OXIDATIVE PHOSPHORYLATION & ETC

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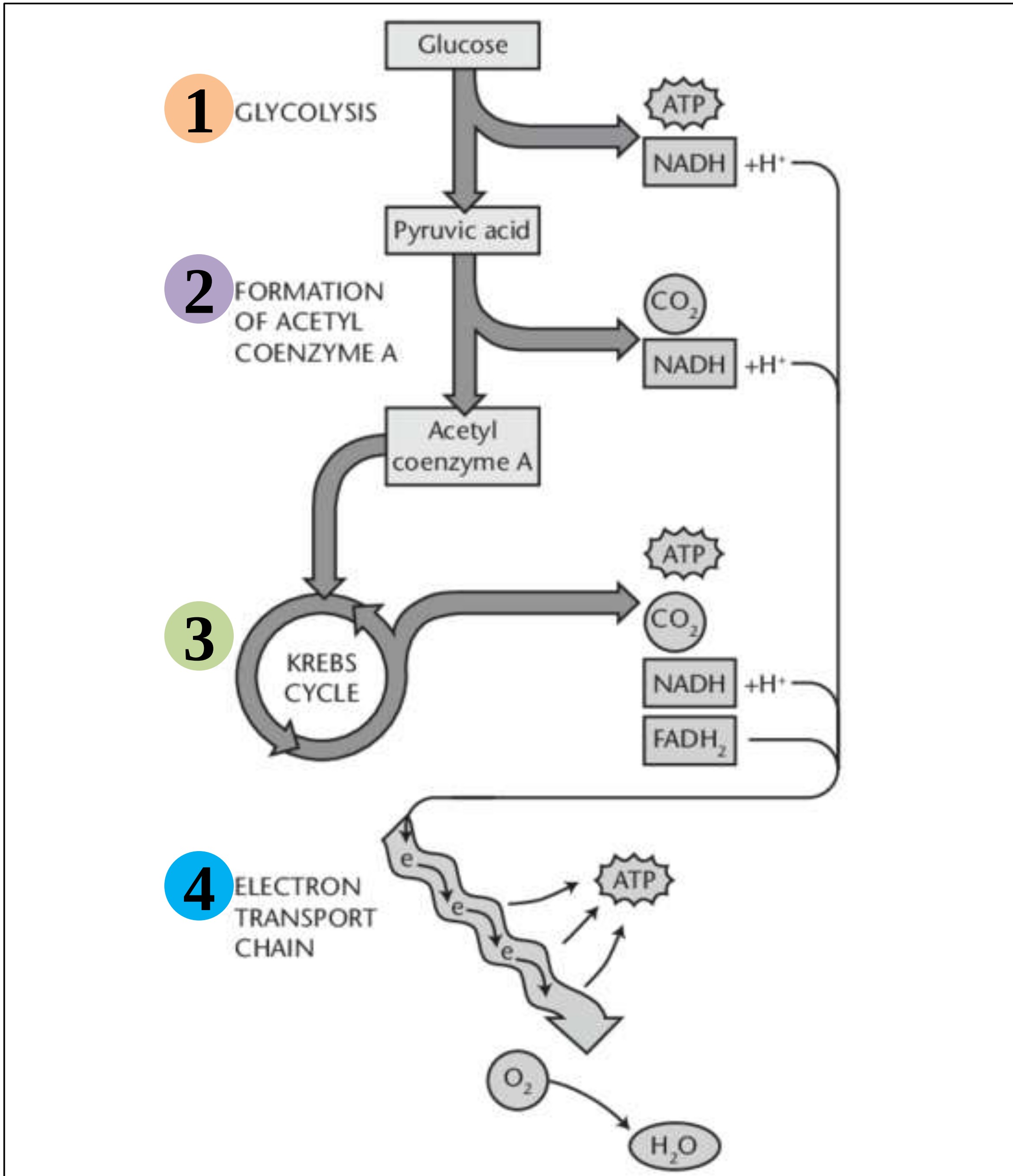
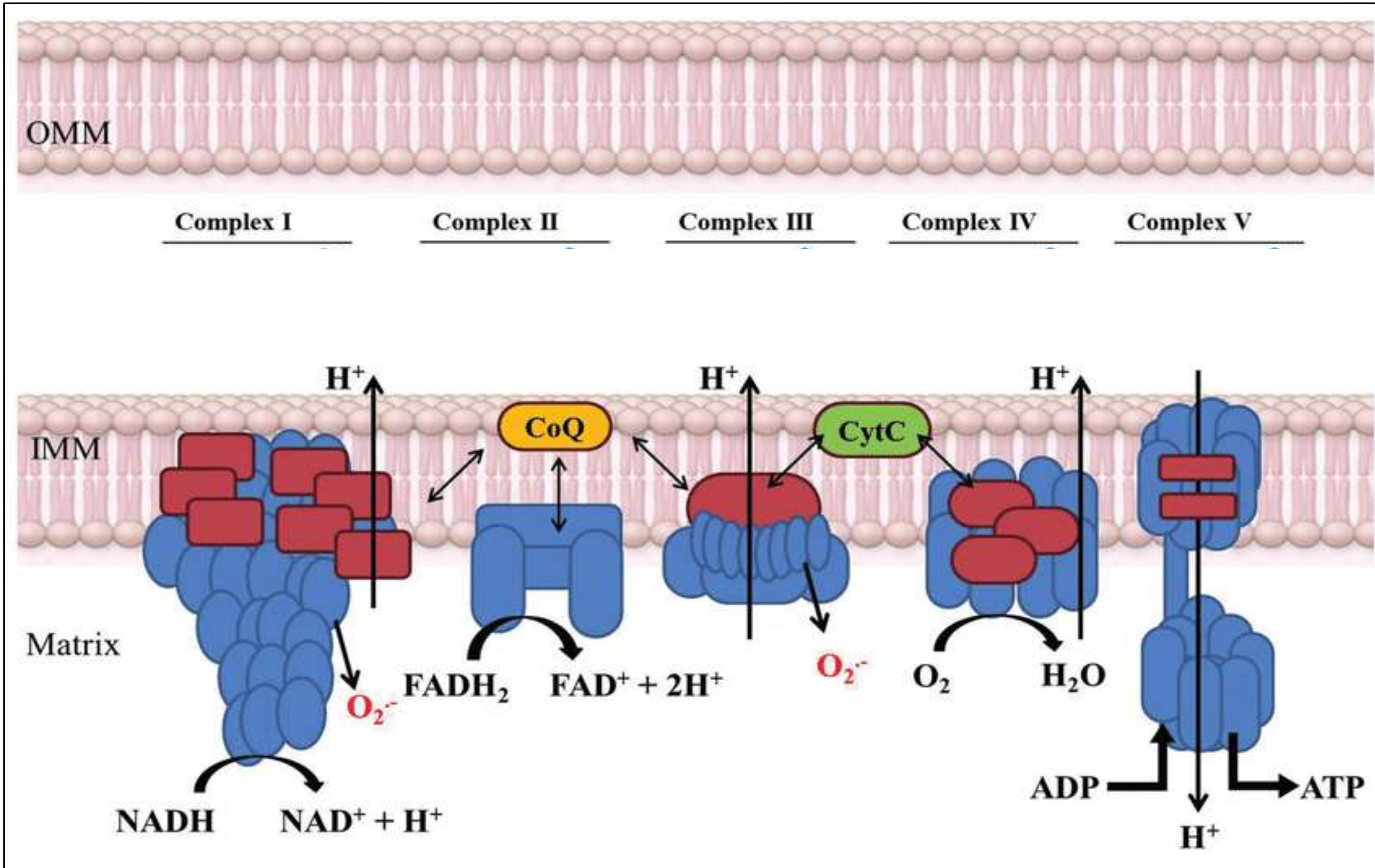
Cellular Respiration Process



