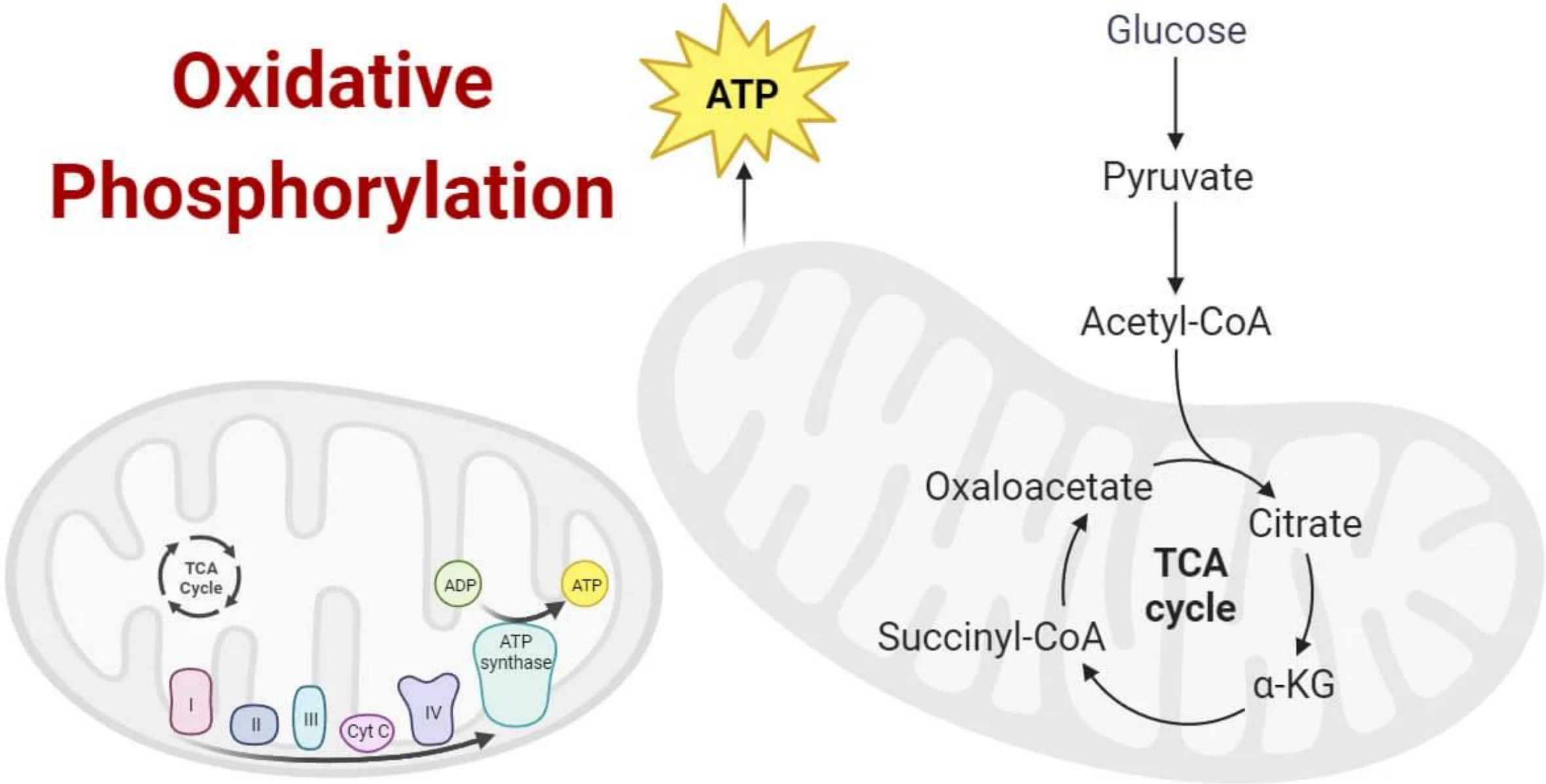
Course: BTBTC301 Biochemistry

Module I-

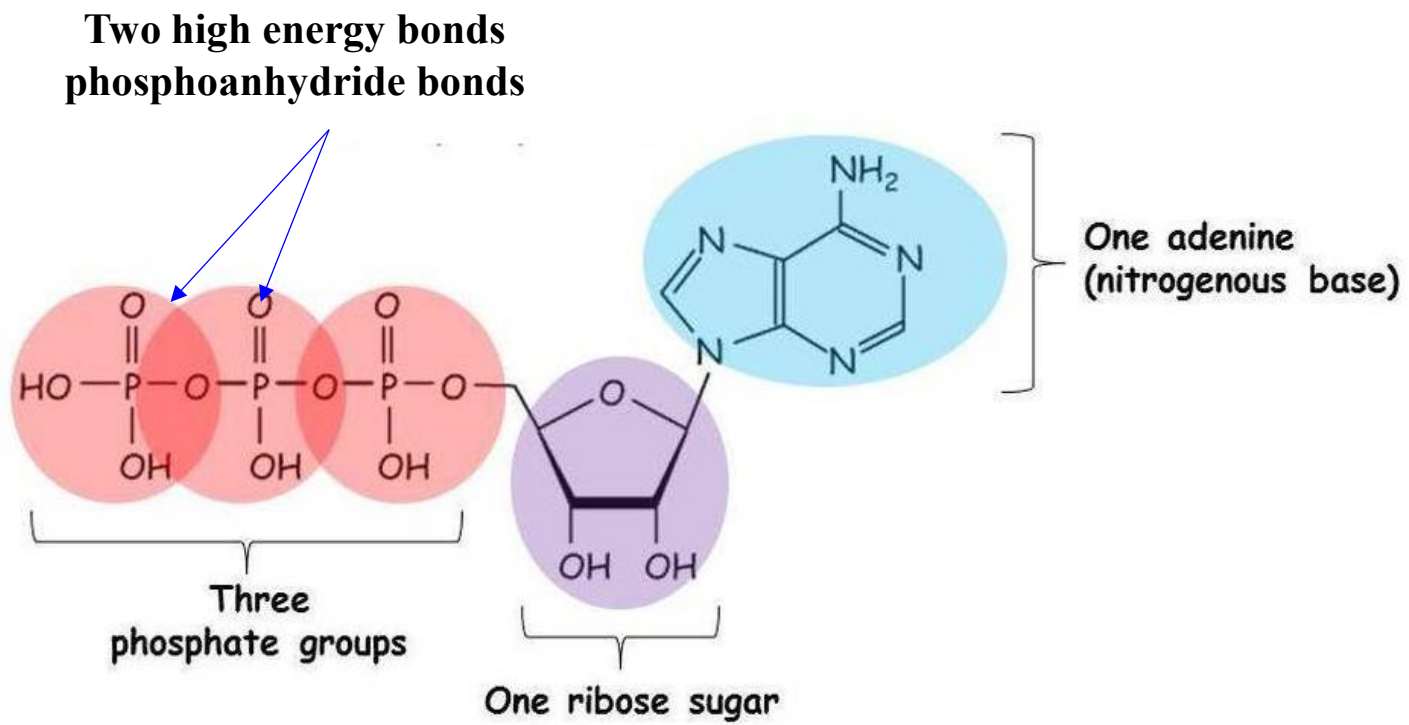
Oxidative phosphorylation and photophosphorylation

Oxidative Phosphorylation



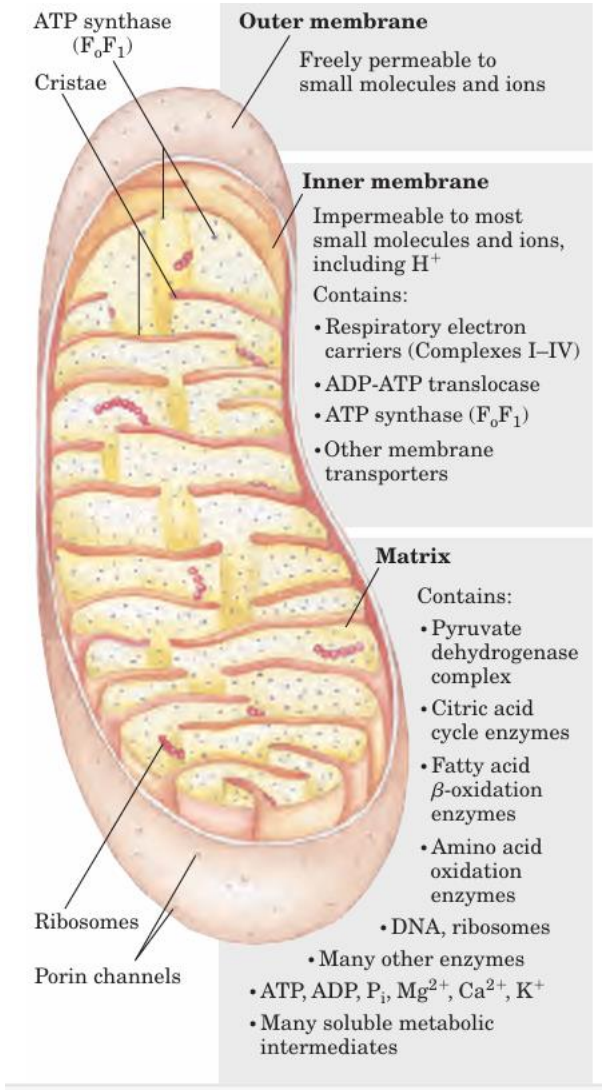
Oxidative Phosphorylation

Oxidative phosphorylation is a process of ATP formation that involves transfer of electron from reducing equivalents like NADH and $FADH_2$, coupled with generation of proton gradient.



