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Subject - Zoology

B.SC -1st year Semester – II
Paper – II: Cell Biology

UNIT- 2

Topic - 2.3 Electron Transport Chain and terminal oxidation

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INTRODUCTION

- The Electron Transport System also called the Electron Transport Chain, is a chain of reactions that converts redox energy available from oxidation of NADH and $FADH_2$, into proton-motive force which is used to synthesize ATP through conformational changes in the ATP synthase complex through a process called oxidative phosphorylation.
- Oxidative phosphorylation is the last step of cellular respiration.
- This stage consists of a series of electron transfer from organic compounds to oxygen while simultaneously releasing energy during the process.

- In aerobic respiration, the final electron acceptor is the molecular oxygen while in anaerobic respiration there are other acceptors like sulfate.
- This chain of reactions is important as it involves breaking down of ATP into ADP and resynthesizing it in the process to ATP, thus utilizing the limited ATPs in the body about 300 times in a day.
- The electron flow takes place in four large protein complexes that are embedded in the inner mitochondrial membrane, together called the respiratory chain or the electron-transport chain.
- This stage is crucial in energy synthesis as all oxidative steps in the degradation of carbohydrates, fats, and amino acids converge at this final stage of cellular respiration, in which the energy of oxidation drives the synthesis of ATP.

Electron Transport Chain Location

- As the citric acid cycle takes place in the mitochondria, the high energy electrons are also present within the mitochondria. As a result, the electron transport chain in eukaryotes also takes place in the mitochondria.
- The mitochondrion is a double-membraned organelle that consists of an outer membrane and an inner membrane that is folded into a series of ridges called cristae.
- There are two compartments in the mitochondria; the matrix and the intermembrane space.

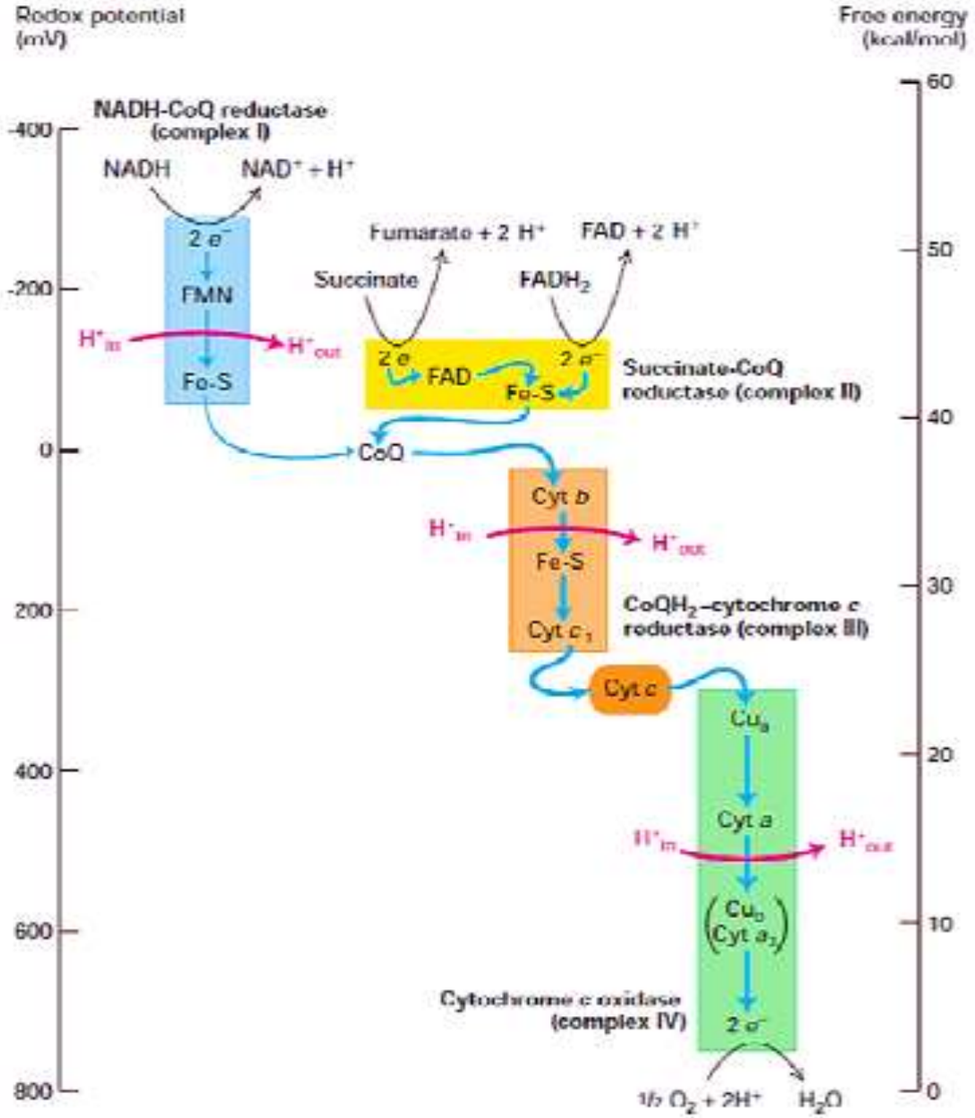
- The outer membrane is highly permeable to ions. It contains enzymes necessary for citric acid cycles while the inner membrane is impermeable to various ions and contains uncharged molecules, electron transport chain and ATP synthesizing enzymes.
- The number of electron transport chains in the mitochondria depends on the location and function of the cell. In the liver mitochondria, there are 10, 000 sets of electron transport chains while the heart mitochondria have three times the number of electron transport chain as in the liver mitochondria.
- The intermembrane space contains enzymes like adenylate kinase, and the matrix contains ATP, ADP, AMP, NAD, NADP, and various ions like Ca^{2+}, Mg^{2+}, etc.

What is Electron Transport Chain (ETC)

- The electron transport chain is a **series of protein complexes** that couple redox reactions, creating an electrochemical gradient that leads to the **creation of ATP.**
- The electron transport chain is the **final component of aerobic respiration** and is the only part of glucose metabolism that **uses atmospheric oxygen.**
- In ETC **electrons move across from NAD^+/NADH to O_2 and form water.**
- Respiratory chain complexes are multi-subunit structures localized to the **inner mitochondrial membrane** comprised of proteins, prosthetic groups such as metal ions and iron-sulfur centers, and cofactors including coenzyme Q10.
- The electron transport chain is an **aggregation of four complexes (I to IV),** together with **associated mobile electron carriers.**
- The electron transport chain is present in **multiple copies in the inner mitochondrial membrane of eukaryotes and the plasma membrane of prokaryotes.**

ETC is a system of electron carrier arranged in their sequential order according to their redox potential from NADH and $FADH_2$ to molecular O_2 and causes the release of energy.

