrightarrow ATP$$

REDOX REACTIONS

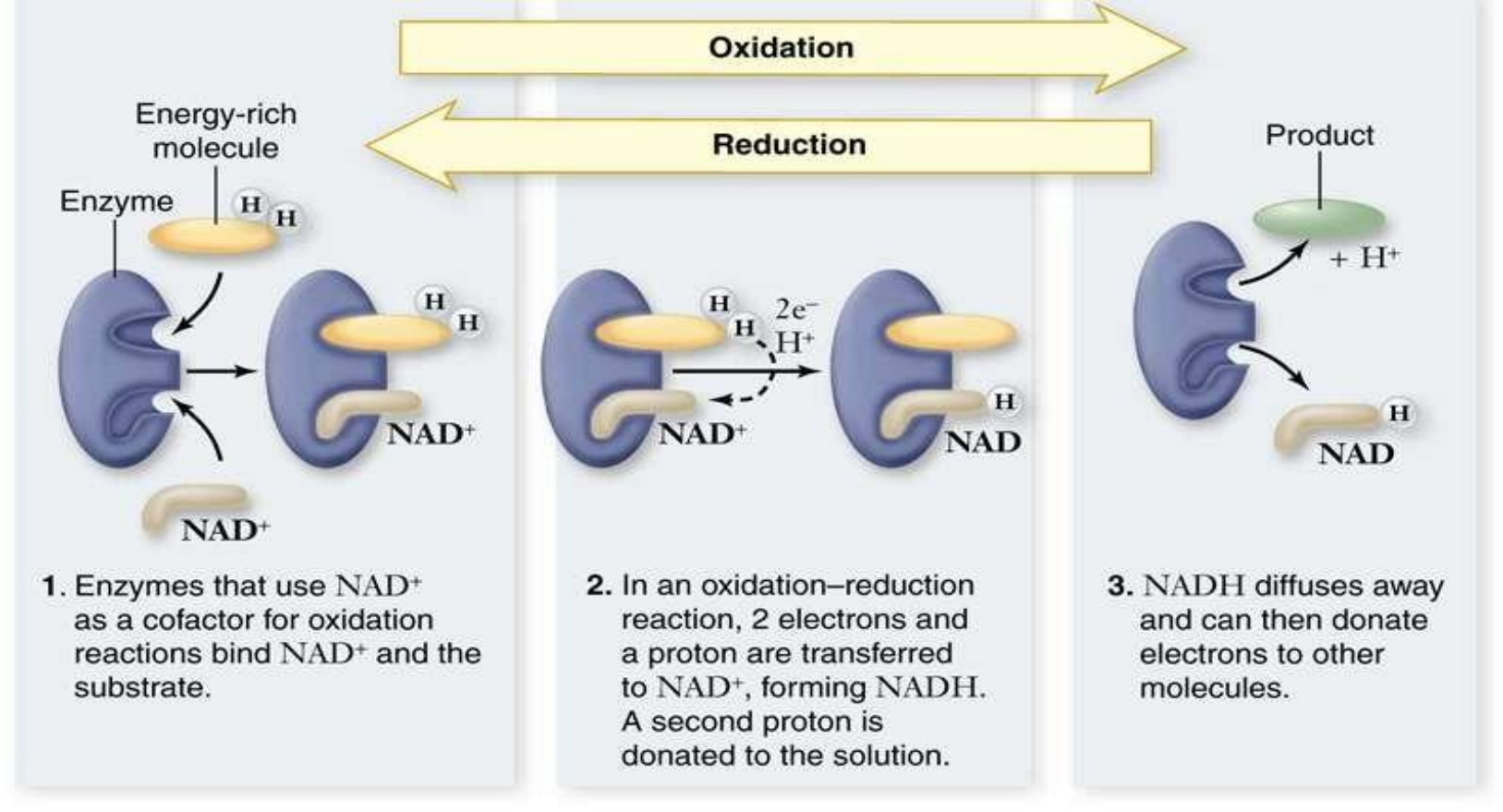
During redox reactions, electrons carry energy from one molecule to another.

1) NAD$^+$ as an electron carrier.

- NAD accepts 2 electrons and 1 proton to become **NADH**
- **T**he reaction is reversible.