Oxidative Phosphorylation (OP)

- It is the last step of cellular respiration process.
- In OP ATP is synthesised from the reduced products, NADH and $FADH_2$, of glycolysis and TCA cycle, and oxygen is utilised.
- Though NADH is sometimes used directly as reducing molecule in biosynthetic pathways such as in gluconeogenesis, both NADH and $FADH_2$ are mostly used to reduce molecular oxygen to water.
- The energy released in the process drives the synthesis of ATP, the energy currency of the cell.
- Process occurs in the presence of oxygen.

Oxidative Phosphorylation (OP)



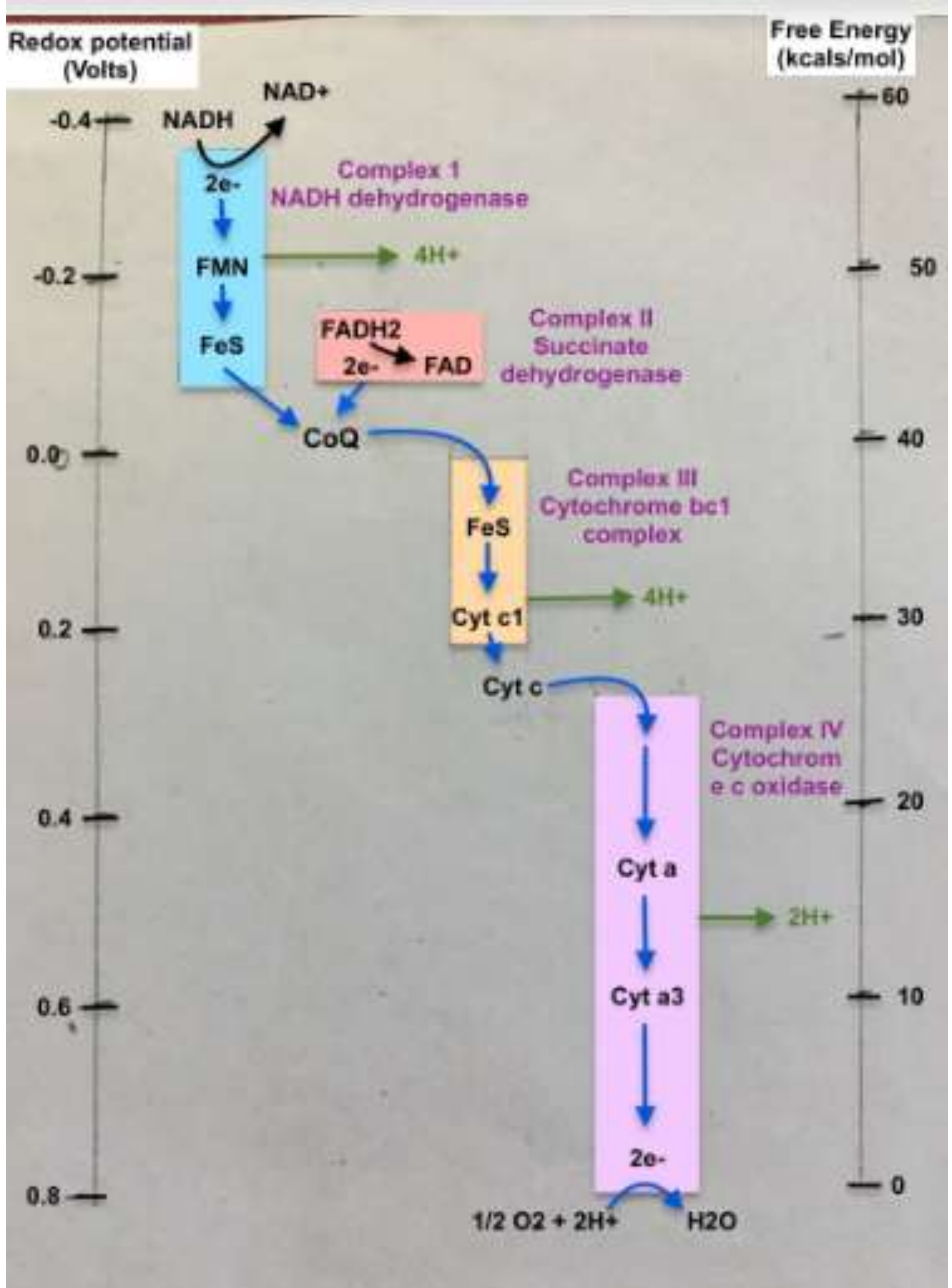
Oxidative Phosphorylation (OP)