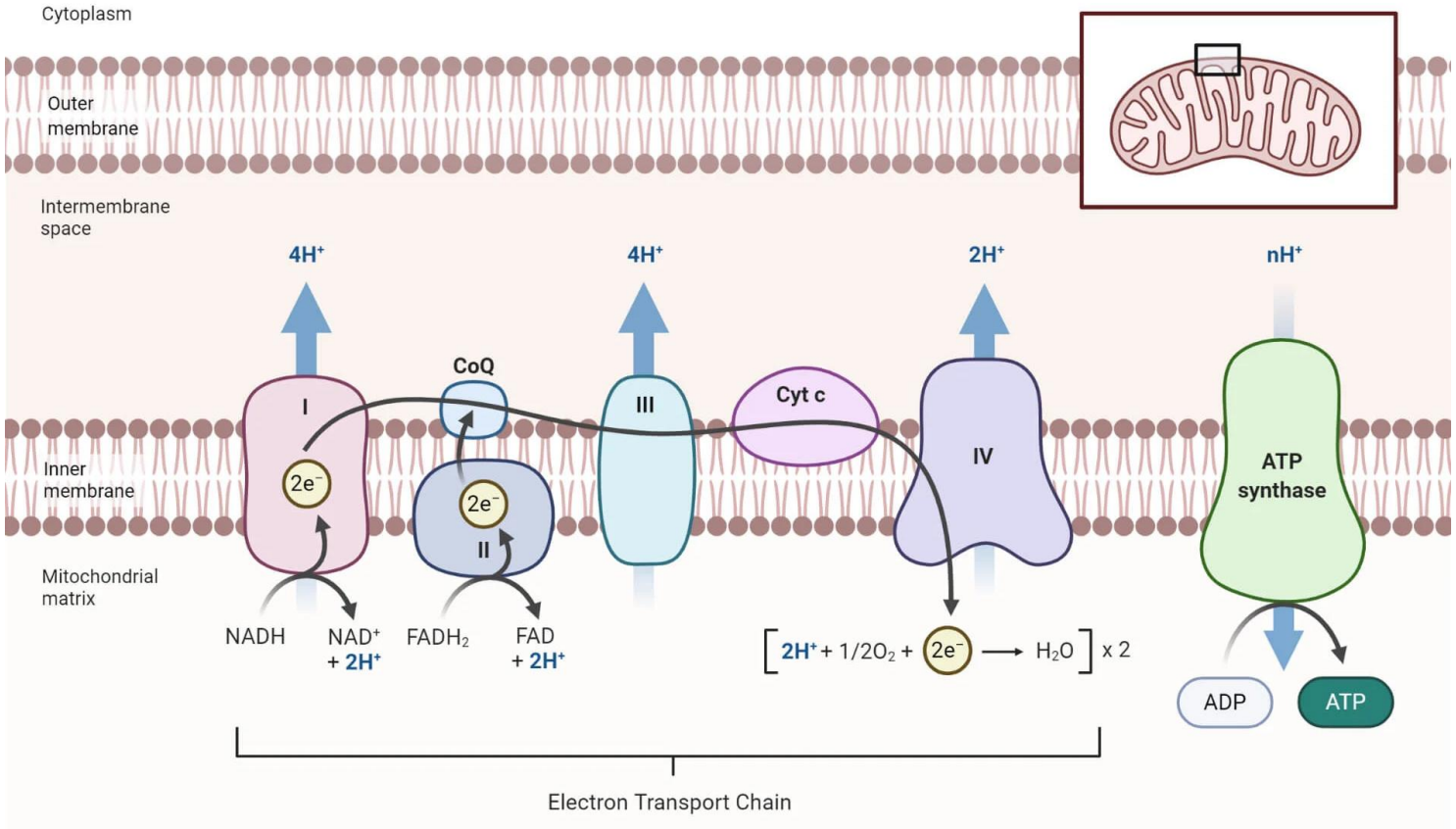
Oxidative Phosphorylation = Electron Transfer Reactions + Chemiosmosis

Site of Electron Transport Chain: Mitochondria



Components of Electron Transfer Chain

- ✓ **Electron donors (Reducing equivalents, NADH, $FADH_2$)**
- ✓ **Multi-subunit enzymes (Complex I-IV)**
- ✓ **Membrane bound Electron carriers (Ubiquinone, Cytochrome C, Fe-S proteins, Rieske iron-sulfur proteins**



The electron flow through the carriers takes place in order of increasing reduction potential.