ETC components present according to their redox potential



- The redox potential is used to describe a system's overall reducing or oxidizing capacity.
- Electrons flow downhill spontaneously moving from molecules that are **strong electron DONORS to strong electron ACCEPTORS = move from high energy state to low energy state**

  NADH = strongest donor

  O2 = strongest acceptor
- **NADH** is very good at donating electrons in redox reactions (that is, its electrons are at a high energy level), so it can **transfer its electrons directly to complex I, turning back into NAD^+.**
- As electrons move through complex I in a series of redox reactions, energy is released, and **the complex uses this energy to pump protons from the matrix into the intermembrane space.**
- **$FADH_2$ is not as good at donating electrons as NADH** (that is, its electrons are at a lower energy level), so it cannot transfer its electrons to complex I. Instead, **it feeds them into the transport chain through complex II, which does not pump protons across the membrane.**

Electrons flow from $FADH_2$ and NADH to O_2 through a series of four multiprotein complexes

- Each of the **four large multiprotein complexes** in the respiratory chain spans the inner mitochondrial membrane and **contains several prosthetic groups that participate in moving electrons.**
- These **small nonpeptide organic molecules or metal ions are tightly and specifically associated** with the multiprotein complexes.

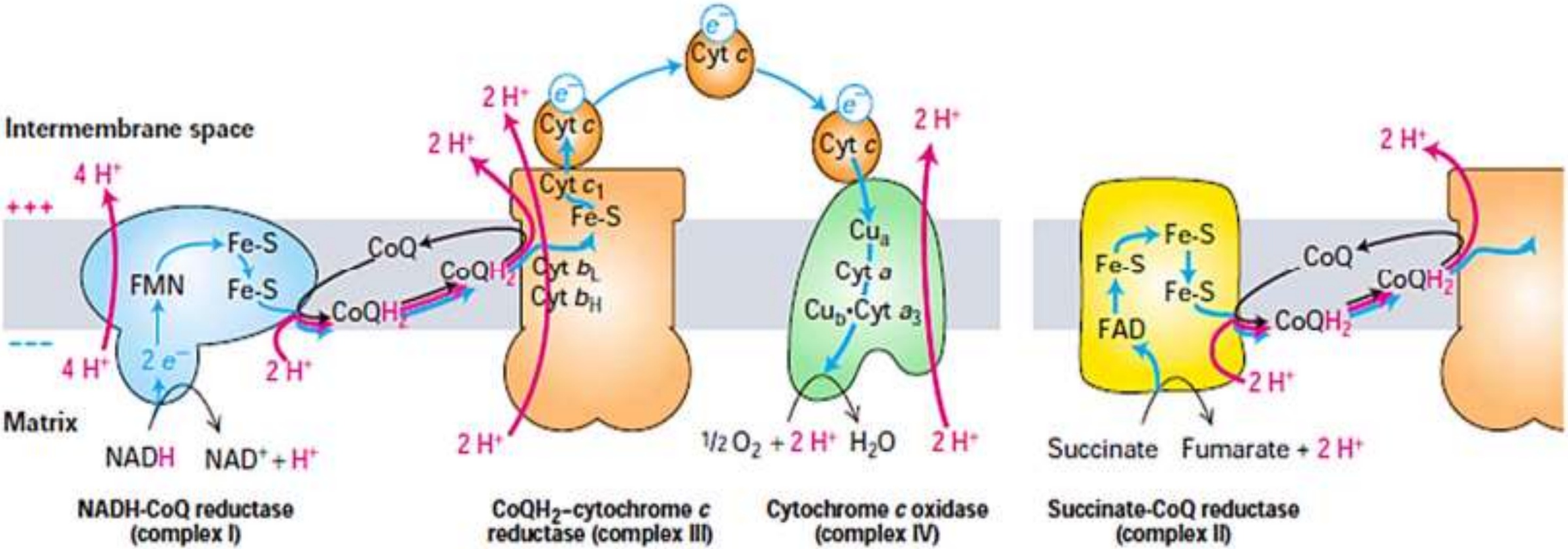
TABLE 8-2 Electron-Carrying Prosthetic Groups in the Respiratory Chain

Protein Component	Prosthetic Groups*
NADH-CoQ reductase (complex I)	FMN Fe-S
Succinate-CoQ reductase (complex II)	FAD Fe-S
$CoQH_2$–cytochrome *c* reductase (complex III)	Heme b_L Heme b_H Fe-S Heme c_1
Cytochrome *c*	Heme *c*
Cytochrome *c* oxidase (complex IV)	Cu_a^{2+} Heme *a* Cu_b^{2+} Heme a_3

*Not included is coenzyme Q, an electron carrier that is not permanently bound to a protein complex.

SOURCE: J. W. De Pierre and L. Ernster, 1977, *Ann. Rev. Biochem.* 46:201.