- NADH and $FADH_2$ get oxidized by donating two electrons each to oxygen and reducing it to water.
- Since the energy for synthesis of ATP molecules is derived from oxidation of NADH or $FADH_2$, which in turn are formed by oxidation of energy-rich molecules such as carbohydrates and fats, this type of phosphorylation or ATP synthesis is called oxidative phosphorylation.
- **Electrons donated by NADH and $FADH_2$ are transported through components of electron transport chain (ETC) sequentially** from one having a more negative electron/reduction potential to the next with less negative potential, till they are accepted by molecular oxygen.

Oxidative Phosphorylation (OP)

- As the electrons move through components of ETC with increasing redox potentials, their free energy is reduced and is released.
- According to the chemi-osmotic theory put forth by Peter Mitchell in 1961, energy released during the movement of electrons through ETC is used in pumping protons across the inner mitochondria membrane and generating a proton concentration gradient.
- It is the electrochemical energy of this gradient that drives the synthesis of ATP.

Increasing Redox Potentials

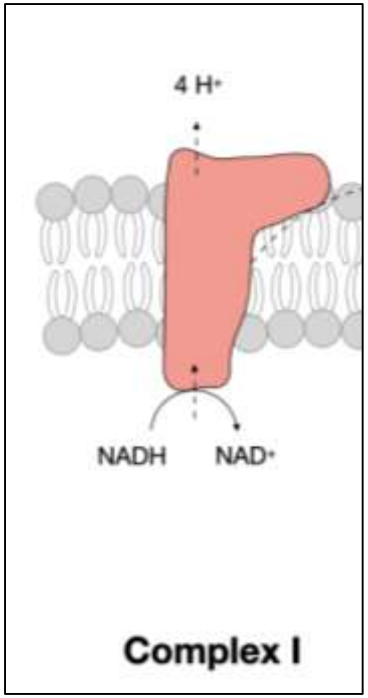


Electron Transport Chain (ETC)

- The components of ETC are electron carriers which are single molecules or multi-subunit protein complexes or enzymes containing prosthetic groups capable of accepting and donating electrons.
- These complexes are associated with the inner mitochondrial membrane.
- In prokaryotes, which lack mitochondria, the complexes are associated with the plasma membrane.
- There are **four complexes/enzymes** of mitochondrial ETC.

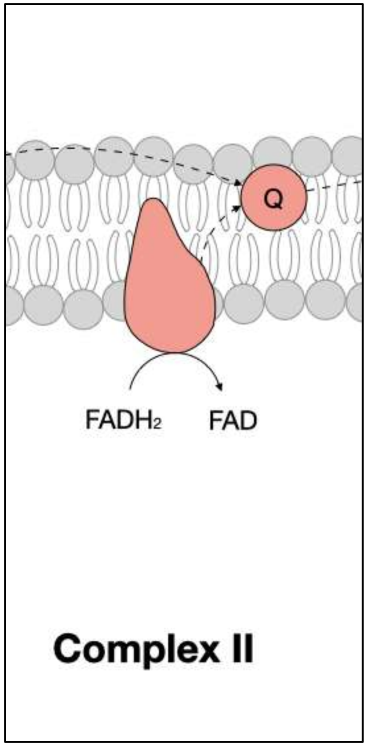
Complex I

- Also called NADH dehydrogenase or NADH:ubiquinone oxidoreductase
- It contains FMN (flavin mononucleotide) and Fe-S (iron-sulfur) centers catalyses the transfer of 2 electrons from NADH to ubiquinone.
- <u>During this transfer a large amount of energy is released which is used to pump protons (H+) from the matrix to the inter-membrane space between the two mitochondrial membranes</u>.
- Approximately 4 protons are pumped for every pair of electrons transferred.