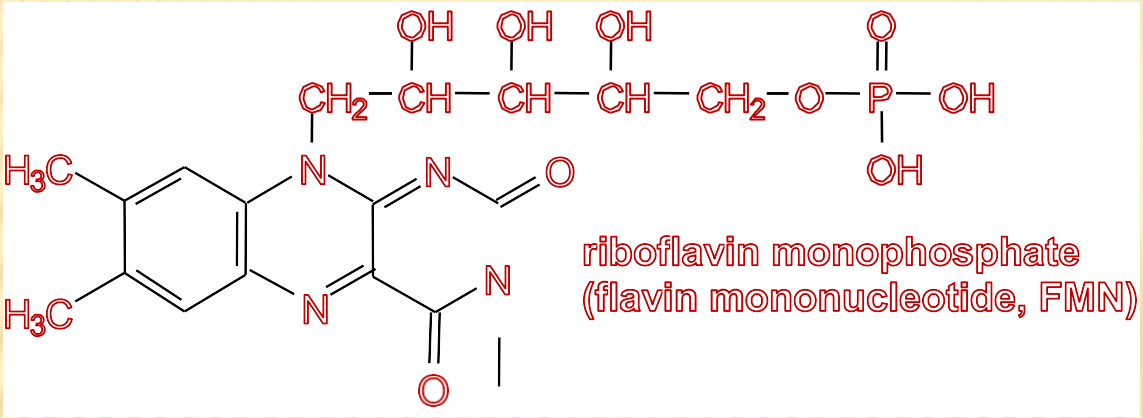


NICOTINAMIDE COENZYME: NAD^+

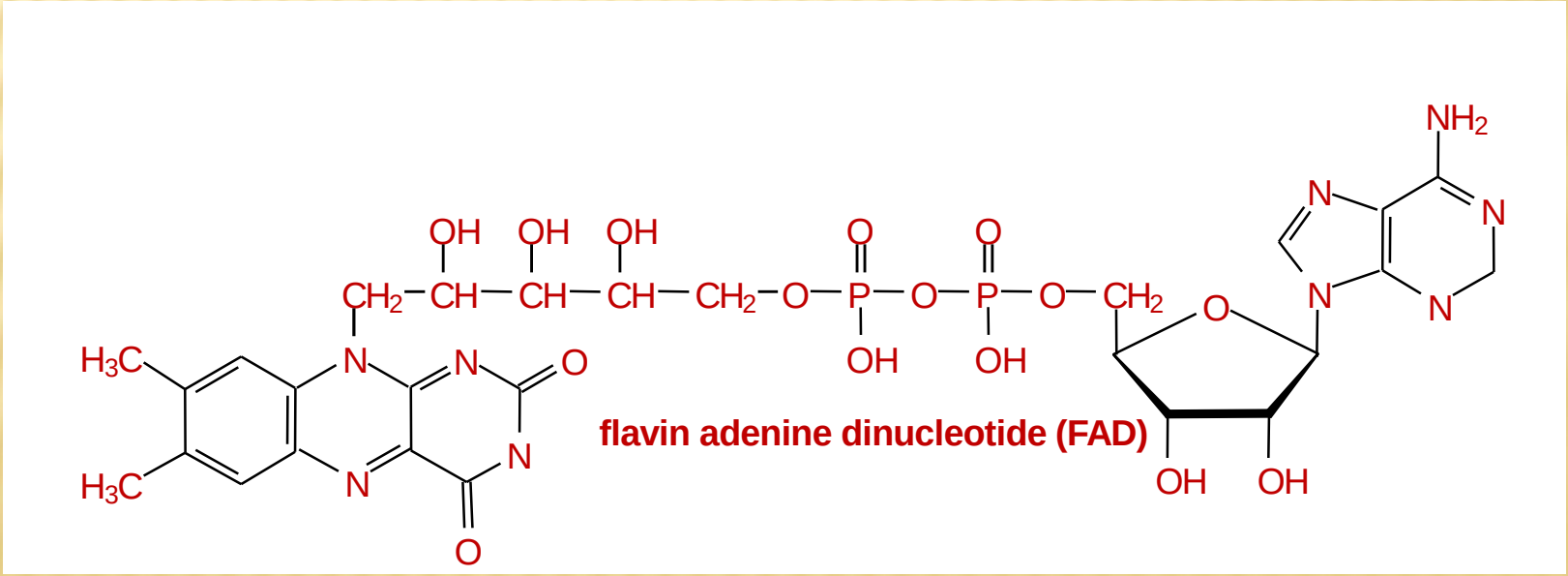


Always a 2-electron reaction transferring 2 e^- and $2H^+$

THE FLAVIN COENZYMES /FLAVOPROTEINS



Always a 2-electron reaction transferring 2 e^- and 2 H^+



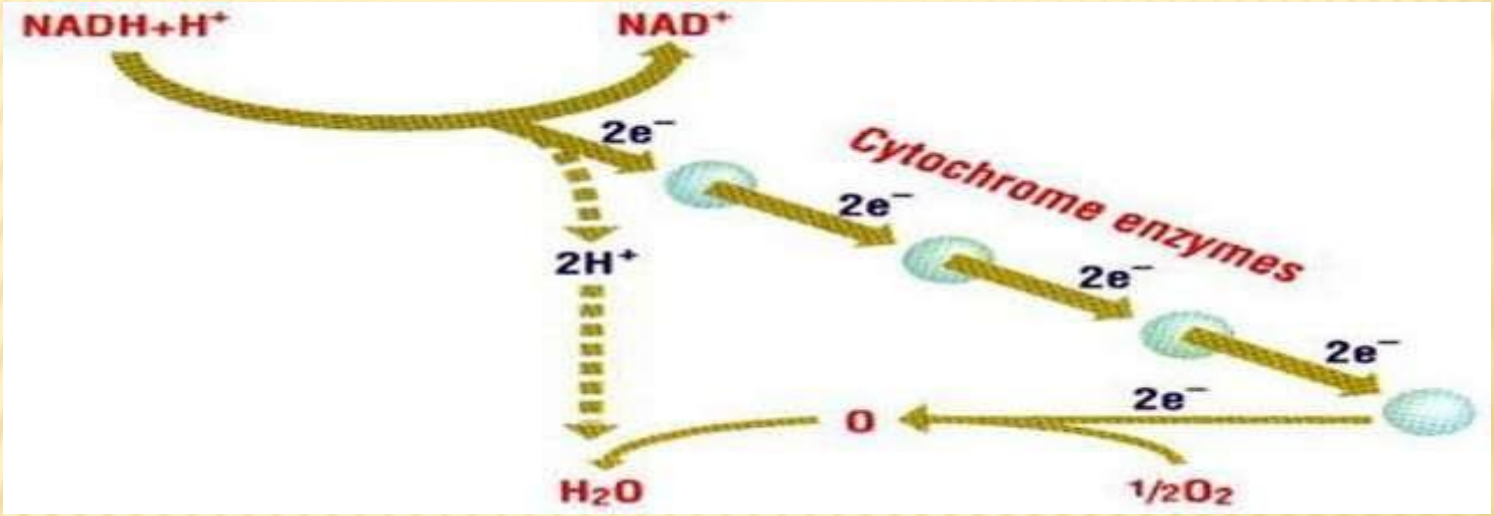
OXIDATION AND REDUCTION OF FLAVIN COENZYMES

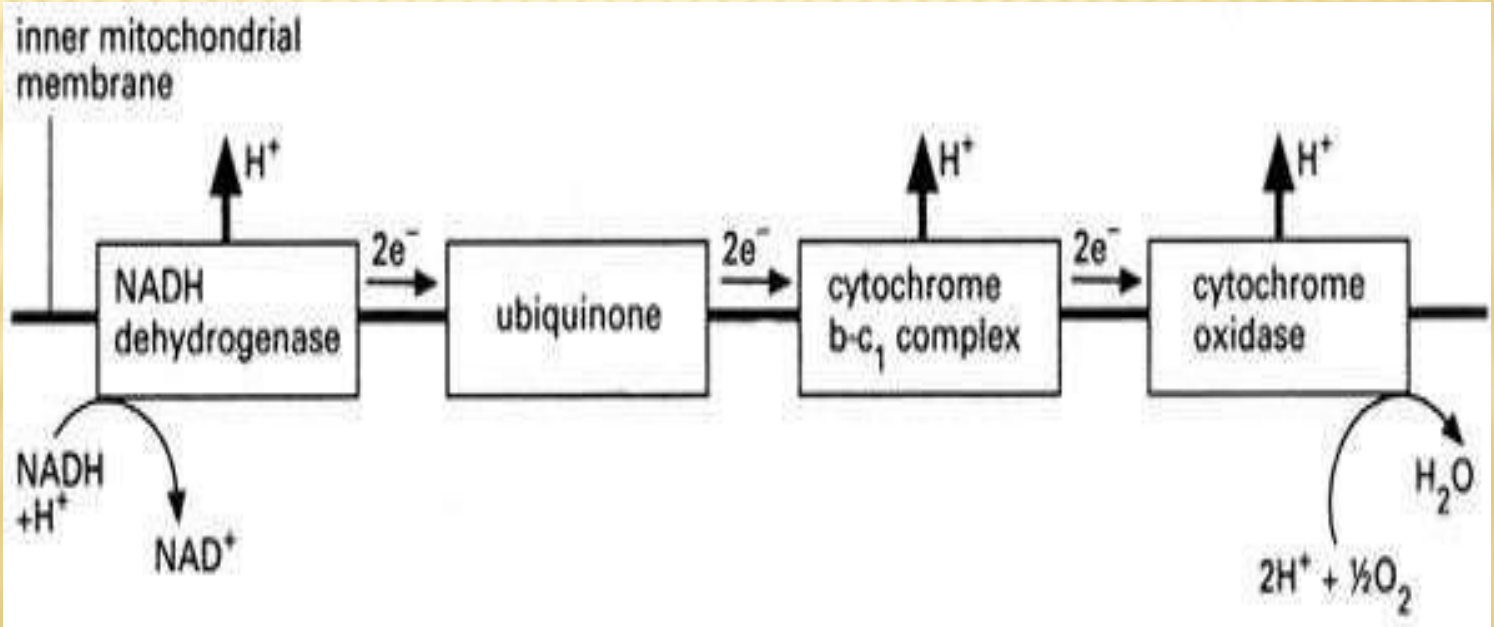
It can accept/donate 1 or 2 e^-. FMN has an important role in mediating e^- transfer between carriers that transfer 2 e^- (e.g., NADH) and those that transfer 1 e^- (e.g., Fe^{+++}).

ELECTRON TRANSPORT CHAIN

- The **electron transport chain (ETC)** is a series of membrane-bound electron carriers.
- Embedded in the mitochondrial inner membrane
- Electrons from NADH and $FADH_2$ are transferred to complexes of the ETC
- Each complex transfers the electrons to the next complex in the chain

ELECTRON TRANSPORT CHAIN - SERIES OF REDOX REACTIONS





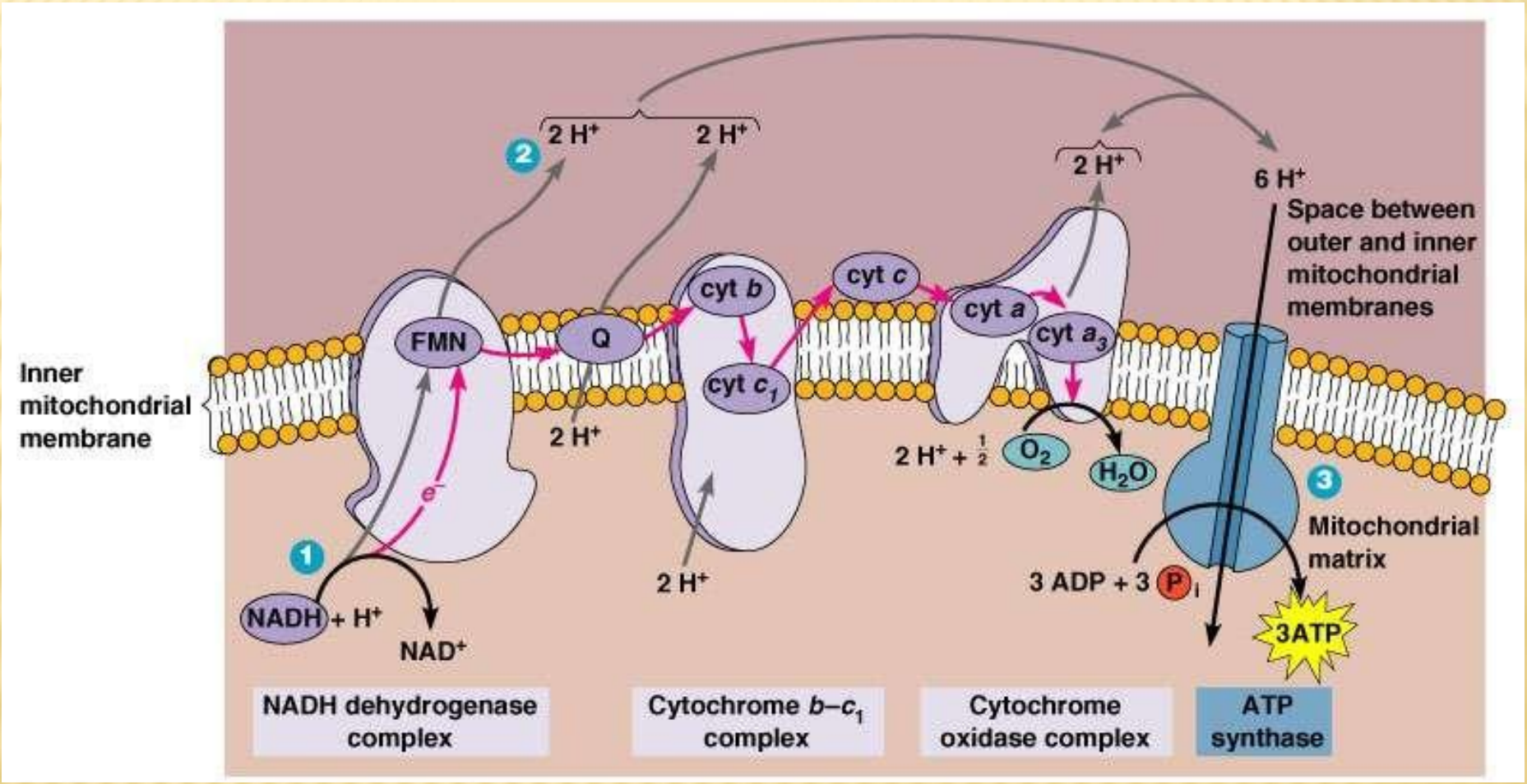
COMPLEXES OF ELECTRON TRANSPORT CHAIN

- Electrons flow through the respiratory chain through a redox span of 1.1 V from NAD^+/NADH to $O_2/2H_2O$ passing through three large protein complexes;
- **NADH-Q oxidoreductase (Complex I),** where electrons are transferred from NADH to coenzyme Q (Q) (also called **ubiquinone**);
- **Q-cytochrome *c* oxidoreductase (Complex III),** which passes the electrons on to cytochrome *c*; and
- **Cytochrome *c* oxidase (Complex IV),** which completes the chain, passing the electrons to O_2 and causing it to be reduced to H_2O .

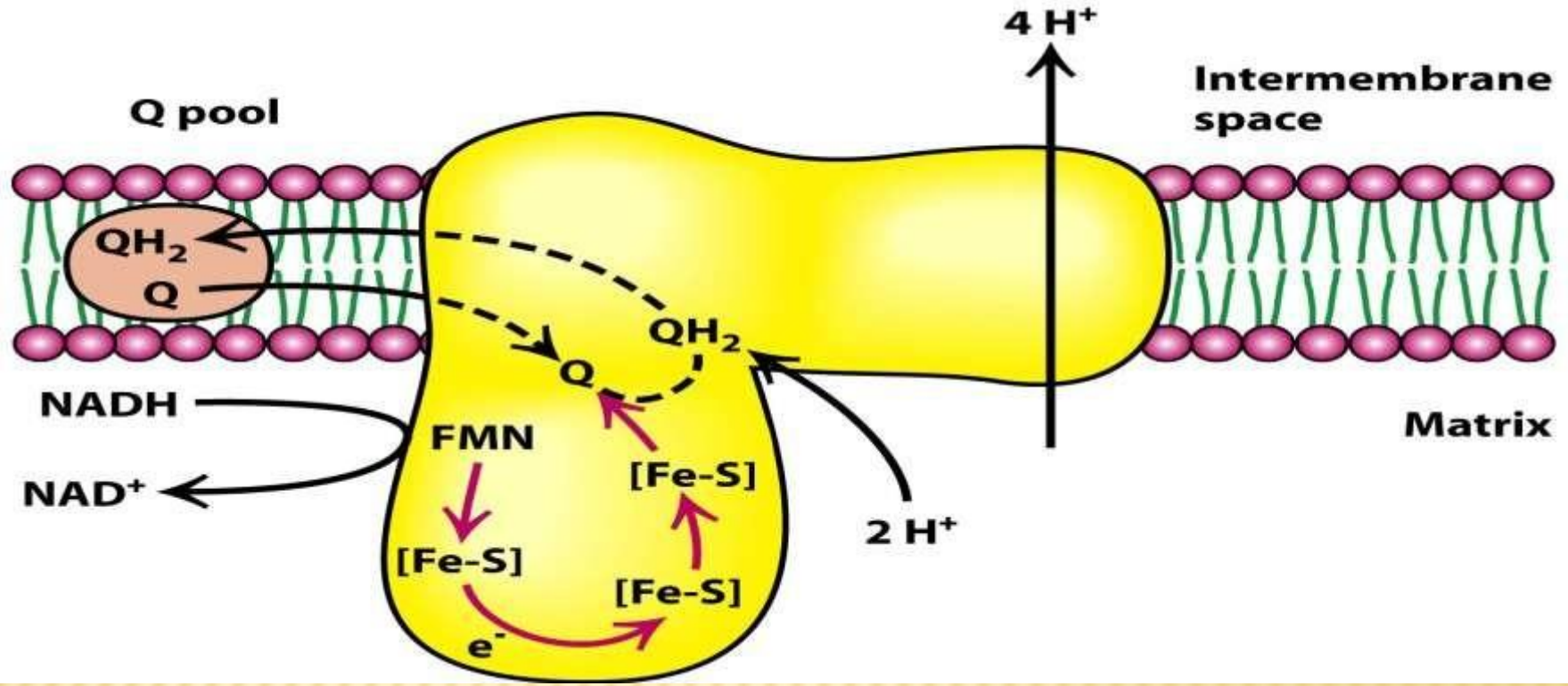
COMPLEXES OF ELECTRON TRANSPORT CHAIN

- Some substrates with more positive redox potentials than NAD^+/NADH (eg, succinate) pass electrons to Q via, **succinate Q reductase (Complex II),** rather than Complex I.
- The four complexes are embedded in the inner mitochondrial membrane, but Q and cytochrome *c* are mobile.
- Q diffuses rapidly within the membrane, while Cytochrome *c* is a soluble protein.
- The flow of electrons through Complexes I, III, and IV results in the pumping of protons from the matrix across the inner mitochondrial membrane into the intermembrane space