Protein complexes



Source of electrons

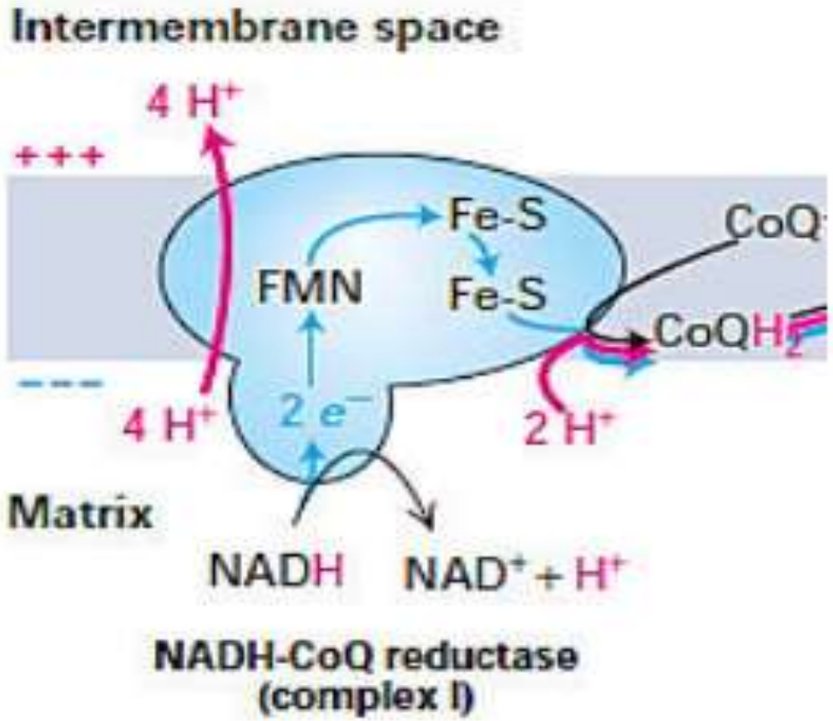
- Aerobic cellular respiration made up of 3 parts: glycolysis, the Krebs cycle, and oxidative phosphorylation.
- In **glycolysis**, glucose is metabolized into 2 molecules of pyruvate, with an output of ATP and **nicotinamide adenine dinucleotide (NADH).**
- The **pyruvate is oxidized** into **acetyl CoA** and **NADH** and carbon dioxide (CO_2).
- The **acetyl CoA is then used in the Krebs cycle**, also known as the citric acid cycle, which is a chain of chemical reactions that produce CO_2, **NADH, flavin adenine dinucleotide ($FADH_2$),** and ATP.
- Fatty acid oxidation also produces NADH and $FADH_2$.
- Reduced electron carriers (NADH and $FADH_2$) from other steps of cellular respiration **transfer their electrons to molecules** near the beginning of the transport chain and get oxidized for reuse.

Complex I

NADH-CoQ Reductase (Complex I)

- Electrons are carried from **NADH to CoQ by the NADH-CoQ reductase complex.**
- NAD is exclusively a **two-electron carrier**: it accepts or releases a pair of electrons at a time. In the NADH-CoQ reductase complex, **electrons first flow from NADH to FMN (flavin mononucleotide), a cofactor related to FAD, then to an iron-sulfur cluster, and finally to CoQ.**
- **FMN, like FAD**, can accept two electrons, but does so **one electron at a time.**
- The **overall reaction** catalyzed by this complex is:

$$\underset{\text{(Reduced)}}{NADH} + \underset{\text{(Oxidized)}}{CoQ} + 2\,H^+ \longrightarrow \underset{\text{(Oxidized)}}{NAD^+} + H^+ + \underset{\text{(Reduced)}}{CoQH_2}$$



NADH-CoQ reductase (complex I)

CoQ/Q/Ubiquitin: Electron carrier or Shuttler

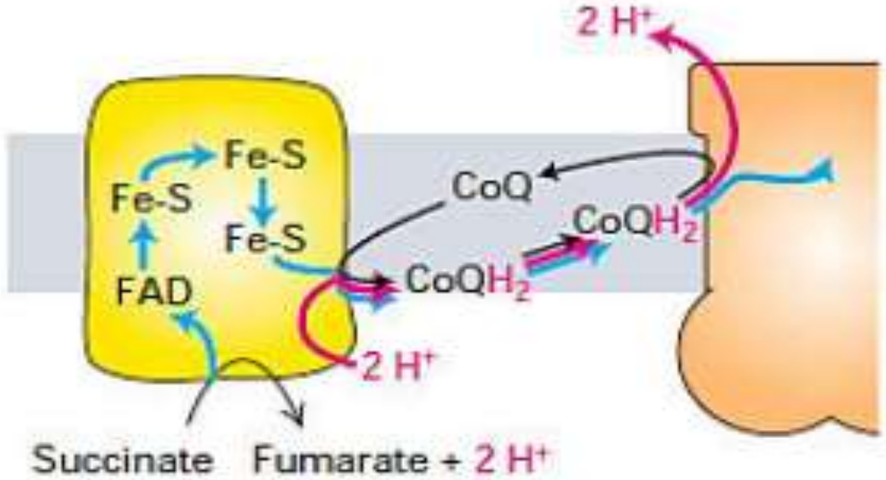
Complex II

Succinate-CoQ Reductase (Complex II)

- Succinate dehydrogenase, the enzyme that **oxidizes a molecule of succinate to fumarate in the citric acid cycle**, is an integral component of the succinate-CoQ reductase complex.
- The **two electrons released in conversion of succinate to fumarate are transferred first to FAD, then to an iron-sulfur cluster, and finally to CoQ.**
- The **overall reaction** catalyzed by this complex is:

$$\underset{\text{(Reduced)}}{\text{Succinate}} + \underset{\text{(Oxidized)}}{\text{CoQ}} \longrightarrow \underset{\text{(Oxidized)}}{\text{fumarate}} + \underset{\text{(Reduced)}}{\text{CoQH}_2}$$

Although the ΔG for this reaction is negative, **the released energy is insufficient for proton pumping. Thus no protons are translocated across the membrane by the succinate-CoQ reductase complex, and no proton-motive force is generated in this part of the respiratory chain.**



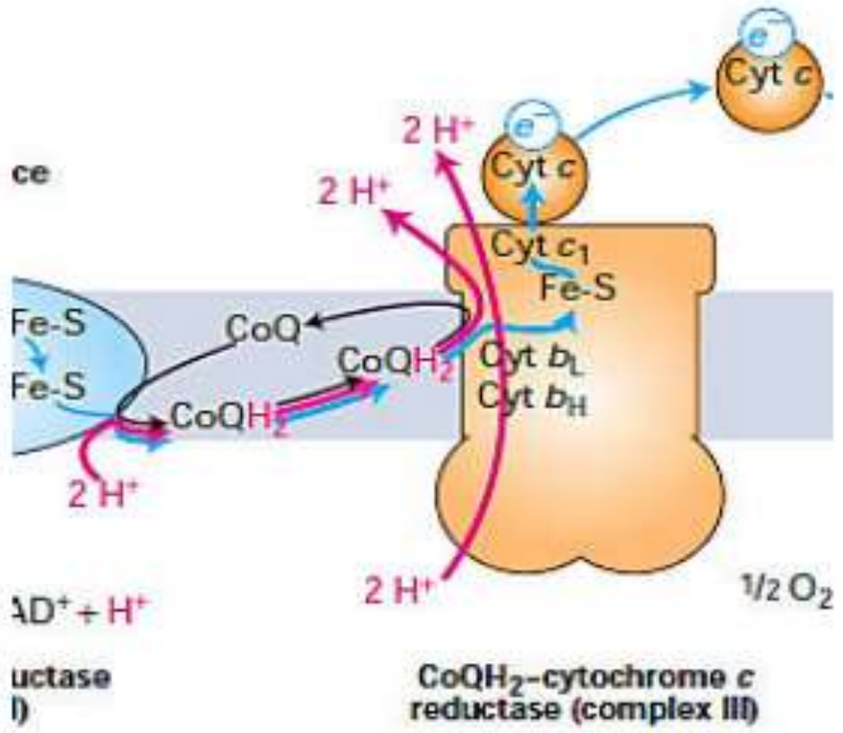
Succinate-CoQ reductase (complex II)