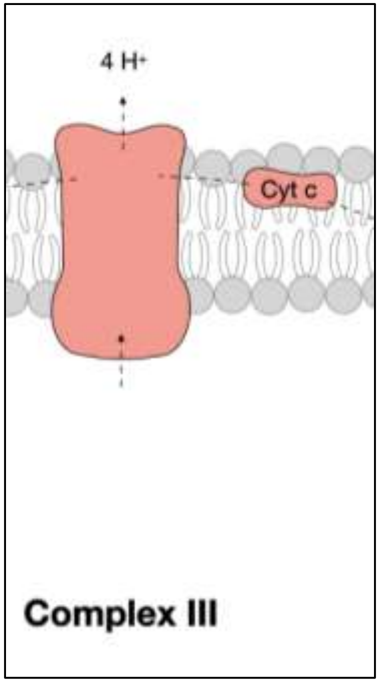
Complex II

- Also called succinate dehydrogenase complex
- An enzyme of TCA cycle with FAD and Fe-S centers catalyzes the transfer of electrons from $FADH_2$ to ubiquinone.
- However, during this electron transfer energy released is not sufficient to transfer any protons.



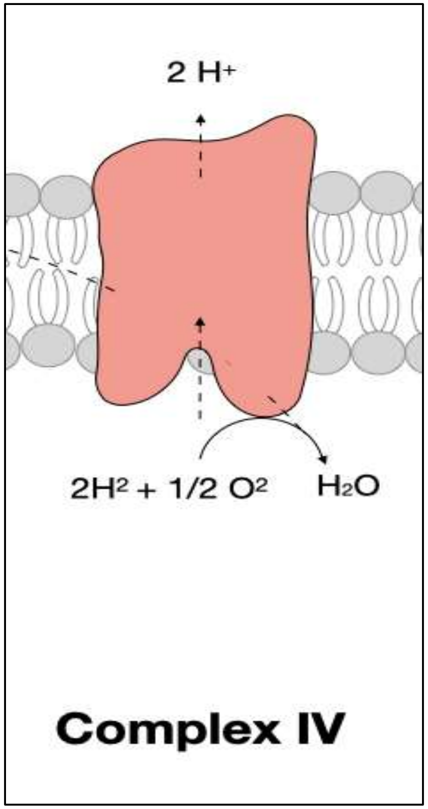
Complex III

- Also called cytochrome bc1 complex or ubiquinol:cytochrome c oxidoreductase.
- It has heme groups of cytochrome b and cytochrome c1 as electron carriers along with Fe-S centers and transfers electrons from ubiquinol (reduced ubiquinone or QH2) , one at a time, to cytochrome c which is present attached on the side of the inner membrane facing the inter-membrane space.
- The transfer results in a drop in the free energy of electrons which is used to pump 4 protons from matrix to the inter-membrane space.