NADH → CoQ → cyt b → cyt c1 → cyt c → cyt a → cyt a3 → O_2

– 0.32 V **0.82 V**

Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

Redox reaction (half-reaction)	*E'° (V)*
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol	0.045
Cytochrome *b* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *c* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome *a* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.8166

Biological Oxidations Often Involve Dehydrogenation

Electrons are transferred from one molecule (electron donor) to another (electron acceptor) in one of four different ways:

1. Directly as electrons.

For example, the Fe^{2+} /Fe^{3+} redox pair can transfer an electron to the Cu^{+} /Cu^{2+} redox pair:

$$Fe^{2+} + Cu^{2+} \rightleftharpoons Fe^{3+} + Cu^{+}$$

2. As hydrogen atoms.

A hydrogen atom consists of a proton (H) and a single electron (e).

In this case we can write the general equation :

$$AH_2 \rightleftharpoons A + 2e^{-} + 2H^{+}$$

AH2 is the hydrogen/electron donor. (Do not mistake the above reaction for an acid dissociation; the H arises from the removal of a hydrogen atom, $H^{+} + e^{-}$.) AH2 and A together constitute a conjugate redox pair (A/AH2), which can reduce another compound B (or redox pair, B/BH2) by transfer of hydrogen atoms:

$$AH_2 + B \rightleftharpoons A + BH_2$$

Electron transfer

3. As a hydride ion(:H), which has two electrons. This occurs in the case of NAD-linked dehydrogenases.

4. Through direct combination with oxygen. In this case, oxygen combines with an organic reductant and is covalently incorporated in the product, as in the oxidation of a hydrocarbon to an alcohol:

$$R{-}CH_3 + \tfrac{1}{2}O_2 \longrightarrow R{-}CH_2{-}OH$$

The hydrocarbon is the electron donor and the oxygen atom is the electron acceptor.

Hydrogen is central to electron transfer because its **different forms (H^+, $H\cdot$, H^-)** provide flexible ways to move electrons in biochemical reactions. This versatility makes it the universal currency of redox chemistry in living systems.

Hydrogen Chemistry & Electron Transfer

✓ Hydrogen (H) is the simplest element: **1 proton + 1 electron**.

✓ It can exist in different oxidation states depending on electron transfer:

Species	**Symbol**	**Oxidation state**	**Composition**
Proton	H^+	+1	No electrons
Hydrogen atom	$H\cdot$	0	1 proton + 1 electron
Hydride ion	H^-	–1	1 proton + 2 electrons

Hydrogen's ability to **gain or lose electrons easily** makes it perfect for redox (reduction–oxidation) reactions.

- ✓ **$H\cdot$ (neutral hydrogen atom)** can donate or accept one electron.
- ✓ **H^- (hydride)** can transfer a pair of electrons in one step.
- ✓ **H^+ (proton)** can move separately, while electrons go through other carriers.

Why Hydrogen Is Used in Electron Transfer ??

- ✓ **Simplicity:** Just one proton and one electron; easy to switch oxidation states.
- ✓ **Versatility :** Can transfer electrons as:

Proton + electron (separate)

Hydrogen atom ($H\cdot$, $1e^- + 1H^+$)

Hydride ion (H^-, $2e^- + 1H^+$)

- ✓ **Abundance in biology** – Comes from water, organic molecules, cofactors (like NADH, $FADH_2$).
- ✓ **Coupling with energy** – Proton (H^+) movement across membranes (proton gradient) drives ATP synthesis in mitochondria and chloroplasts.
- ✓ **Controlled reactivity** – Free electrons are unstable, but moving them with hydrogen (as $H\cdot$ or H^-) stabilizes the transfer.

- ✓ **Biological Application** :

NADH/NADPH: Transfer hydride ($H^- \rightarrow 2e^- + H^+$)

FAD/FMN: Accept or donate hydrogen atoms ($H\cdot$)

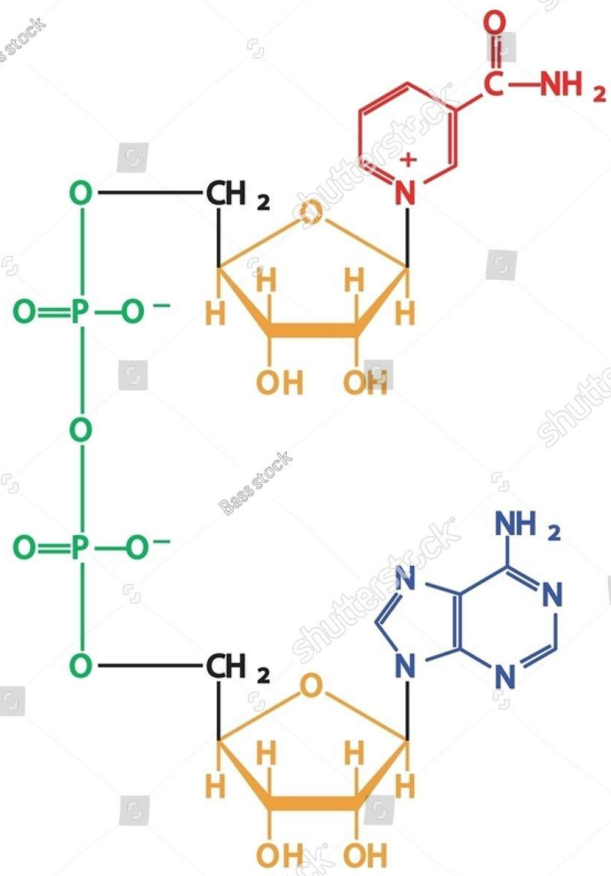
Cytochromes: Transfer electrons separately from protons