Complex III

- **$CoQH_2$–Cytochrome c Reductase (Complex III)**

- A **$CoQH_2$ generated either by complex I or complex II donates two electrons to the $CoQH_2$–cytochrome c reductase complex, regenerating oxidized CoQ.**
- Concomitantly it releases **two protons picked up on the cytosolic face into the intermembrane space**, generating part of the proton-motive force.
- Within complex III, the **released electrons first are transferred to an iron-sulfur cluster within complex III and then to two b-type cytochromes (b_L and b_H) or Cytochrome c_1.**
- Finally, the two electrons are **transferred to two molecules of the oxidized form of Cytochrome c,** a water-soluble peripheral protein that diffuses in the intermembrane space.
- For each pair of electrons transferred, the **overall reaction** catalyzed by the $CoQH_2$–cytochrome c reductase complex is:

$$\underset{\text{(Reduced)}}{CoQH_2} + 2\ \underset{\text{(Oxidized)}}{\text{Cyt } c^{3+}} \longrightarrow \underset{\text{(Oxidized)}}{CoQ} + 2\,H^+ + 2\ \underset{\text{(Reduced)}}{\text{Cyt } c^{2+}}$$



Cyt c: Electron carrier or Shuttler

Complex IV

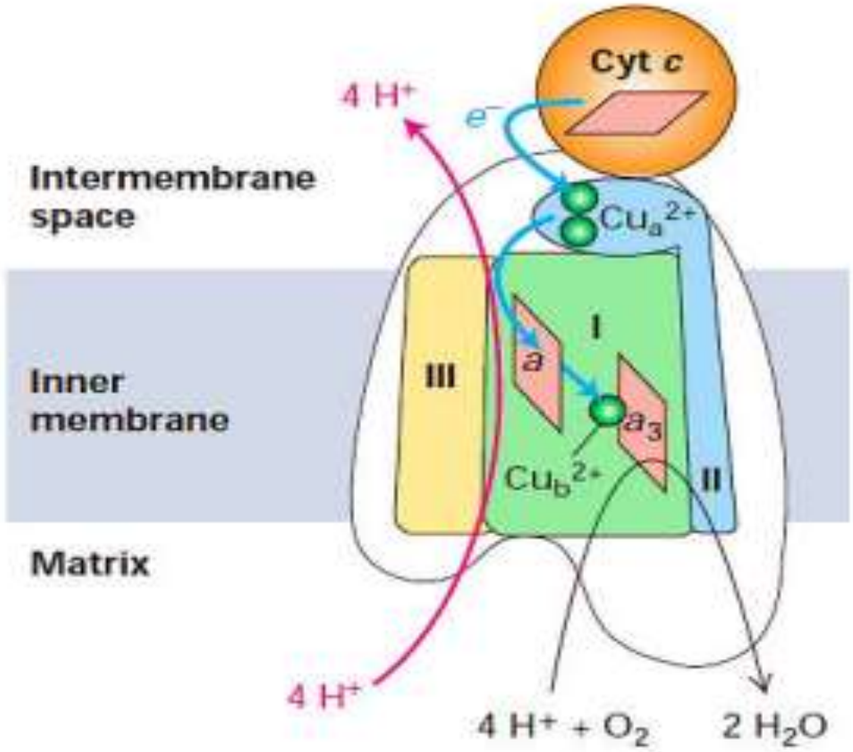
Cytochrome c Oxidase (Complex IV)

- Cytochrome c, after being reduced by the $CoQH_2$–cytochrome c reductase complex, **transports electrons, one at a time, to the cytochrome c oxidase complex.**
- Within this complex, **electrons are transferred, again one at a time, first to a pair of copper ions called Cu_a^{2+}, then to cytochrome a, next to a complex of another copper ion (Cu_b^{2+}) and cytochrome a_3, and finally to O_2,** the ultimate electron acceptor, yielding H_2O.
- For each pair of electrons transferred, **the overall reaction** catalyzed by the cytochrome c oxidase complex is:

$$2\ \text{Cyt}\ c^{2+} + 2\ H^+ + \tfrac{1}{2}\ O_2 \longrightarrow 2\ \text{Cyt}\ c^{3+} + H_2O$$

(Reduced) (Oxidized)

During transport of each pair of electrons through the cytochrome c oxidase complex, two protons are translocated across the membrane.



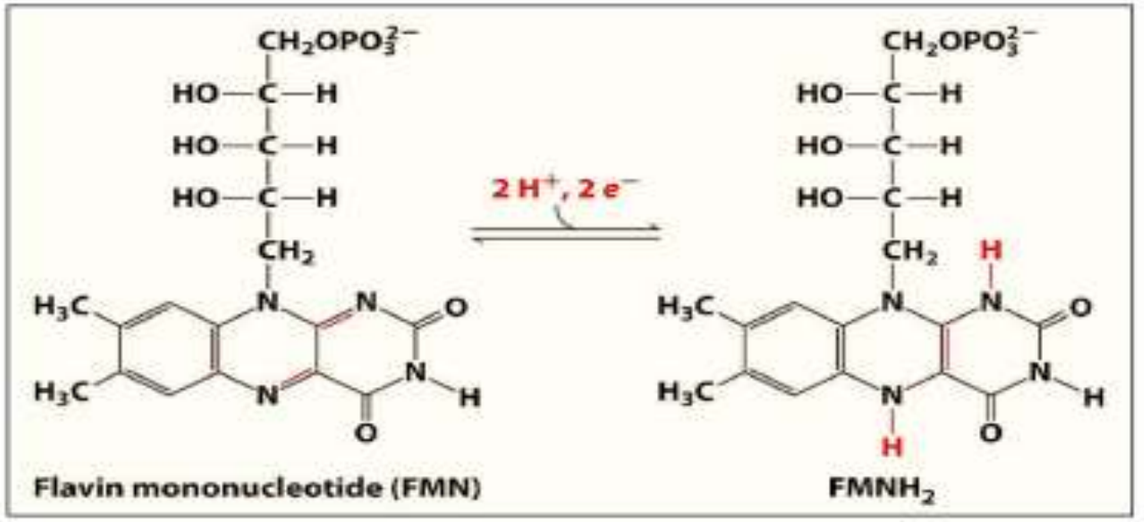
Cytochrome *c* oxidase (complex IV)