ELECTRON CARRIERS IN ETC

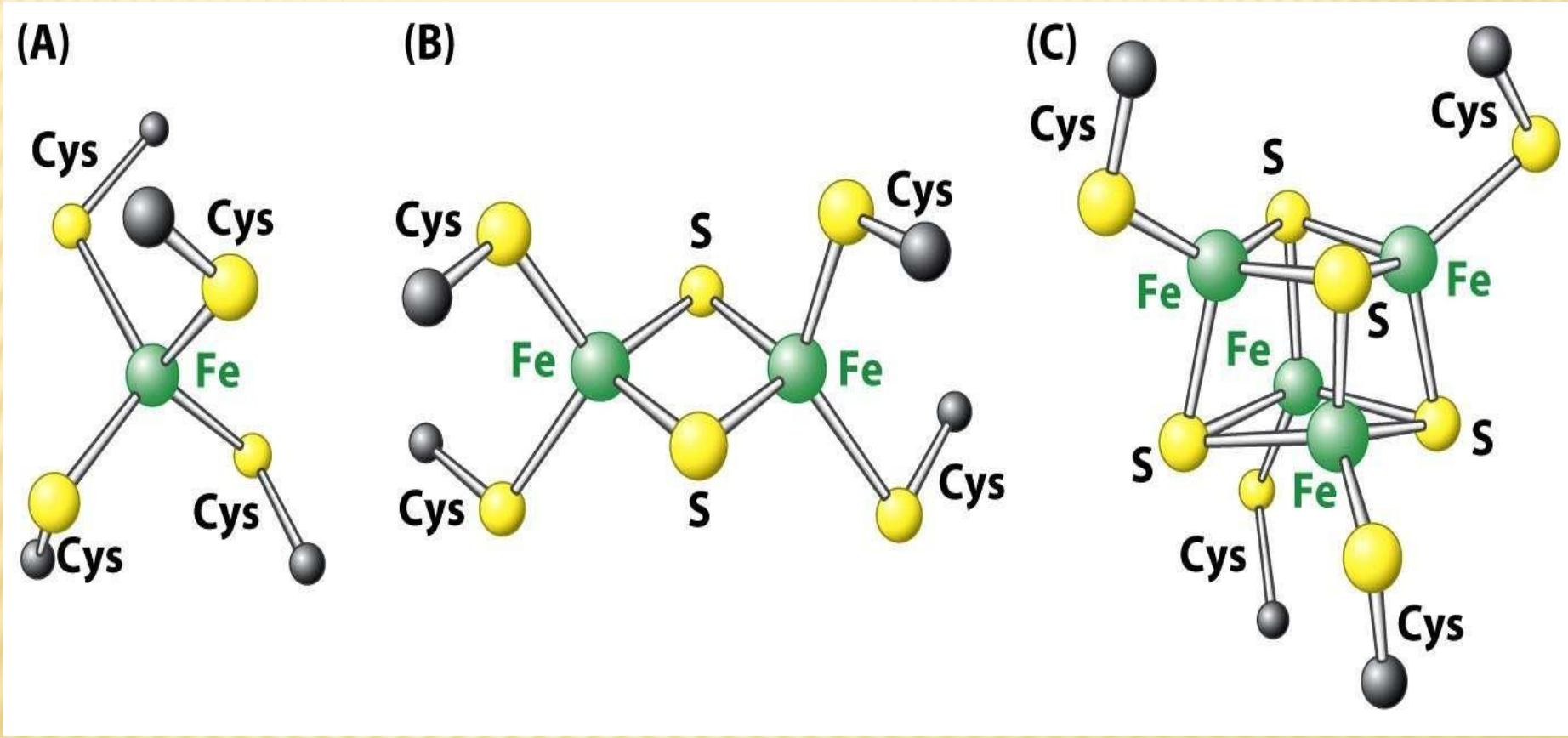


FLAVOPROTEINS



Flavoproteins are important components of Complexes I and II. The oxidized flavin nucleotide (FMN or FAD) can be reduced in reactions involving the transfer of two electrons (to form $FMNH_2$ or $FADH_2$), but they can also accept one electron to form the semiquinone

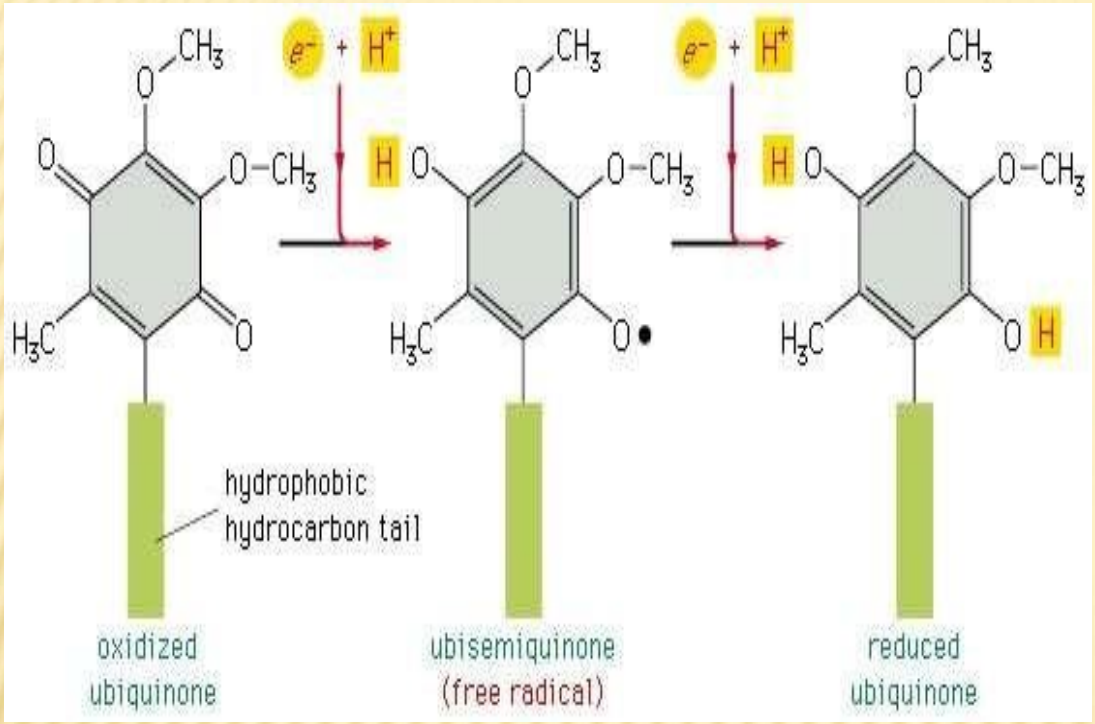
IRON-SULFUR CENTERS (CLUSTERS)



Iron-sulfur centers (Fe-S) are prosthetic groups containing 1-4 iron atoms

Iron-sulfur centers **transfer only one electron**, even if they contain two or more iron atoms.

UBIQUINONE



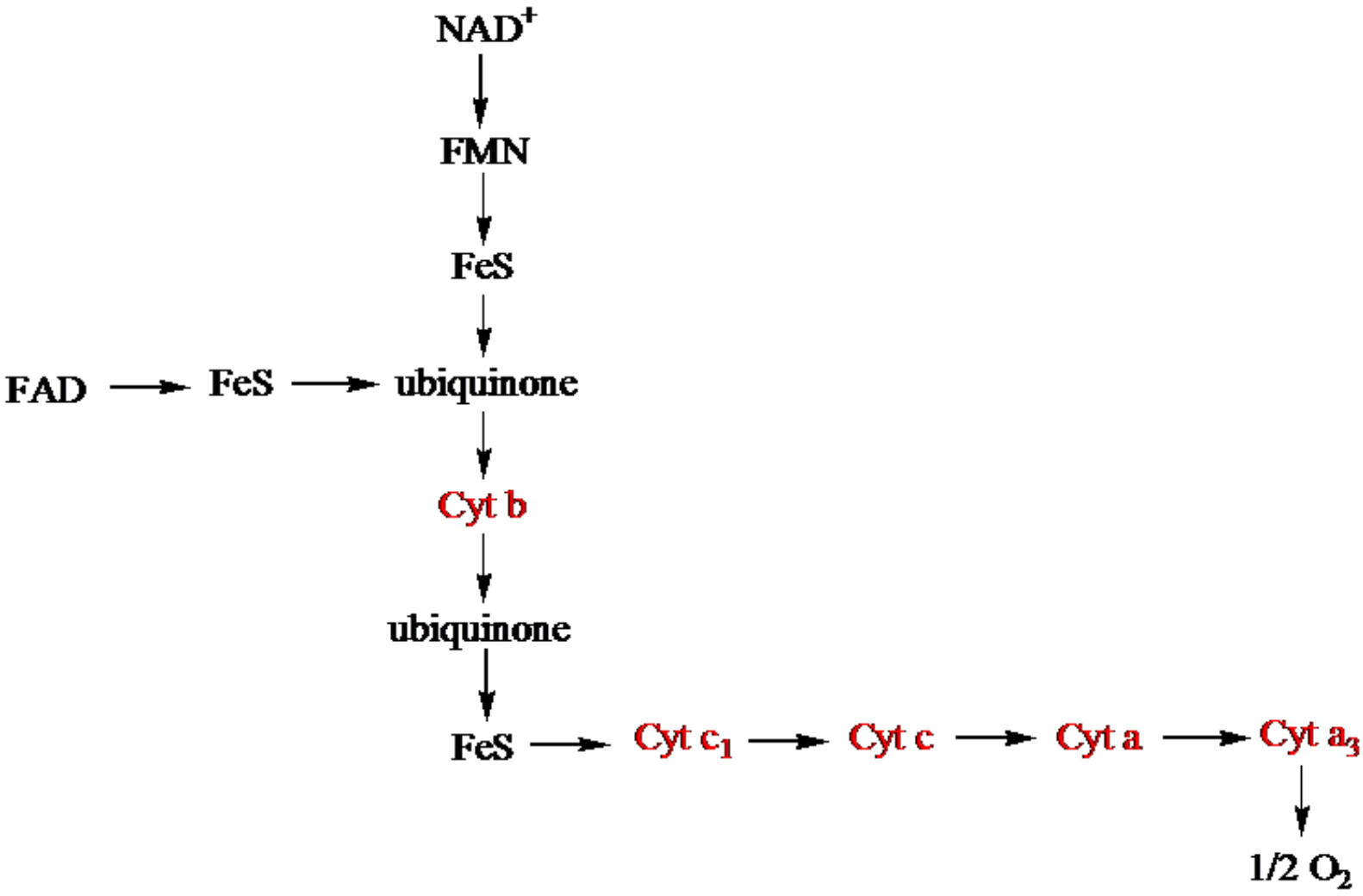
Coenzyme Q (CoQ, Q or ubiquinone) is lipid-soluble. It dissolves in the hydrocarbon core of a membrane.

•the only electron carrier not bound to a protein.
it can accept/donate 1 or 2 e^-. Q can mediate e^- transfer between 2 e^- that transfer and 1 e^- carriers

Free CoQ can undergo a 2 e^- oxidation/reduction:
$Q + 2\ e^- + 2\ H^+ \rightleftharpoons QH_2$.

When bound to special sites in respiratory complexes, **CoQ** can accept **1 e^-** to form a **semiquinone radical ($Q^{\bullet -}$)**.

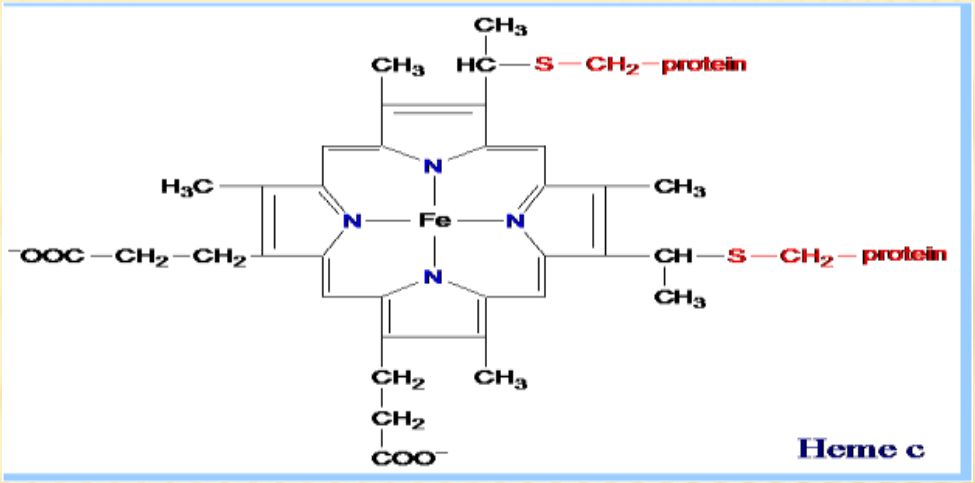
CYTOCHROMES



CYTOCHROMES

- **Cytochromes are electron carriers containing heme .**
- Heme in the 3 classes of cytochromes (**a**, **b**, **c**) differ in substituents on the porphyrin ring.
- Some cytochromes(b,c1,a,a3) are part of large **integral membrane protein complexes**.
- **Cytochrome c** is a small, water-soluble protein.