Water splitting in photosynthesis: $2H_2O \rightarrow O_2 + 4H^+ + 4e^-$

Electrons Are Funneled to Universal Electron Acceptors

- ✓ Oxidative phosphorylation begins with the entry of electrons into the respiratory chain.
- ✓ Most of these electrons arise from the action of dehydrogenases that collect electrons from catabolic pathways and funnel them into universal electron acceptors—**nicotinamide nucleotides (NAD or NADP) or flavin nucleotides (FMN or FAD).**
- ✓ NAD-linked dehydrogenases remove **two hydrogen atoms** from their substrates.
  One of these is transferred as a hydride ion (: H) to NAD ; the other is released as H in the medium
- ✓ NADH and NADPH are **water-soluble electron carriers** that associate reversibly with dehydrogenases.
- ✓ NADH carries electrons from catabolic reactions to their point of entry into the respiratory chain.
- ✓ NADPH generally supplies electrons to anabolic reactions.
- ✓ Neither NADH nor NADPH can cross the inner mitochondrial membrane, but the electrons they carry can be shuttled across in directly

Nicotinamide adenine dinucleotide (NAD $^{+}$)

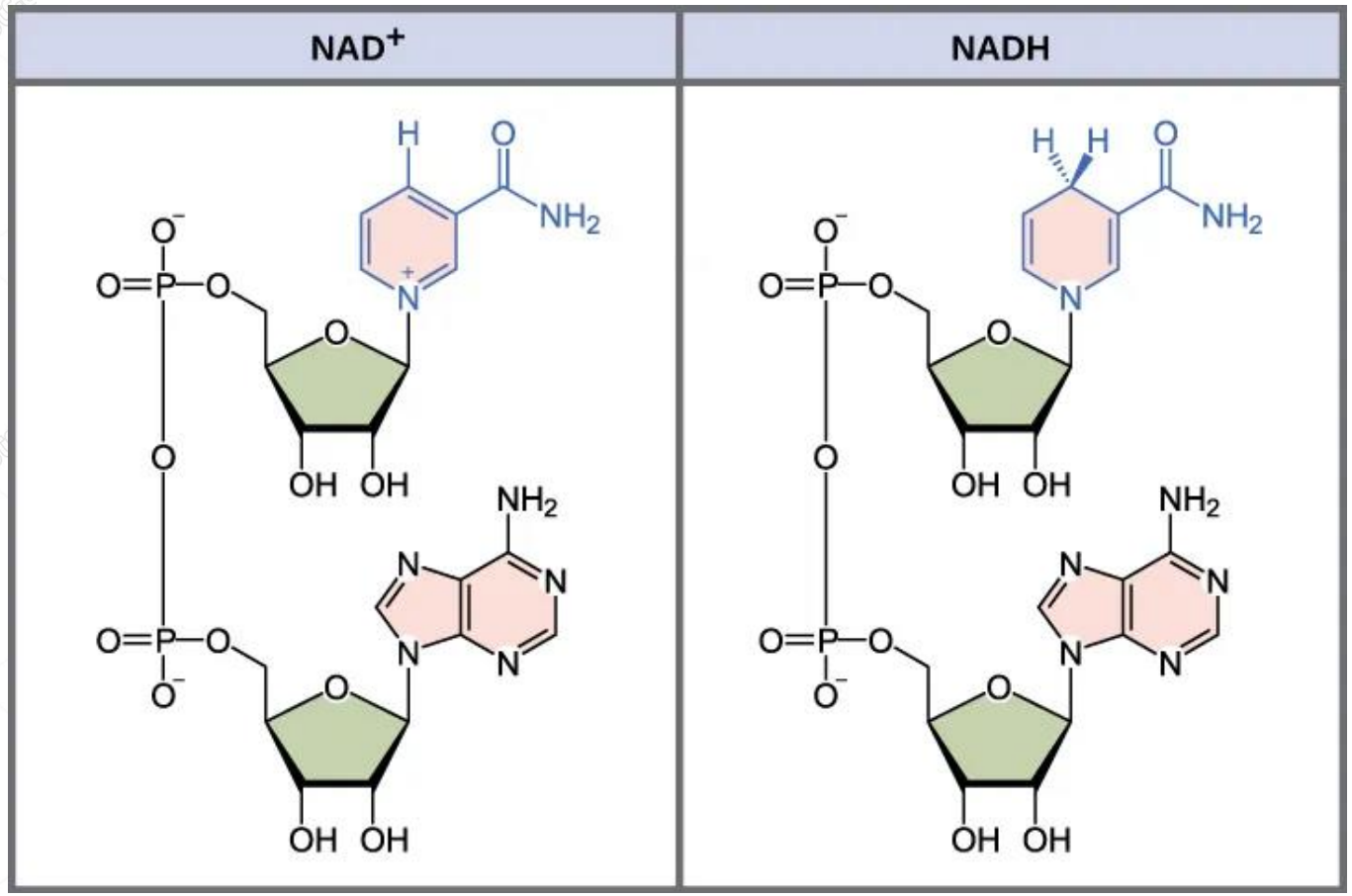


Adenine

Phosphates

Ribose

Nicotinamide



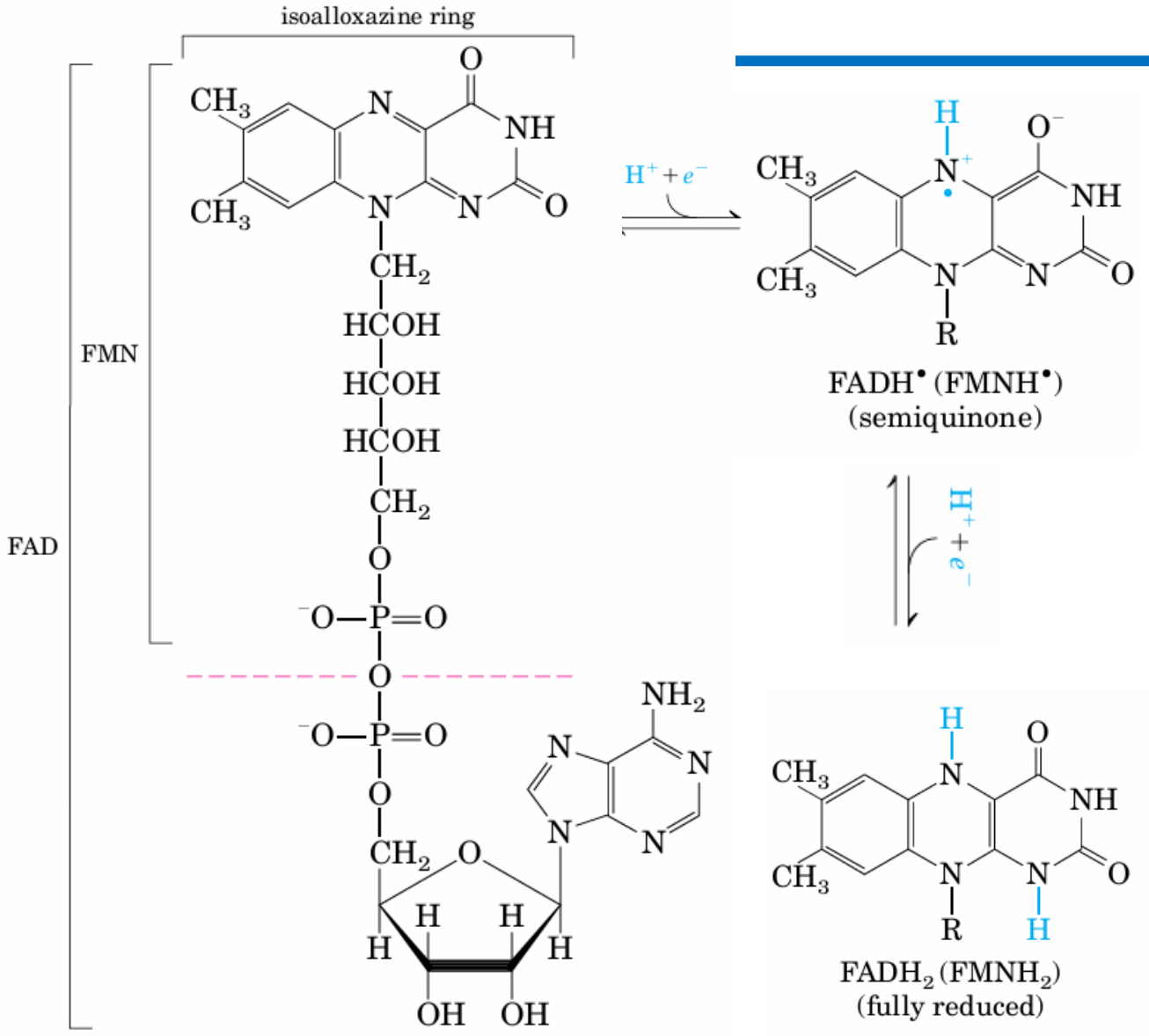
Nicotinamide nucleotide–linked dehydrogenases catalyze reversible reactions