Flavins

- Complexes contain enzymes with **electron carrying groups** or **oxidation – reduction components.**
- Protein components use **metal containing prosthetic groups** or **flavins** to carry electrons.
- Metal-containing groups such **as iron-sulfur clusters, copper ions, hemes.**
- **Flavins**:

  (Complex I) FMN - $FMNH_2$

  (Complex II) FAD - $FADH_2$

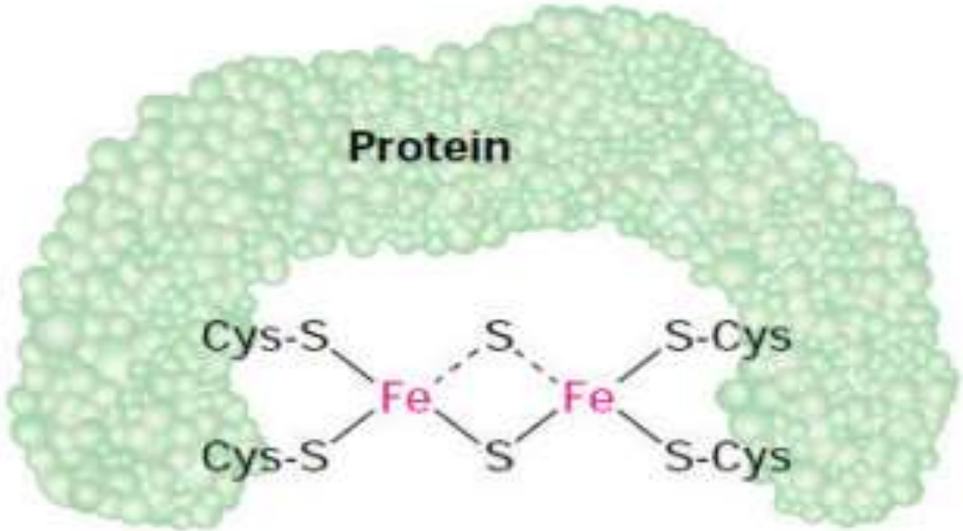


Flavin mononucleotide (FMN) $FMNH_2$

In different enzymes flavins may be present as covalently-bound riboflavin, or tightly (but non-covalently) bound riboflavin monophosphate (which is sometimes known as flavin mononucleotide, FMN) or flavin adenine dinucleotide (FAD).

Iron-sulfur clusters

- They are **non-heme, iron-containing prosthetic groups** consisting of Fe atoms bonded both to **inorganic S atoms and to S atoms on cysteine residues on a protein.**
- **Some Fe atoms in the cluster bear a +2 charge; others have a +3 charge.**
- However, the **net charge of each Fe atom is actually between +2 and +3** because electrons in the outermost orbits are dispersed among the Fe atoms and move rapidly from one atom to another.
- Iron-sulfur clusters **accept and release electrons one at a time;** the additional electron is also dispersed over all the Fe atoms in the cluster.



Cytochromes

Several **types of heme, an iron-containing prosthetic group** similar to that in hemoglobin and myoglobin, are tightly bound or covalently linked to mitochondrial proteins, forming the **cytochromes.**

Electron flow through the cytochromes **occurs by oxidation and reduction of the Fe atom** in the center of the heme molecule:

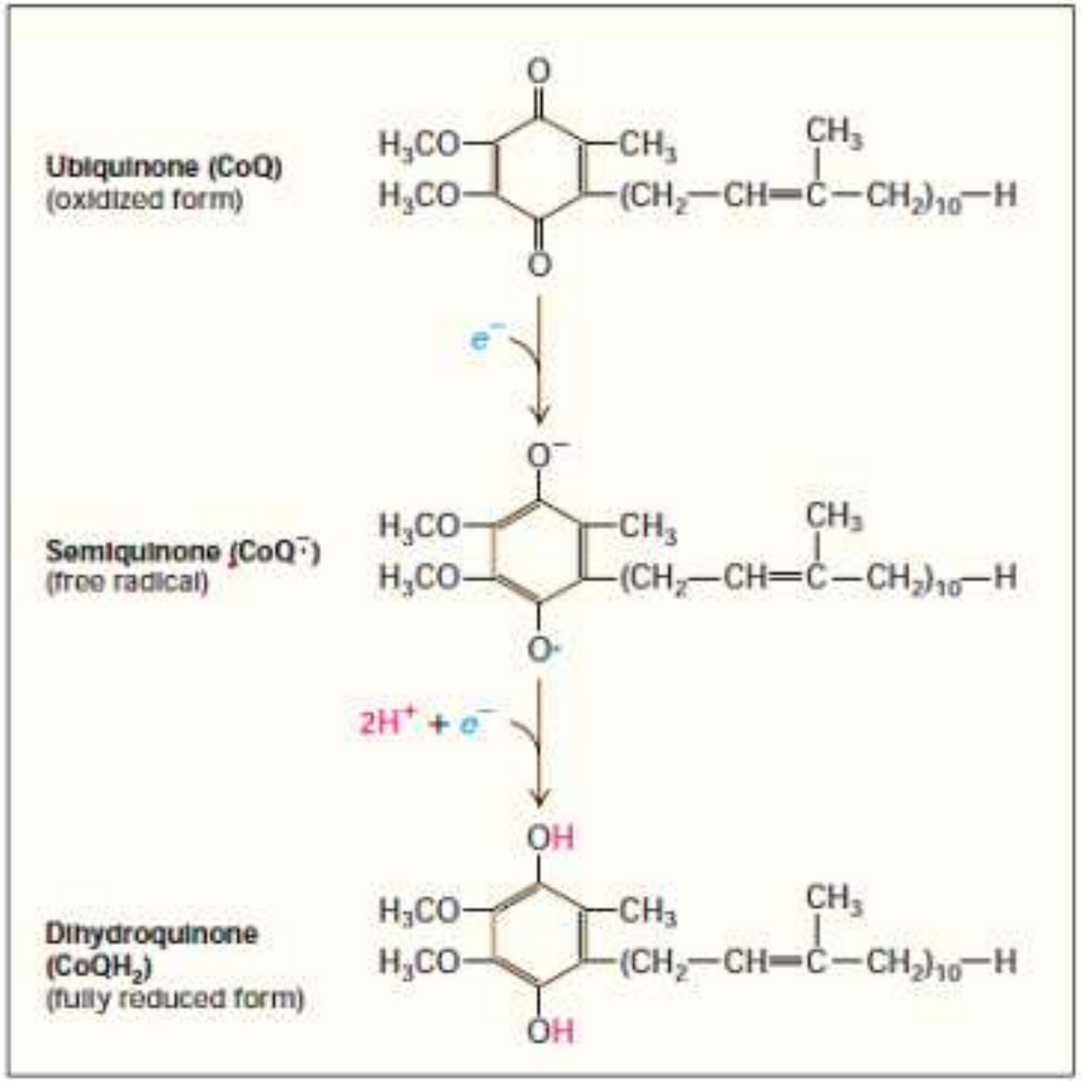
$$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$$