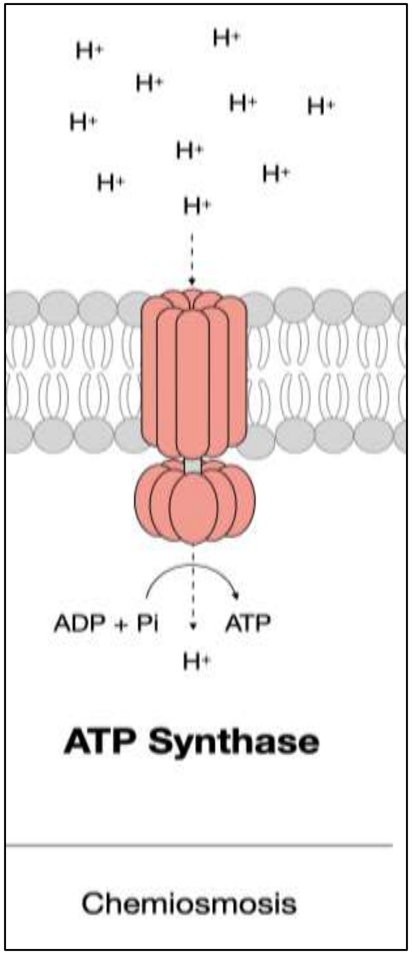
Complex IV

- Also called cytochrome c oxidase or cytochrome aa3 complex
- It has heme a and a3 along with copper and iron-copper centers and transfers four electrons, one at a time, from cytochrome c to molecular oxygen, reducing it to water.
- Enough energy is released to pump 2 protons from matrix to inter-membrane space.