STRUCTURE OF CYTOCHROME

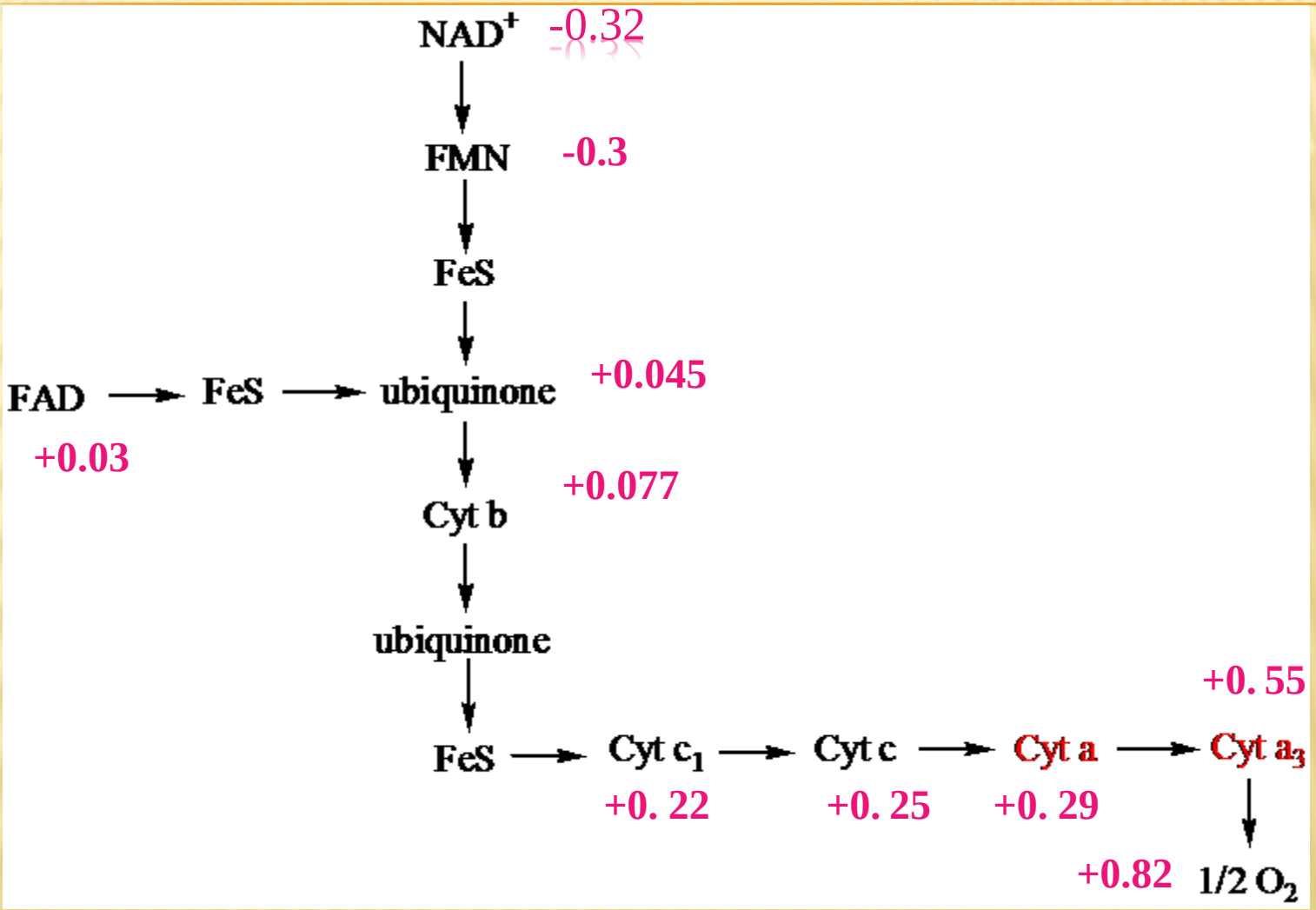


- **Heme** is a prosthetic group of **cytochromes**.
- Heme contains an iron atom in a porphyrin ring system.
- The heme iron can undergo 1 e^- transition between ferric and ferrous states: $Fe^{3+} + e^- \leftrightarrow Fe^{2+}$
- Copper ions besides two heme A groups (*a* and a_3) act as electron carriers in Cyta,a3
$Cu^{2+} + e^- \leftrightarrow Cu^+$

ELECTRON CARRIERS

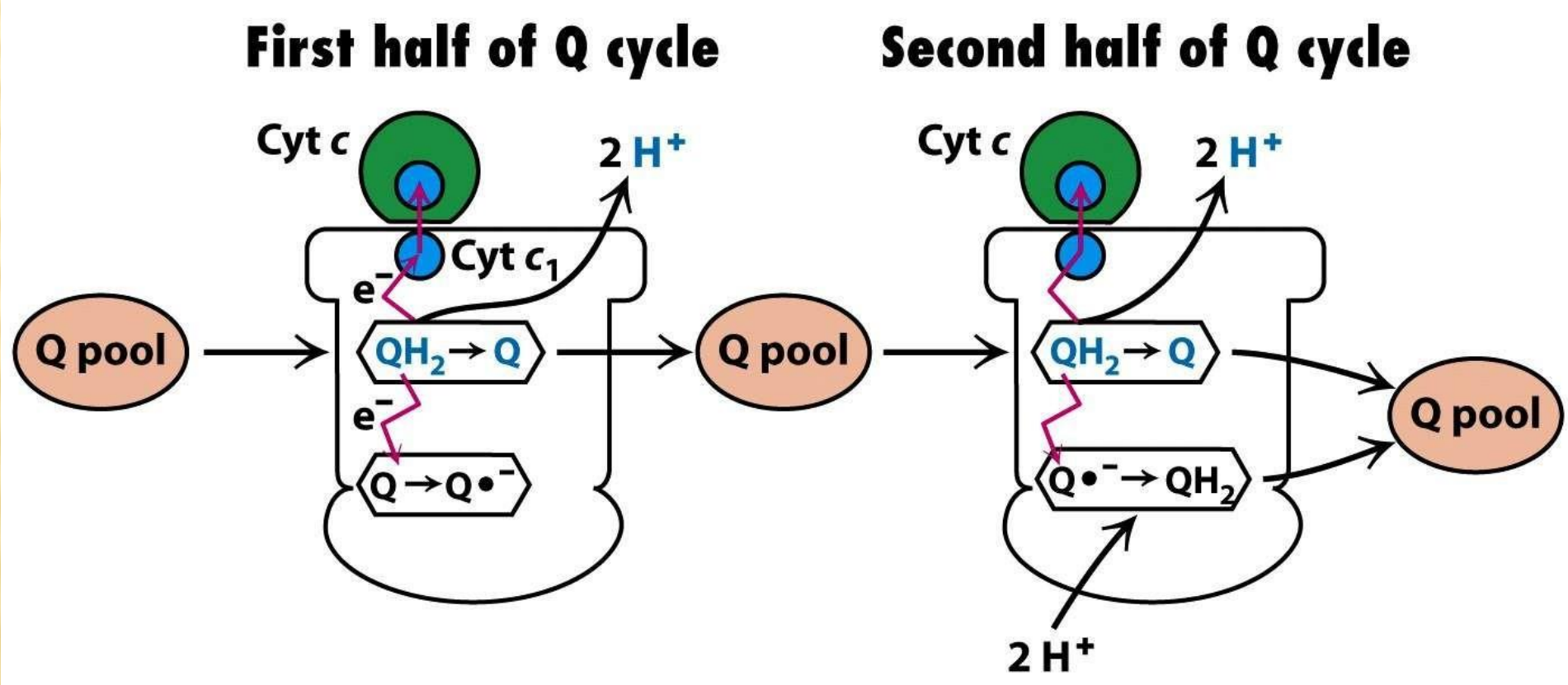
- NAD^+, flavins andCo Q carry electrons and H^+
- Cytochromes and non-haem iron proteins carry only electrons.
- NAD^+ FAD undergoes only a 2 e^- reaction;
- Cytochromes undergo only 1e^- reactions
- FMN Q undergoes 1e^- and 2 e^- reaction

ORDER AND REDOX POTENTIALS

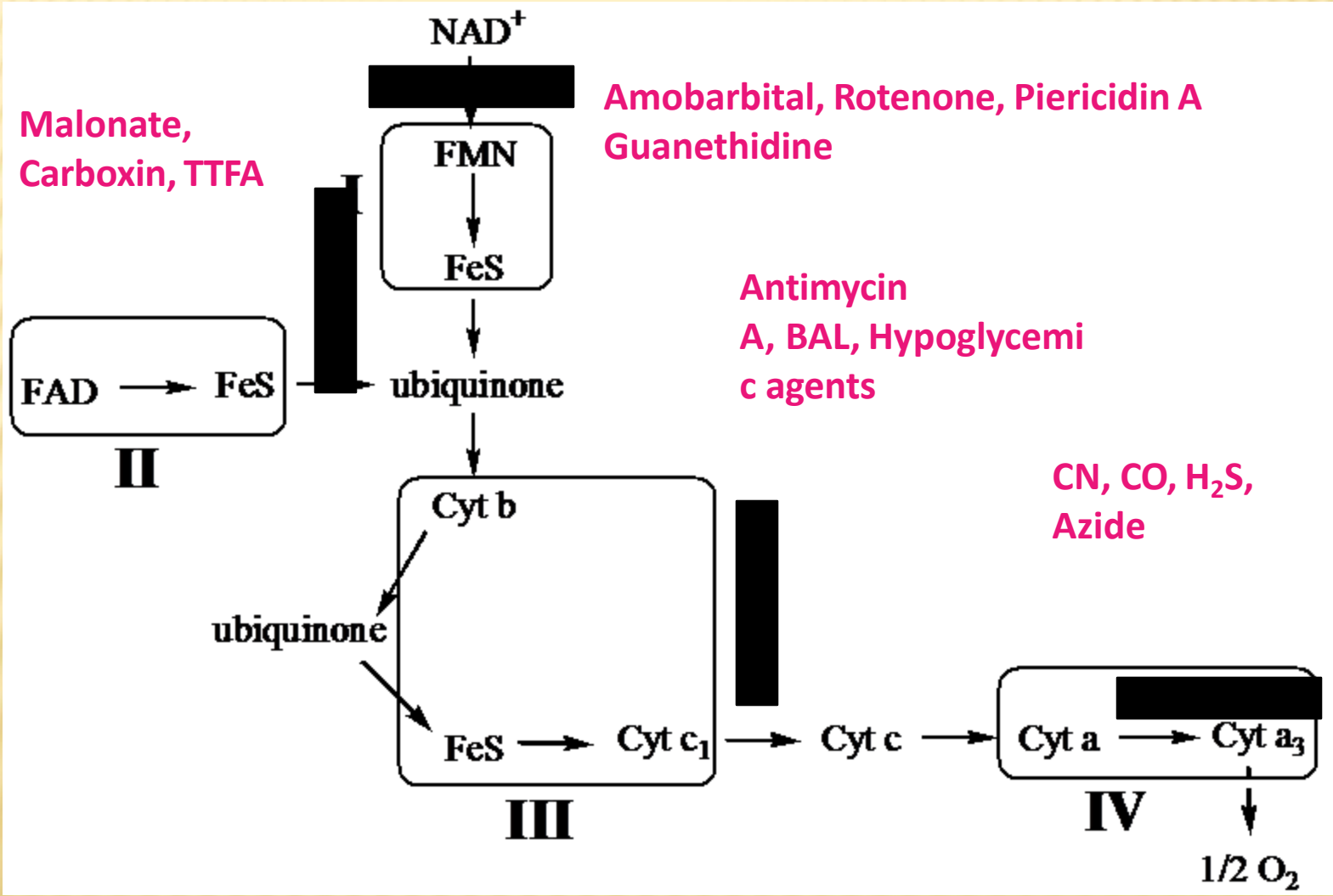


The electron carriers are arranged in terms of rising redox potential in the electron transport chain.