In the respiratory chain, **electrons move through the cytochromes in the following order: b, c1, c, a, and a3.**

- The various cytochromes have slightly different heme groups and axial ligands, which generate different environments for the Fe ion.
- Therefore, each cytochrome has a different reduction potential, or tendency to accept an electron—an important property dictating the unidirectional electron flow along the chain.

Coenzyme Q (CoQ)

- It is also called **ubiquinone,** is the only electron carrier in the respiratory chain that is not a proteinbound prosthetic group.
- It is a **carrier of hydrogen atoms, that is, protons plus electrons.**
- The **oxidized quinone form of CoQ can accept a single electron to form a semiquinone, a charged free radical denoted by CoQ^{-}.**
- Addition of a **second electron and two protons to CoQ^{-} forms dihydroubiquinone (CoQH2), the fully reduced form.**
- Both **CoQ and CoQH2 are soluble in phospholipids and diffuse freely in the inner mitochondrial membrane.**



Overall, what does the electron transport chain do for the cell?

Regenerates electron carriers

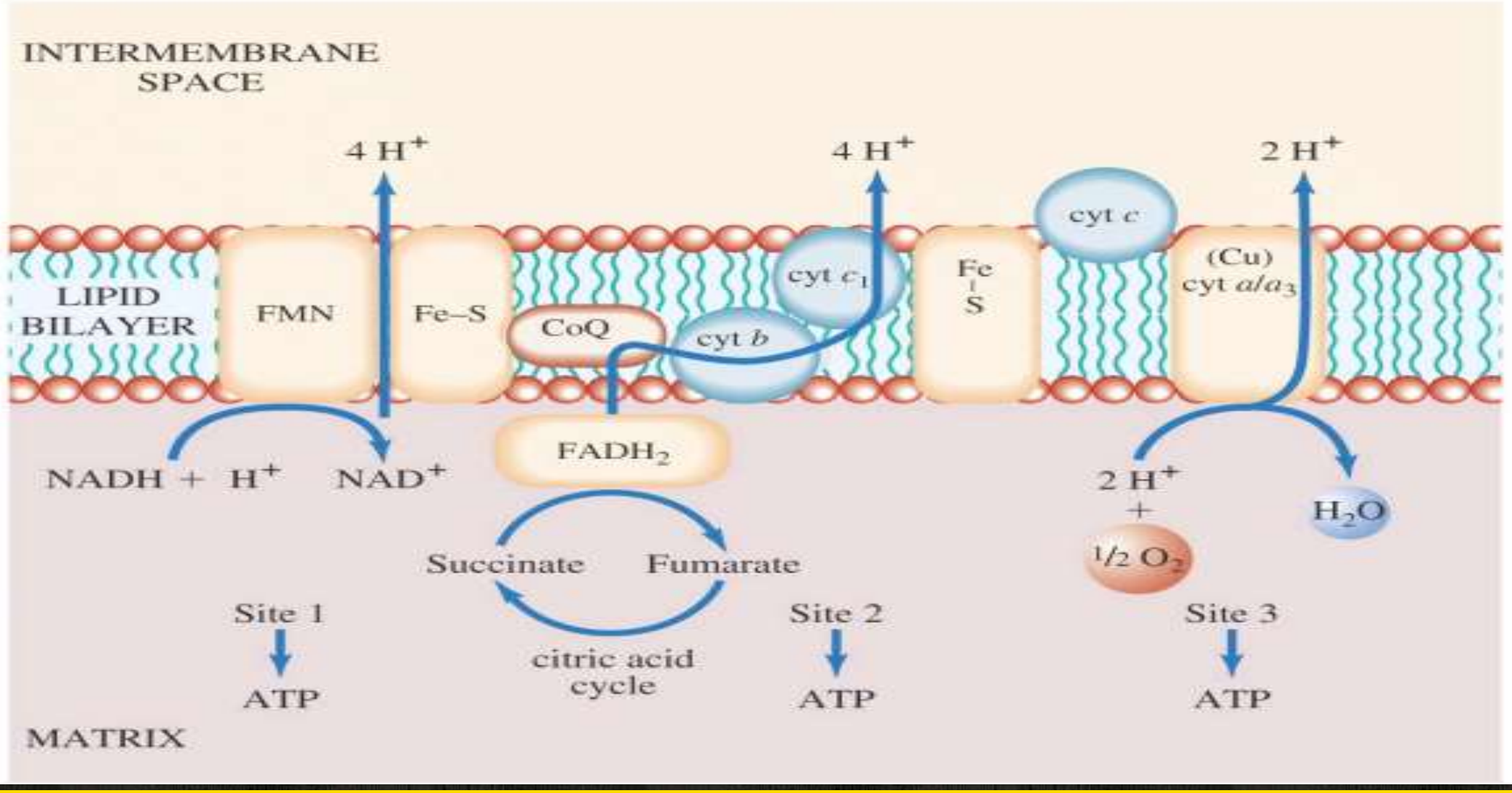
NADH and $FADH_2$, pass their electrons to the electron transport chain, turning back into NAD^+ and FAD. This is important because the oxidized forms of these electron carriers are used in glycolysis and the citric acid cycle and must be available to keep these processes running.