TABLE 19–1 Some Important Reactions Catalyzed by NAD(P)H-Linked Dehydrogenases

Reaction[*]	*Location*[†]
NAD-linked	
α-Ketoglutarate + CoA + $NAD^+ \rightleftharpoons$ succinyl-CoA + CO_2 + NADH + H^+	M
L-Malate + $NAD^+ \rightleftharpoons$ oxaloacetate + NADH + H^+	M and C
Pyruvate + CoA + $NAD^+ \rightleftharpoons$ acetyl-CoA + CO_2 + NADH + H^+	M
Glyceraldehyde 3-phosphate + P_i + $NAD^+ \rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	C
Lactate + $NAD^+ \rightleftharpoons$ pyruvate + NADH + H^+	C
β-Hydroxyacyl-CoA + $NAD^+ \rightleftharpoons$ β-ketoacyl-CoA + NADH + H^+	M
NADP-linked	
Glucose 6-phosphate + $NADP^+ \rightleftharpoons$ 6-phosphogluconate + NADPH + H^+	C
NAD- or NADP-linked	
L-Glutamate + H_2O + $NAD(P)^+ \rightleftharpoons$ α-ketoglutarate + NH_4^+ + NAD(P)H	M
Isocitrate + $NAD(P)^+ \rightleftharpoons$ α-ketoglutarate + CO_2 + NAD(P)H + H^+	M and C

Flavoproteins

- ✓ Flavoproteins contain a very tightly, sometimes covalently, bound flavin nucleotide, either FMN or FAD.
- ✓ The oxidized flavin nucleotide can accept either one electron (yielding the semiquinone form) or two (yielding FADH2 or FMNH2).
- ✓ Electron transfer occurs because the flavoprotein has a higher reduction potential than the compound oxidized
- ✓ The standard reduction potential of a flavin nucleotide, unlike that of NAD or NADP, depends on the protein with which it is associated.

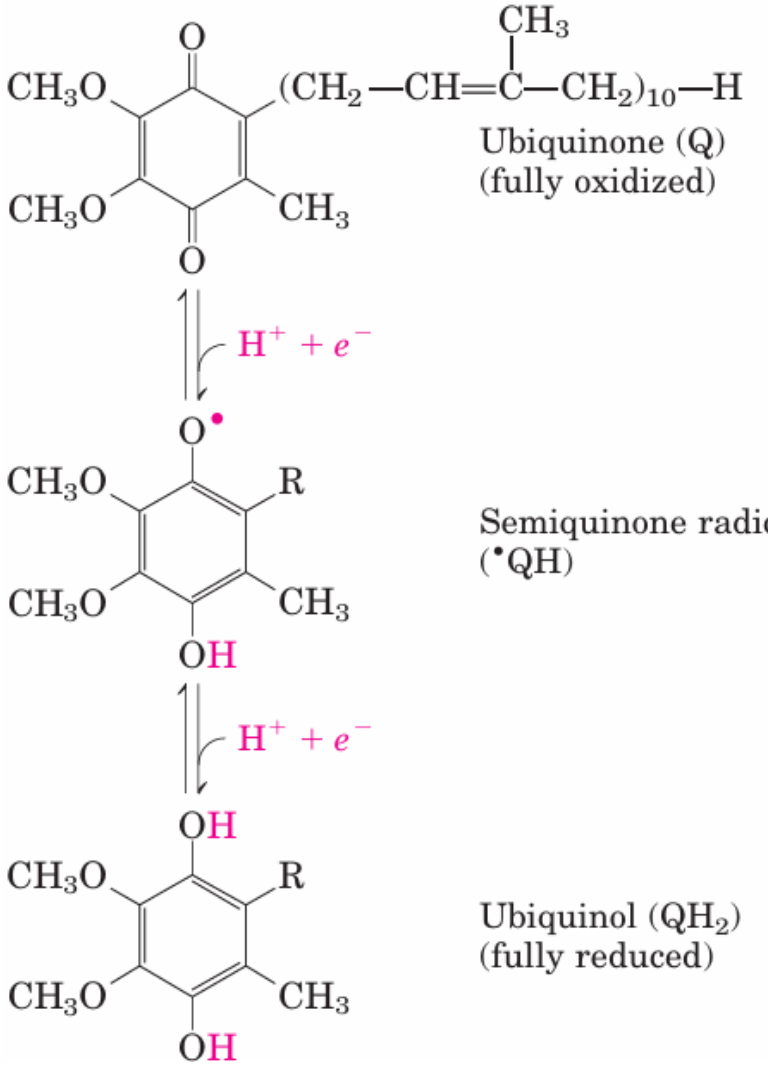


Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

Membrane-Bound Carriers

In addition to NAD and flavoproteins, three other types of electron-carrying molecules function in the respiratory chain:

- ✓ a hydrophobic quinone (ubiquinone) and,
- ✓ two different types of iron-containing proteins (cytochromes and iron-sulfur proteins).