Q CYCLE :THE MECHANISM OF H^+ TRANSPORT IN COMPLEX III



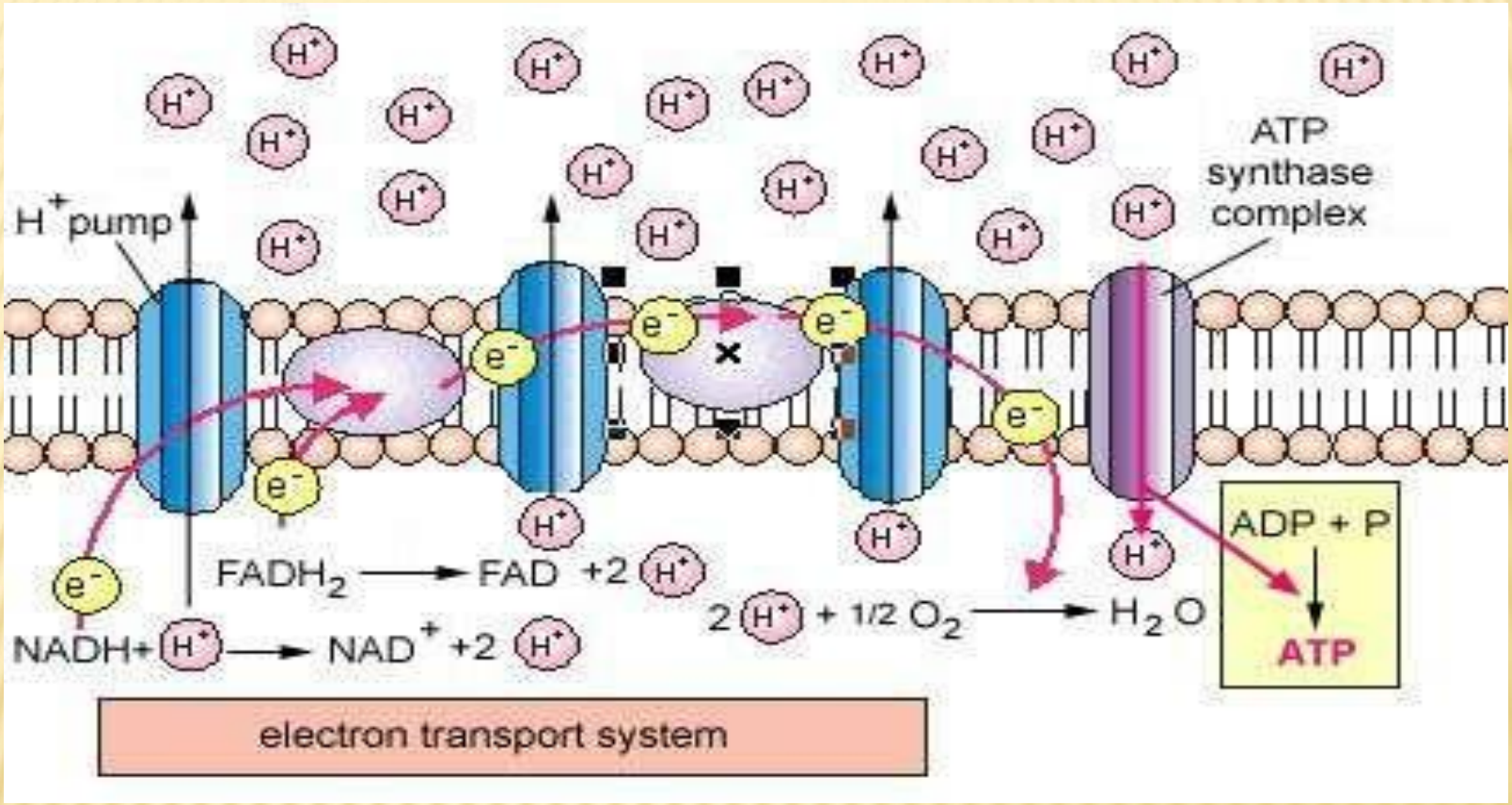
SITE SPECIFIC INHIBITORS OF ETC



INHIBITORS OF ETC

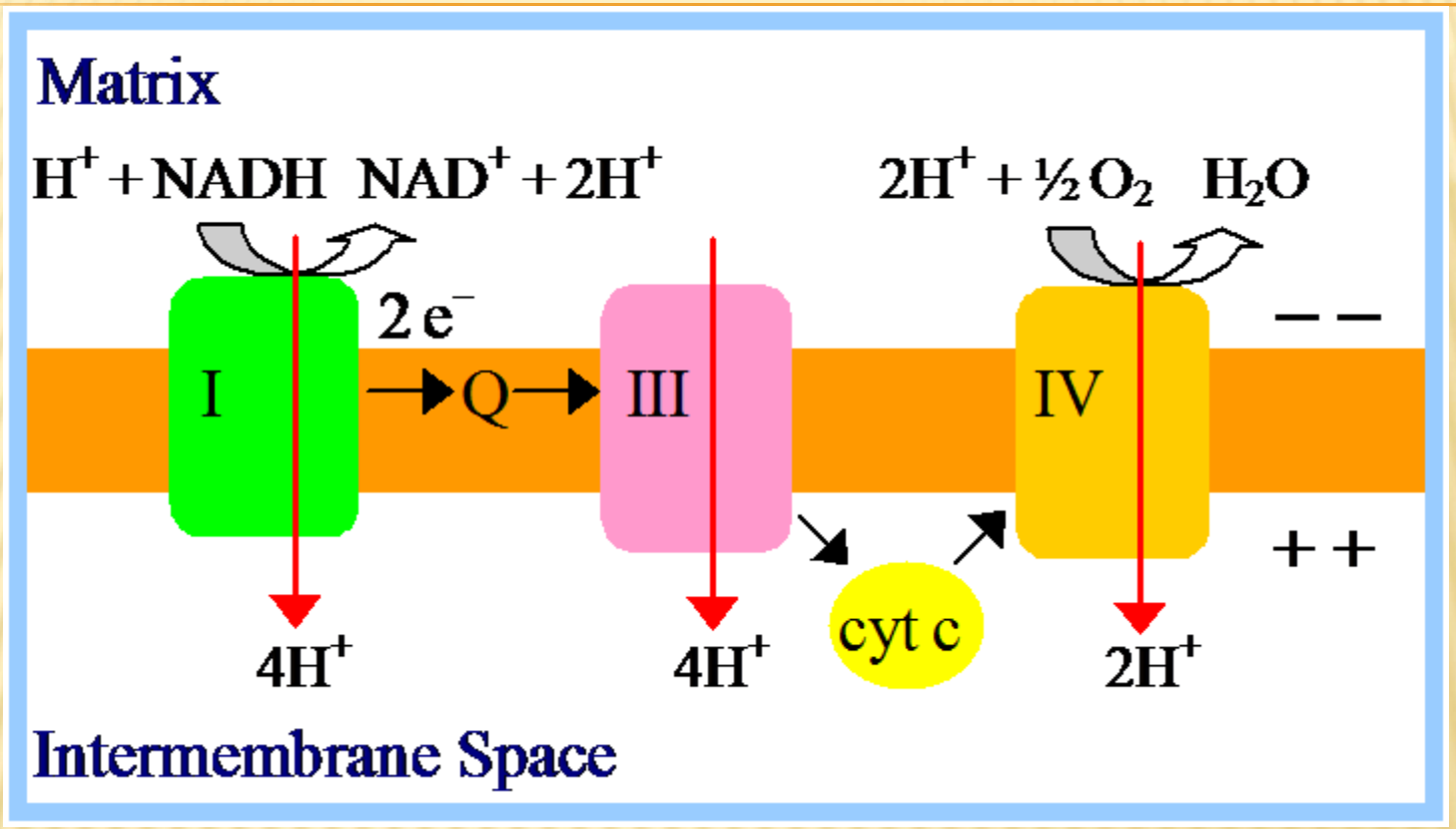
- **Barbiturates** such as Amobarbital inhibit electron transport via Complex I by blocking the transfer from Fe-S to Q. At sufficient dosage, they are fatal in vivo.
- **Antimycin A** and **dimercaprol** inhibit the respiratory chain at Complex III.
- The classic poisons **H_2S**, **carbon monoxide,** and **cyanide** inhibit Complex IV and can therefore totally arrest respiration.
- **Malonate** is a competitive inhibitor of Complex II.
- **Atractyloside** inhibits oxidative phosphorylation by inhibiting the transporter of ADP into and ATP out of the mitochondrion .

H^+ /PROTON TRANSPORT



Complexes I, III, and IV act as **proton pumps.** Since the inner mitochondrial membrane is impermeable to ions in general and particularly to protons, these accumulate in the intermembrane space, creating the proton motive force predicted by the chemiosmotic theory.

PROTON TRANSPORT



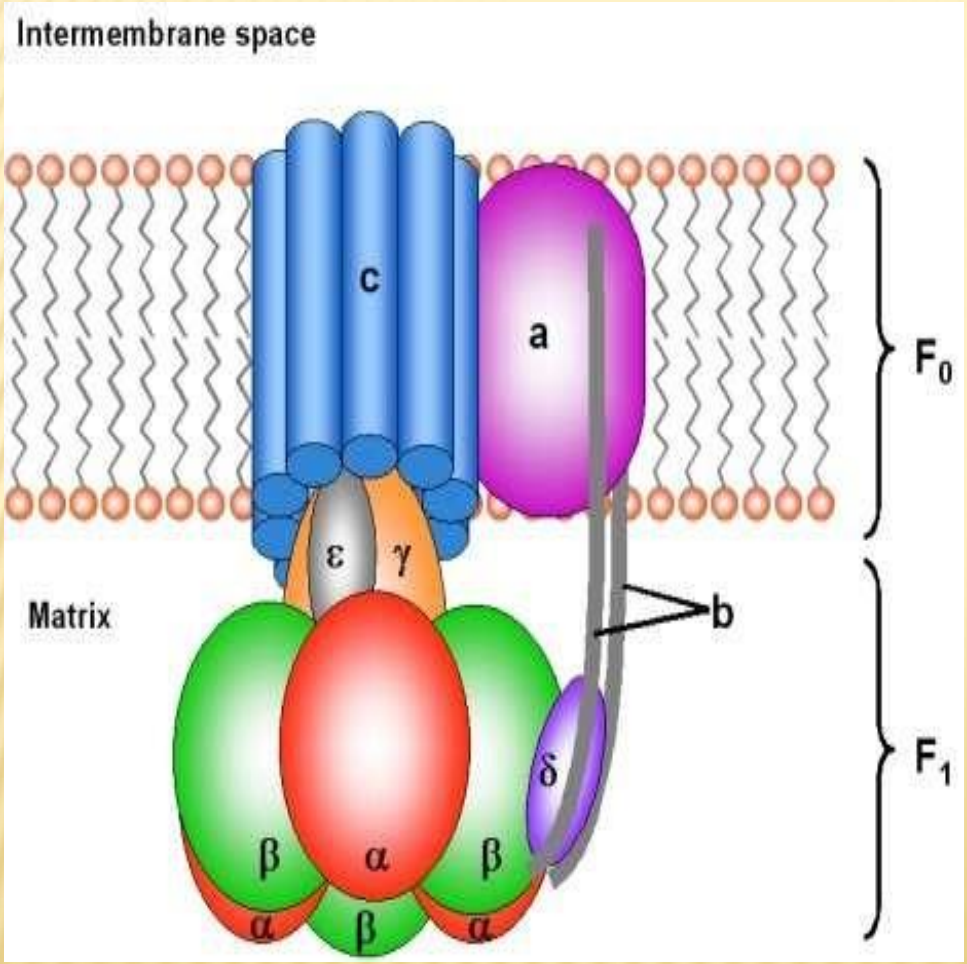
$4H^+$ are pumped per 2e- passing through complex III.

The H^+/e- ratio is less certain for the other complexes: probably $4H^+$/2e- for complex I; $2H^+/2e^-$ for complex IV.