Makes a proton gradient

The transport chain builds a proton gradient across the inner mitochondrial membrane, with a higher concentration of H^+ in the intermembrane space and a lower concentration in the matrix. This gradient represents a stored form of energy, and, as we'll see, it can be used to make ATP.

ATP synthase uses the proton gradient across the mitochondrial membrane to form ATP. It is made up of F_0 and F_1 subunits which act as a rotational motor system. F0 portion is embedded in the mitochondrial membrane and is protonated and deprotonated repeatedly causing it to rotate. This rotation catalyzes the formation of ATP from ADP and Pi.

SUMMARY OF ELECTRON TRANSPORT CHAIN



Electron Transport Chain Products

The end products of the electron transport chain are:

30-32 ATPs and 44 moles of H2O

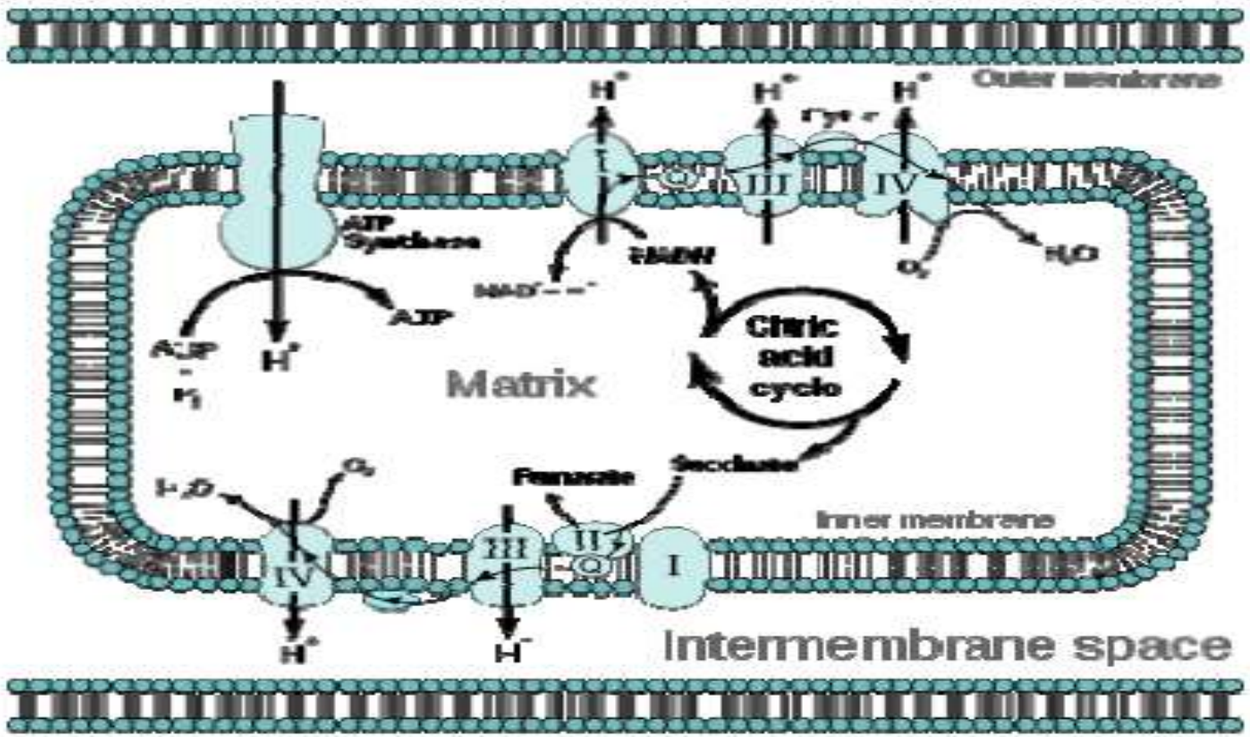
Stage	Direct products (net)	Ultimate ATP yield (net)
Glycolysis	2 ATP	2 ATP
	2 NADH	3-5 ATP
Pyruvate oxidation	2 NADH	5 ATP
Citric acid cycle	2 ATP/GTP	2 ATP
	6 NADH	15 ATP
	2 $FADH_2$	3 ATP
Total		**30-32 ATP**

Table Source: Khan Academy

Note: In some cases, we can see the production of 38 ATPs also.

Terminal Oxidation

Oxidative phosphorylation



The electron transport chain in the mitochondrion is the site of oxidative phosphorylation in eukaryotes. The NADH and succinate generated in the citric acid cycle are oxidized, releasing energy to power the ATP synthase.

Oxidative phosphorylation (or OXPHOS in short) is a metabolic pathway that uses energy released by the oxidation of nutrients to produce adenosine triphosphate (ATP). Although the many forms of life on earth use a range of different nutrients, almost all aerobic organisms carry out oxidative phosphorylation to produce ATP, the molecule that supplies energy to metabolism. This pathway is probably so pervasive because it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis.

During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP. In eukaryotes, these redox reactions are carried out by a series of protein complexes within the cells intermembrane wall mitochondria, whereas, in prokaryotes, these proteins are located in the cells' intermembrane space. These linked sets of proteins are called electron transport chains. In eukaryotes, five main protein complexes are involved, whereas in prokaryotes many different enzymes are present, using a variety of electron donors and acceptors.