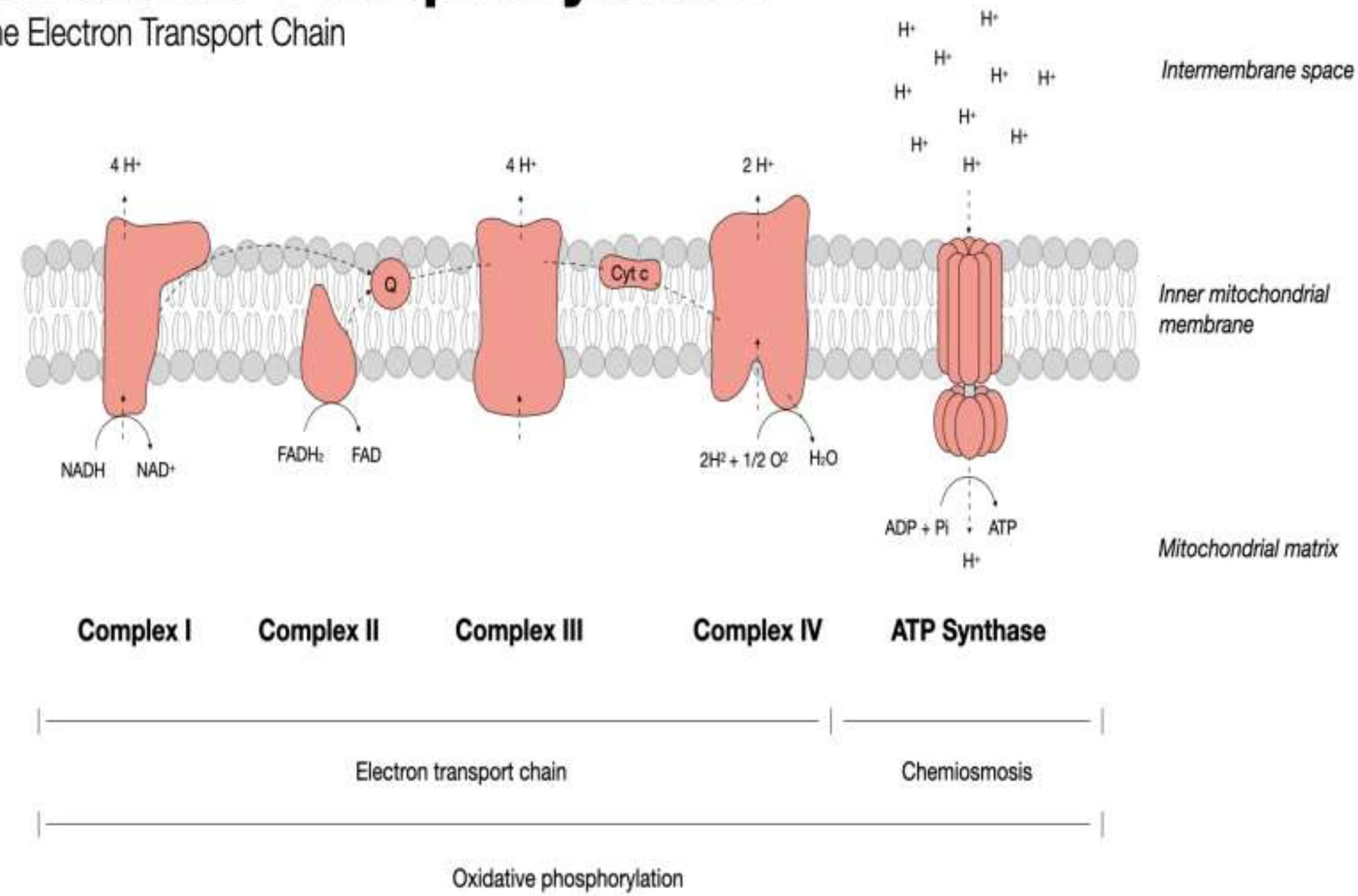
Chemiosmosis

- Also called ATP synthase
- It is a multi-subunit protein complex consisting of two major components,
  - F1 – the headpiece which projects into the matrix and contains the site for synthesis of ATP.
  - F0 – the stalk-like integral membrane protein complex forming a pore or channel through which protons pass from the inter-membrane space to the matrix.



Oxidative Phosphorylation

The Electron Transport Chain



Alternative Pathways

- **Plant mitochondria** have alternative pathways for oxidising NADH and NADPH through alternative oxidase and dehydrogenases.
- These pathways are protective mechanisms to dissipate excess energy, and to prevent over-reduction of ETC and formation of severely damaging reactive oxygen species.
- There are 4 alternative pathways.

Alternative Pathways

I. An alternative NADH dehydrogenase present on the matrix side of the inner membrane transfers electrons from NADH directly to ubiquinone bypassing the rotenone sensitive NADH dehydrogenase complex I and proton pumping at the site.

As a result fewer protons are pumped.

This pathway proceeds only when the ratio of NADH/ NAD+ is exceptionally high, especially when photorespiration is occurring at a very high rate.

Alternative Pathways

ii. Similarly, an alternative NADPH dehydrogenase transfers electrons from NADPH in the matrix to ubiquinone directly.

iii. External NADH dehydrogenase and NADPH dehydrogenase situated on the outer side of inner membrane toward the inter-membrane space oxidise cytosolic NADH and NADPH, respectively.

Here, too, the pathway becomes active only when the cytosolic NAD(P)H / NAD(P)+ ratio is high.

These enzymes too are rotenone resistant.

Alternative Pathways

iv. Alternative oxidase, catalyses the transfers of electrons from ubiquinol to oxygen directly.

Thus, electron flow through complex III and the cyanide sensitive complex IV, and their proton pumps is bypassed.

The unused energy is released as heat.

This process occurs when the pool of ubiquinone is highly reduced.

ETC

- The ETC ensures that energy of oxidation of NADH and $FADH_2$ is released in small packets at three stages (complex I, complex III, and complex IV) rather than in one large burst, and therefore, is conserved more efficiently.
- Since membranes have a dynamic fluid structure, these complexes are randomly arranged and can move within the membranes; their position is neither fixed nor sequentially arranged.
- However, many of the complexes assemble into supramolecular or super molecular complexes called repirasomes.
- The functional significance of such aggregation is not clear but is believed to reduce oxidative damage and increase efficiency of electron transfer.

Mechanism of ATP synthesis

- Electron transfer through ETC is coupled to proton pumping.
- A total of 10 protons are pumped out for each pair of electrons transferred to O2, 4 protons by Complex I, 4 by Complex III, and 2 by Complex IV.
- The pumping out of protons from the matrix to the inter membrane space as electrons are transferred through the ETC, builds up an electrochemical proton concentration gradient across the inner mitochondrial membrane (the inner mitochondrial membrane is impermeable to protons).
- The electrochemical energy stored in the proton gradient is called the proton-motive force.

Mechanism of ATP synthesis

- The release of protons by the spontaneous and passive flow down the electrochemical gradient through the channel provided by F0 of ATP synthase makes available the proton motive force.
- This force powers the rotational movement and conformational changes in the F1 subunits of ATP synthase, resulting in the synthesis of ATP from ADP and Pi.
- Approximately, transfer of 3 or 4 protons are required to drive synthesis of one ATP molecule.

THANK YOU