ATP SYNTHASE COMPLEX

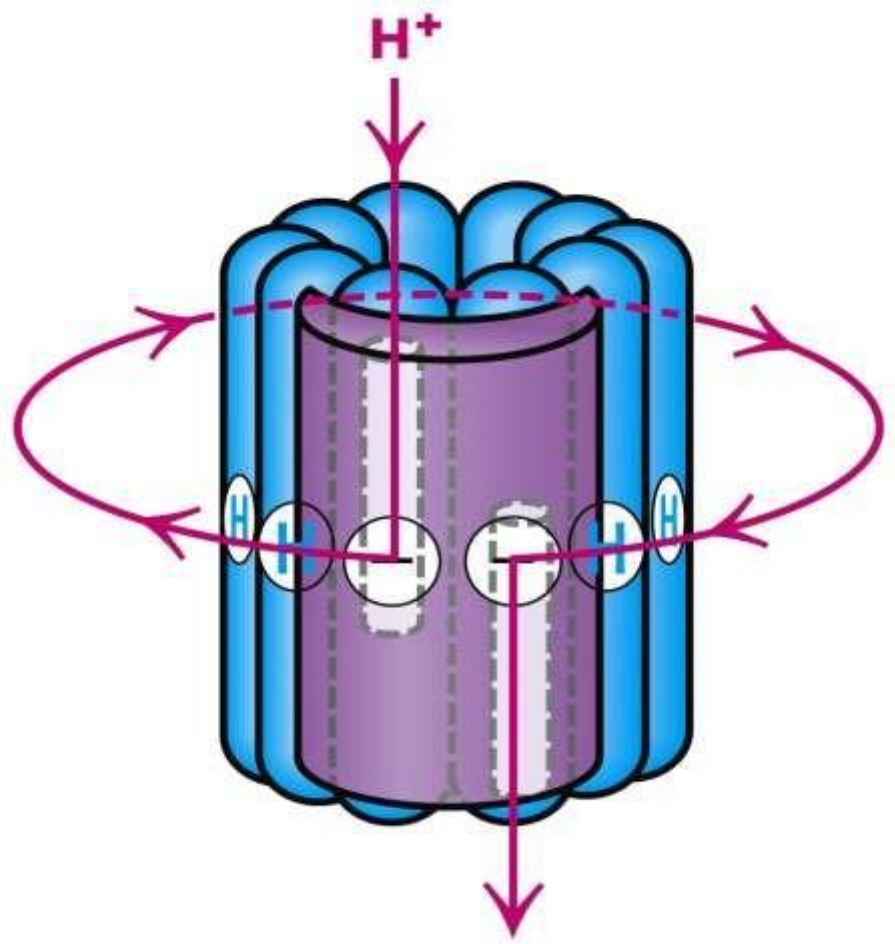
- ATP synthase is embedded in the inner membrane, together with the respiratory chain complexes .
- Several subunits of the protein form a ball-like shape arranged around an axis known as F_1, which projects into the matrix and contains the phosphorylation mechanism .
- F_1 is attached to a membrane protein complex known as F_0, which also consists of several protein subunits.
- F_0 spans the membrane and forms a proton channel.
- The flow of protons through F_0 causes it to rotate, driving the production of ATP in the F_1 complex.

ATP SYNTHASE COMPLEX



- The enzyme complex consists of an F_0 subcomplex which is a disk of "C" protein subunits.
- Attached is a Ysubunit in the form of a "bent axle." Protons passing through the disk of "C" units cause it and the attached Y subunit to rotate.
- The Ysubunit fits inside the F_1 sub complex of three α and three βsubunits, which are fixed to the membrane and do not rotate.

PROTON MOTION AND ROTATION OF C RING



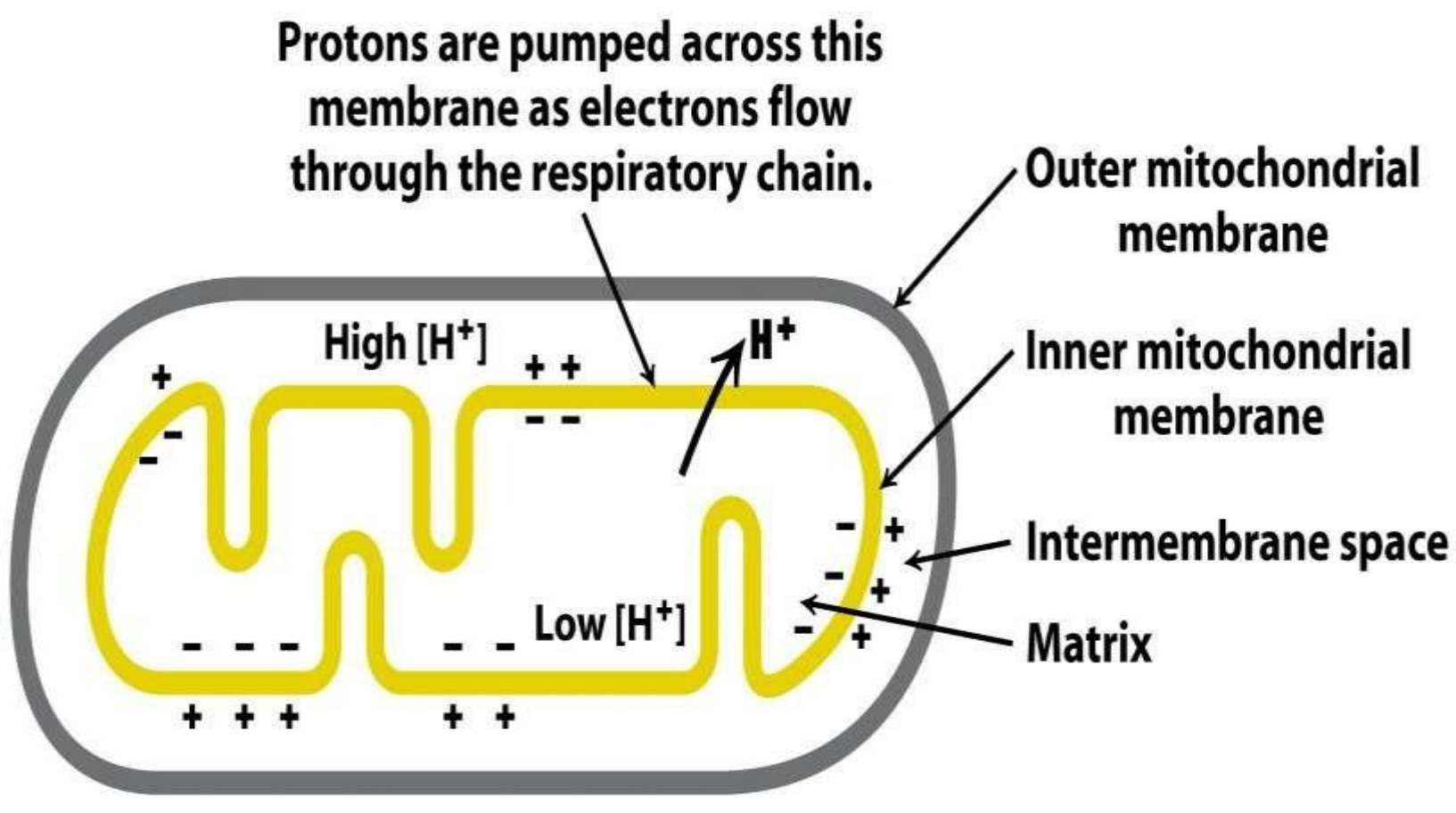
OXIDATIVE PHOSPHORYLATION- CHEMIOSMOSIS

- As the electrons are transferred, some electron energy is lost with each transfer.
- This energy is used to pump protons (H^+) across the membrane from the matrix to the inner membrane space.
- A **proton gradient** is established.

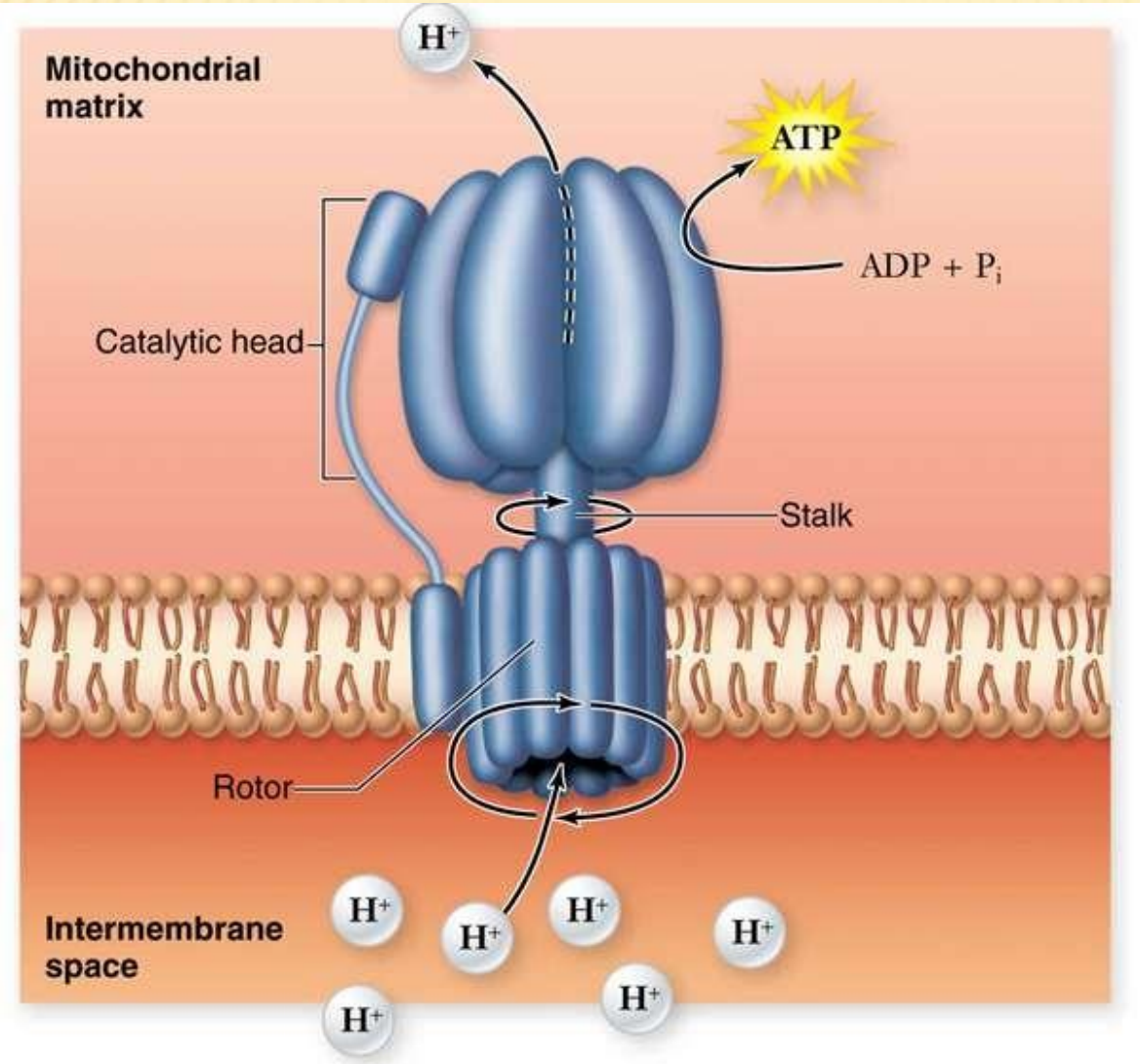
OXIDATIVE PHOSPHORYLATION-CHEMIOSMOSIS

- The higher negative charge in the matrix attracts the protons (H^+) back from the intermembrane space to the matrix.
- The accumulation of protons in the intermembrane space drives protons into the matrix via diffusion.
- Most protons move back to the matrix through **ATP synthase**.
- ATP synthase uses the energy of the proton gradient to synthesize ATP from ADP + P_i.