The energy released by electrons flowing through this electron transport chain is used to transport protons across the inner mitochondrial membrane, in a process called *chemiosmosis*. This generates potential energy in the form of a pH gradient and an electrical potential across this

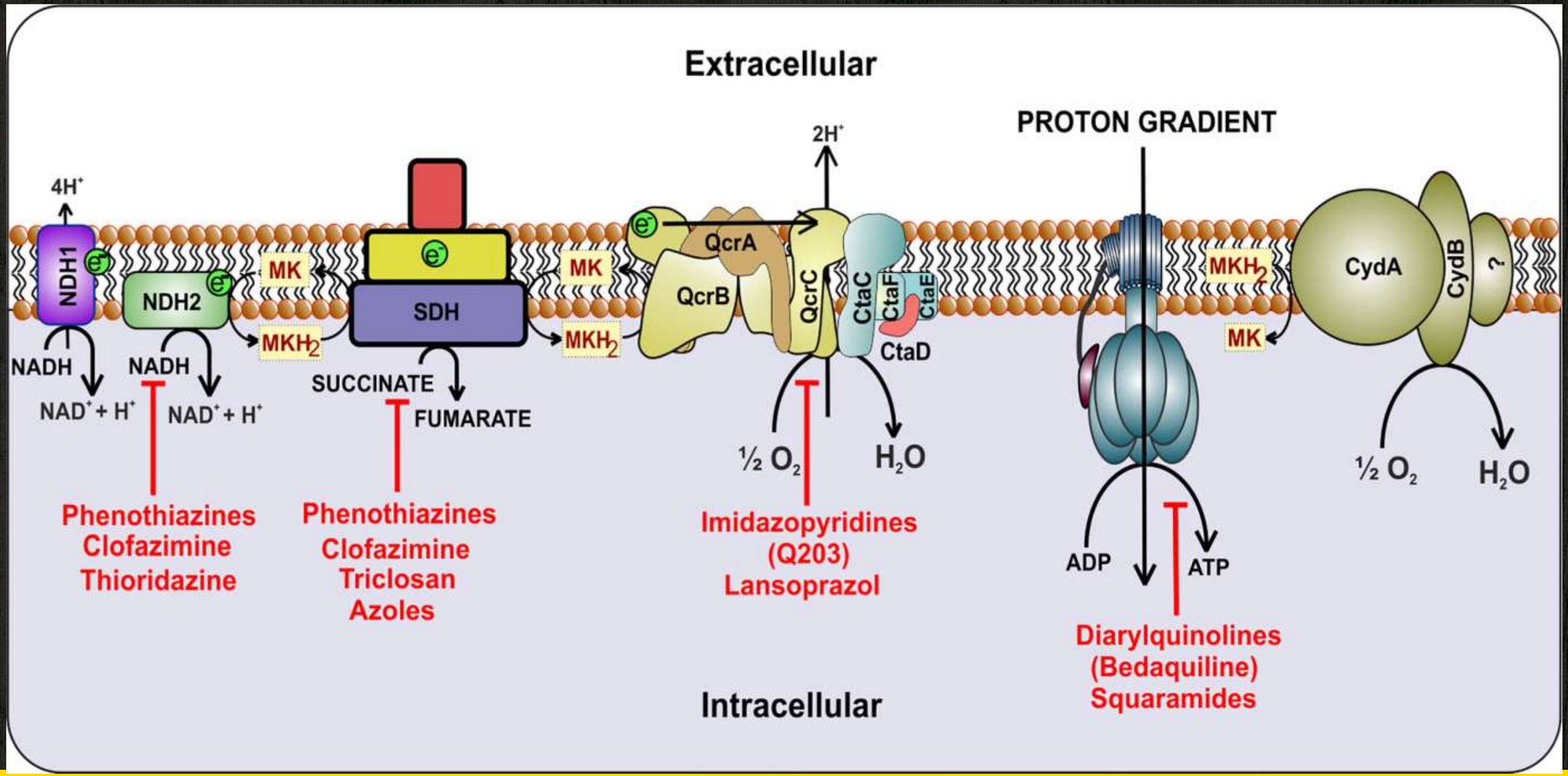
membrane. This store of energy is tapped by allowing protons to flow back across the membrane and down this gradient, through a large enzyme called ATP synthase. This enzyme uses this energy to generate ATP from adenosine diphosphate (ADP), in a phosphorylation reaction. This reaction is driven by the proton flow, which forces the rotation of a part of the enzyme; the ATP synthase is a rotary mechanical motor.

Although oxidative phosphorylation is a vital part of metabolism, it produces reactive oxygen species such as superoxide and hydrogen peroxide, which lead to propagation of free radicals, damaging cells and contributing to disease and, possibly, aging (senescence). The enzymes carrying out this metabolic pathway are also the target of many drugs and poisons that inhibit their activities.

Inhibitors

There are several well-known drugs and toxins that inhibit oxidative phosphorylation. Although any one of these toxins inhibits only one enzyme in the electron transport chain, inhibition of any step in this process will halt the rest of the process. For example, if oligomycin inhibits ATP synthase, protons cannot pass back into the mitochondrion. As a result, the proton pumps are unable to operate, as the gradient becomes too strong for them to overcome. NADH is then no longer oxidized and the citric acid cycle ceases to operate because the concentration of NAD^+ falls below the concentration that these enzymes can use.

Compounds	Use	Effect on oxidative phosphorylation
Cyanide Carbon monoxide Azide	Poisons	Inhibit the electron transport chain by binding more strongly than oxygen to the Fe–Cu center in cytochrome c oxidase, preventing the reduction of oxygen.
Oligomycin	Antibiotic	Inhibits ATP synthase by blocking the flow of protons through the F_o subunit.
CCCP 2,4-Dinitrophenol	Poisons	Ionophores that disrupt the proton gradient by carrying protons across a membrane. This ionophore uncouples proton pumping from ATP synthesis because it carries protons across the inner mitochondrial membrane.
Rotenone	Pesticide	Prevents the transfer of electrons from complex I to ubiquinone by blocking the ubiquinone-binding site.
Malonate and oxaloacetate		Competitive inhibitors of succinate dehydrogenase (complex II).


Extracellular
PROTON GRADIENT
4H+
NDH1
NDH2
NADH
NAD+ + H+
NADH
NAD+ + H+
MK
MKH2
SDH
SUCCINATE
FUMARATE
MK
MKH2
2H+
QcrA
QcrB
QcrC
CtaC
CtaF
CtaE
CtaD
½ O2
H2O
MKH2
MK
CydA
CydB
?
½ O2
H2O
ADP
ATP
Phenothiazines
Clofazimine
Thioridazine
Phenothiazines
Clofazimine
Triclosan
Azoles
Imidazopyridines
(Q203)
Lansoprazol
Diarylquinolines
(Bedaquiline)
Squaramides
Intracellular