CHEMIOSMOTIC HYPOTHESIS

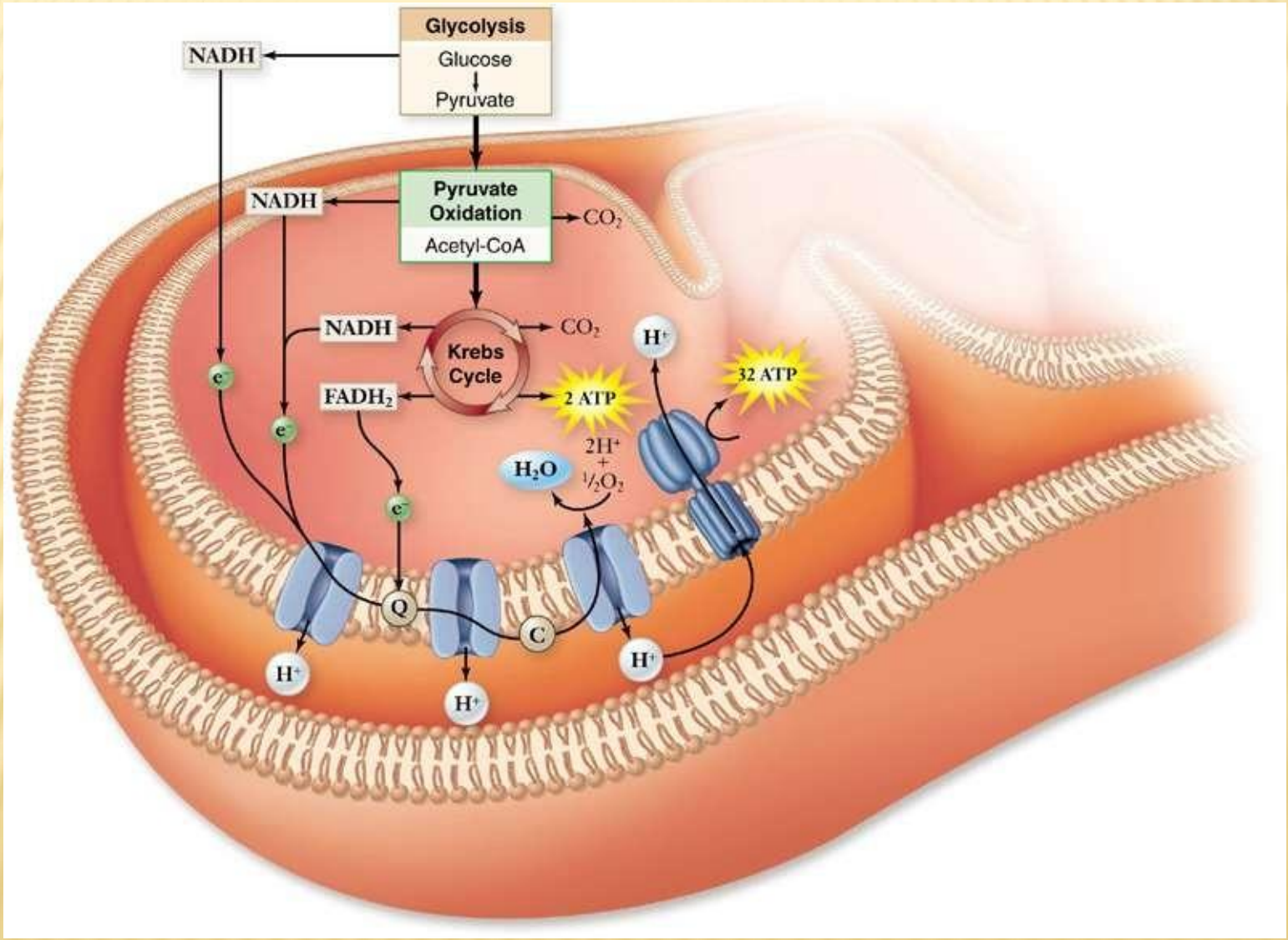


OXIDATIVE PHOSPHORYLATION

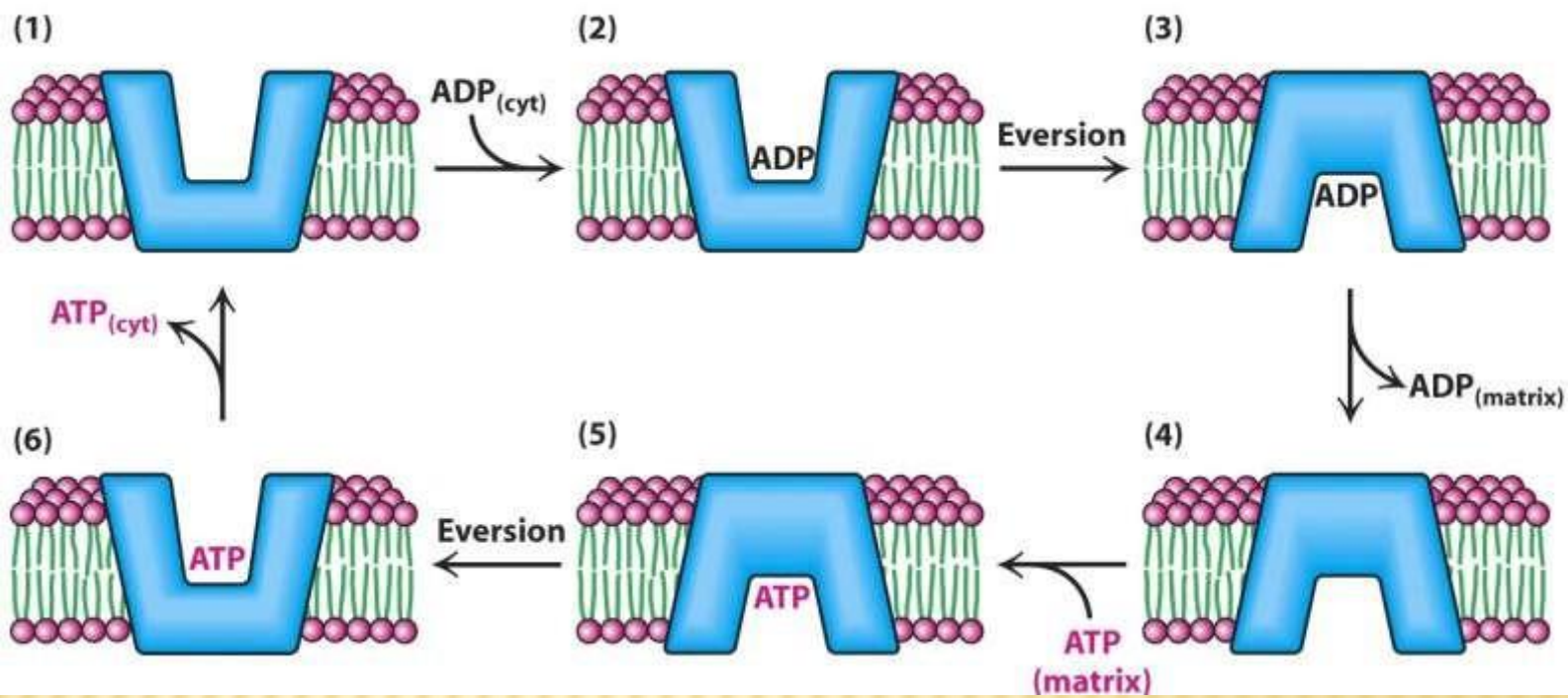


The chemiosmotic theory, proposed by Peter Mitchell in 1961, postulates that the two processes are coupled by a proton gradient across the inner mitochondrial membrane so that **the proton motive force** caused by the electrochemical potential difference (negative on the matrix side) drives the mechanism of ATP synthesis

OXIDATIVE PHOSPHORYLATION



ATP/ ADP EXCHANGE



- ATP/ADPexchange transporter is inhibited by Atractyloside and Bongregate

UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION

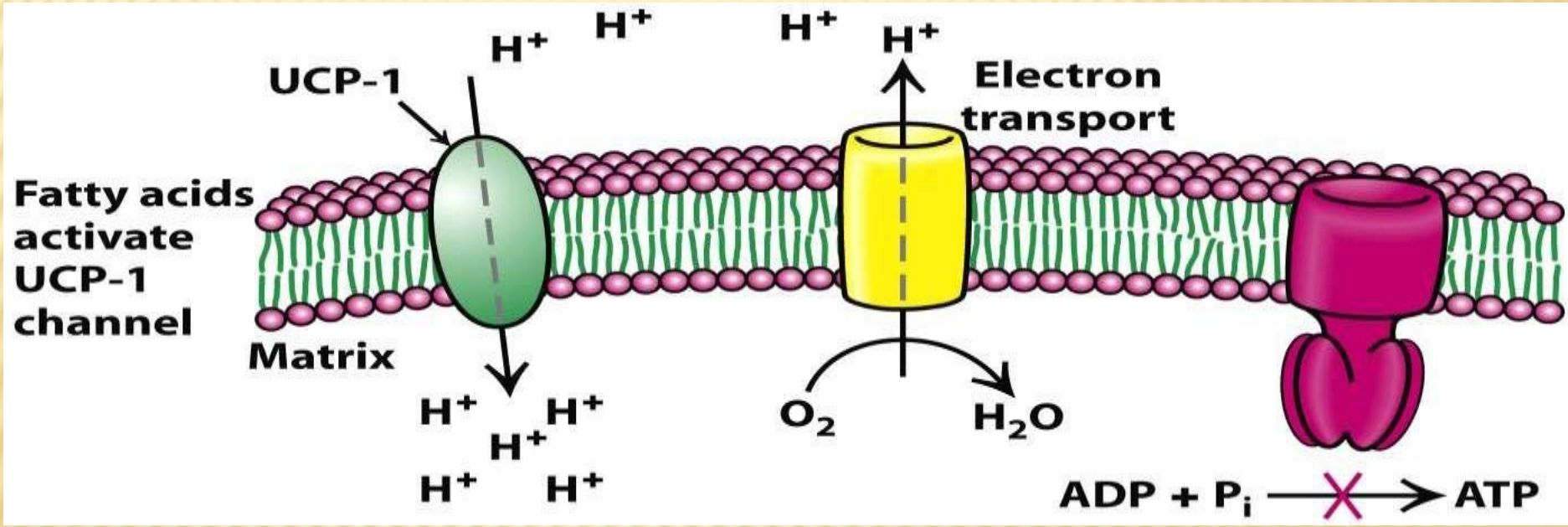
- **Uncouplers** dissociate oxidation in the respiratory chain from phosphorylation.
- These compounds are toxic in vivo, causing respiration to become uncontrolled, since the rate is no longer limited by the concentration of ADP or P_i.
- **2,4-dinitrophenol**
- **2, 4- dinitrocresol**
- **CCCP**
- **TCCP**
- **Valinomycin**
- **High dose of Aspirin**
- The antibiotic **oligomycin** completely blocks oxidation and phosphorylation by blocking the flow of protons through ATP synthase

UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION

Physiological Uncouplers

- Long chain fatty acids
- Thyroxin
- Brown Adipose tissue-**Thermogenin (or the uncoupling protein)** is a physiological uncoupler found in brown adipose tissue that functions to generate body heat, particularly for the newborn and during hibernation in animals
- Calcium ions

MECHNISM OF ACTION OF UNCOUPLING PROTEIN-THERMOGENIN



- Proton gradient is dissipated, no ATP formation

P: O RATIO

- Defined as the number of inorganic phosphate molecules incorporated in to ATP for every atom of oxygen consumed.
- Oxidation of NADH yields 3 ATP molecules(P: O ratio 3, Latest concept 2.5)
- Oxidation of FADH2 yields 2 ATP molecules (P: O ratio 2, Latest concept 1.5)

REGULATION OF ATP SYNTHESIS

- The rate of respiration of mitochondria can be controlled by the availability of ADP.
- This is because oxidation and phosphorylation are tightly coupled; ie, oxidation cannot proceed via the respiratory chain without concomitant phosphorylation of ADP.
- This is called respiratory control or acceptor control.

SUBSTRATE SHUTTLES

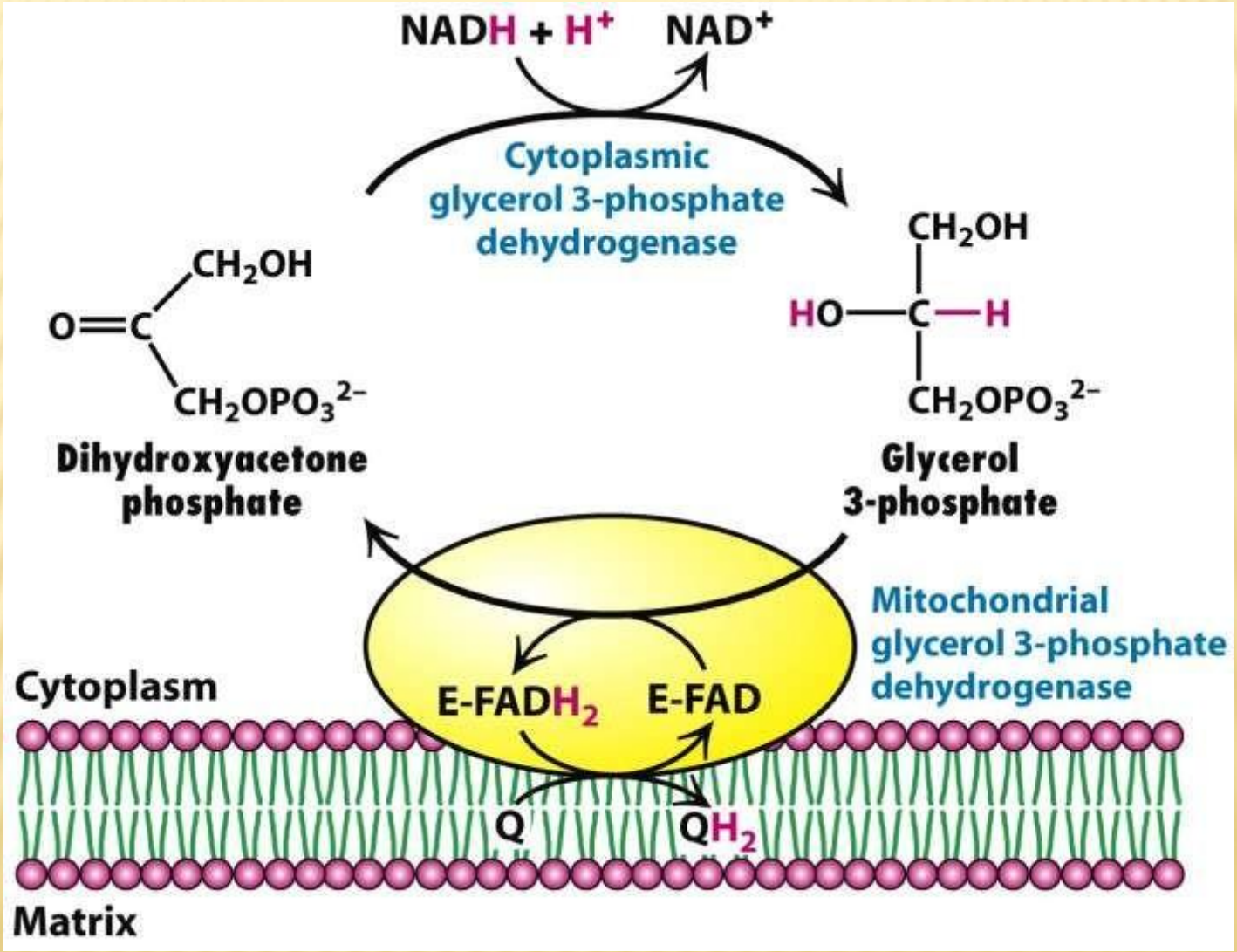
- Oxidation of Extra mitochondrial NADH Is Mediated by Substrate Shuttles.
- NADH cannot penetrate the mitochondrial membrane, but it is produced continuously in the cytosol **by 3-phosphoglyceraldehyde dehydrogenase, an enzyme in the glycolysis sequence.**
- However, under aerobic conditions, extra mitochondrial NADH does not accumulate and is presumed to be oxidized by the respiratory chain in mitochondria.
- The transfer of reducing equivalents through the mitochondrial membrane requires substrate pairs, linked by suitable dehydrogenases on each side of the mitochondrial membrane

SUBSTRATE SHUTTLES

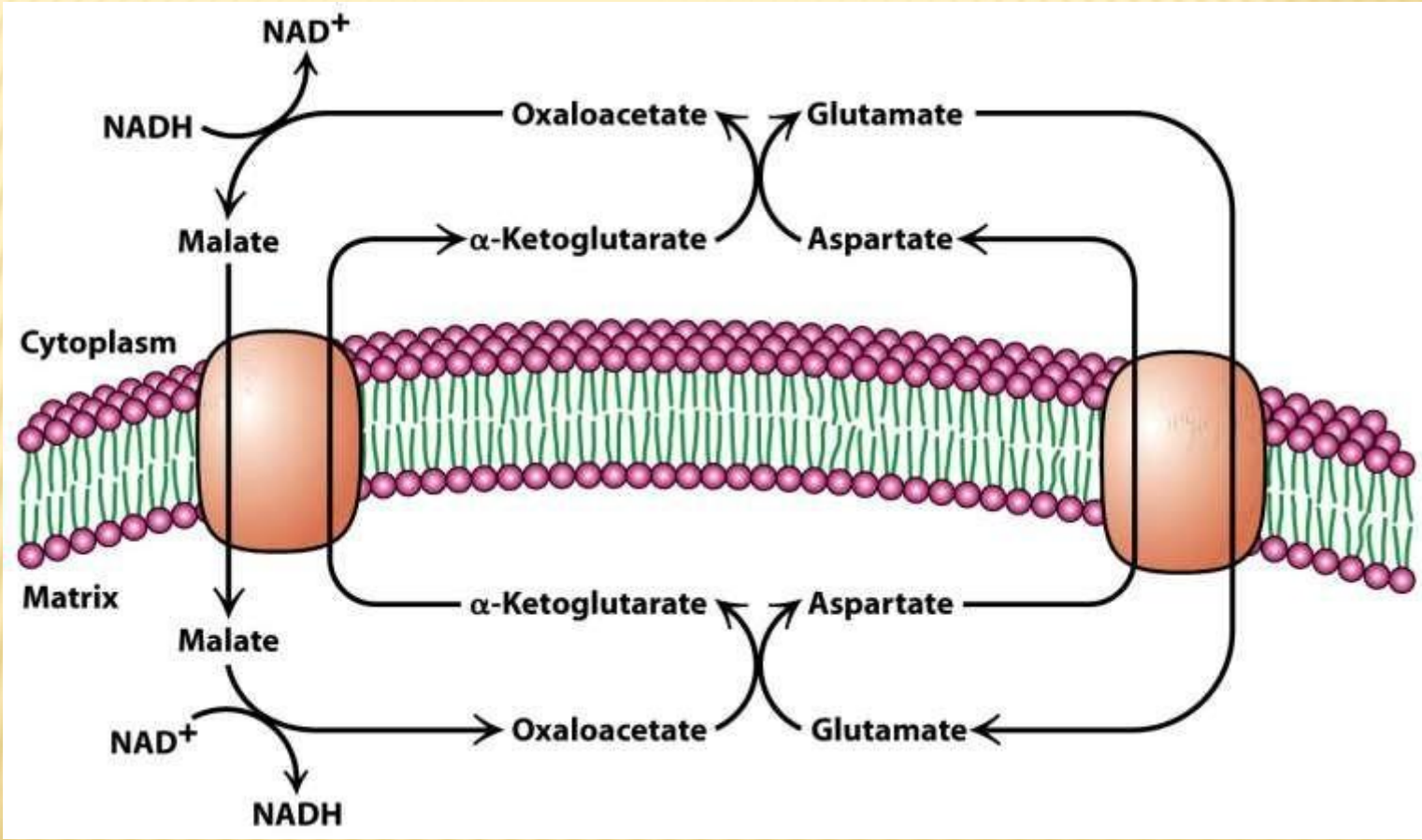
Two main shuttle systems are of importance

- **Glycerophosphate shuttle** – present in skeletal muscle and brain
- **Malate shuttle** – present in liver, kidney and of more universal utility

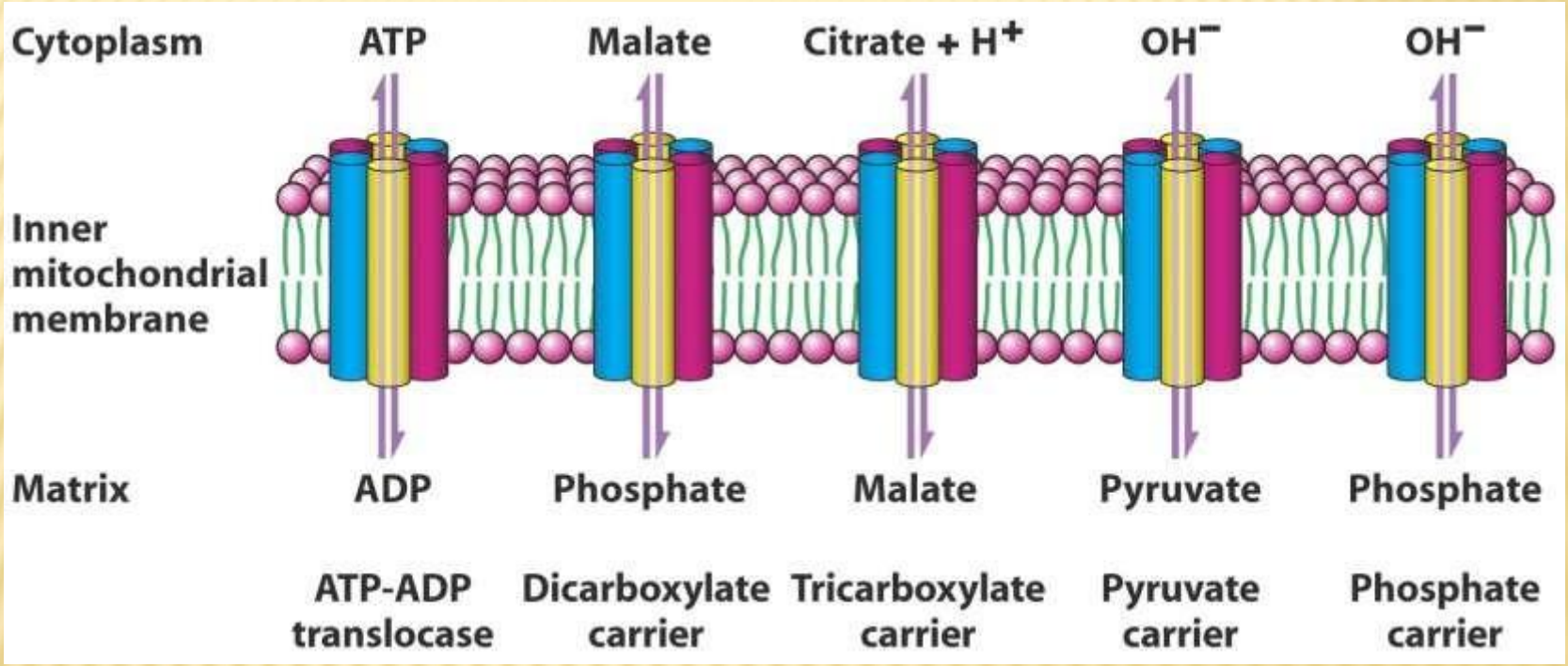
GLYCEROL-3-PHOSPHATE SHUTTLE



MALATE ASPARTATE SHUTTLE



MITOCHONDRIAL INNER MEMBRANE TRANSPORTERS



- Mitochondrial inner membrane is selectively permeable. The movement of biomolecules is mediated through specific transporters

CLINICAL ASPECT

- The condition known as **fatal infantile mitochondrial myopathy and renal dysfunction** involves severe diminution or absence of most oxidoreductases of the respiratory chain.
- **MELAS** (mitochondrial encephalopathy, lactic acidosis, and stroke) is an inherited condition due to NADH:Q oxidoreductase (Complex I) or cytochrome oxidase (Complex IV) deficiency.
- It is caused by a mutation in mitochondrial DNA and may be involved in Alzheimer's disease and diabetes mellitus.
- A number of drugs and poisons act by inhibition of oxidative phosphorylation.

SUMMARY

- Virtually all energy released from the oxidation of carbohydrate, fat, and protein is made available in mitochondria as reducing equivalents (—H or e^-). These are funneled into the respiratory chain, where they are passed down a redox gradient of carriers to their final reaction with oxygen to form water.
- The redox carriers are grouped into four respiratory chain complexes in the inner mitochondrial membrane. Three of the four complexes are able to use the energy released in the redox gradient to pump protons to the outside of the membrane, creating an electrochemical potential between the matrix and the inner membrane space.
- ATP synthase spans the membrane and acts like a rotary motor using the potential energy of the proton gradient or proton motive force to synthesize ATP from ADP and P_i. In this way, oxidation is closely coupled to phosphorylation to meet the energy needs of the cell.