(A) Ubiquinone (Q, or coenzyme Q)

- ✓ It is a lipid-soluble benzoquinone with a long isoprenoid side chain
- ✓ It is both small and hydrophobic and freely diffusible within the lipid bilayer of the inner mitochondrial membrane shuttle reducing equivalents between other, less mobile electron carriers in the membrane.
- ✓ Ubiquinone can accept one electron to become the semi quinone radical (QH) or two electrons to form ubiquinol (QH2), carries both electron and proton

Cytochromes

- **Cytochromes** are the proteins with hemeprosthetic groups. They absorb light at characteristic wavelengths. It carries one electron at a time to complex IV.

- There are three classes of cytochromes involved in mitochondria

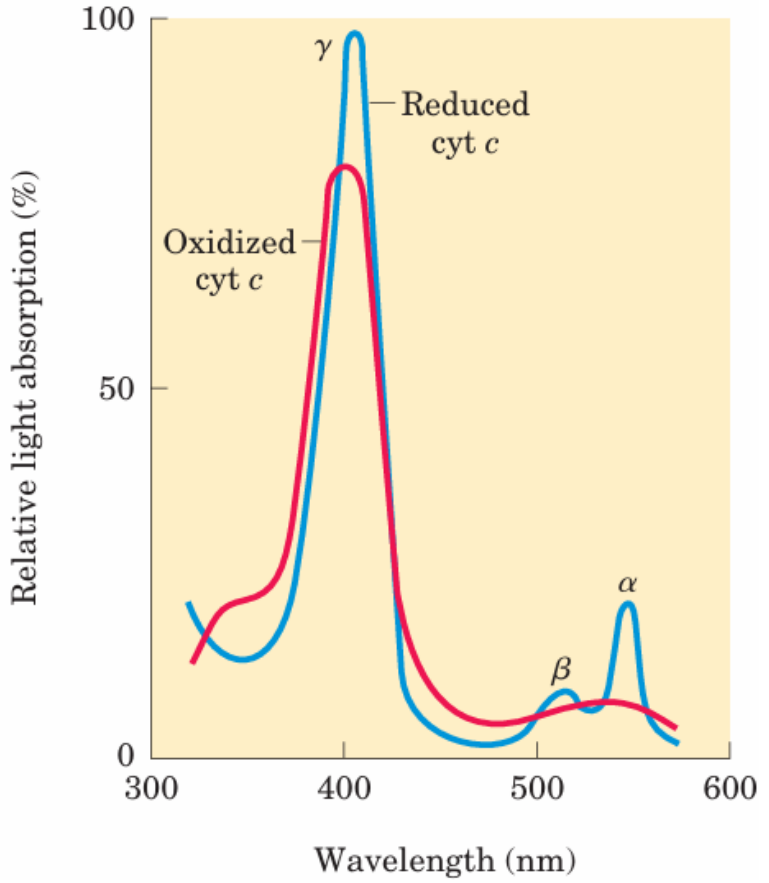
  Two types of cytochrome a i.e. cyt a and a_3

  Two types of cytochrome b i.e. cyt b_1 and b_2

  Two types of cytochrome c i.e. cyt c and c_1

- Each type of cytochrome in its reduced (Fe^{2+}) state has three absorption bands in the visible range.

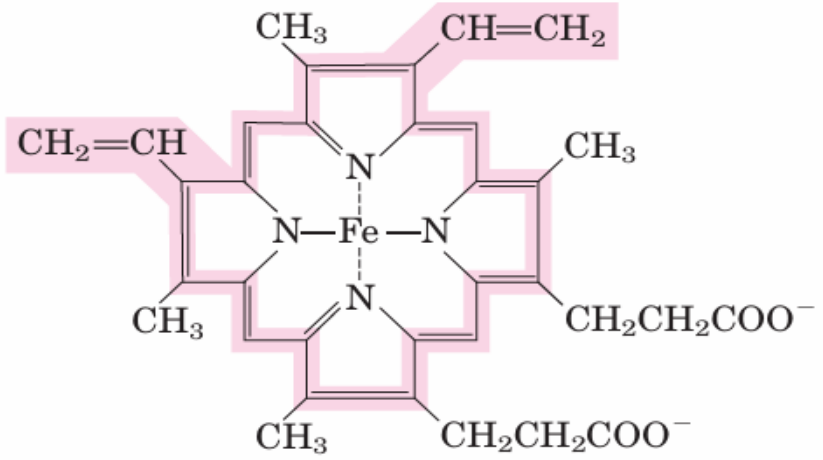
- The longest wavelength band is near 600 nm in type a cytochromes, near 560 nm in type b, and near 550 nm in type c.



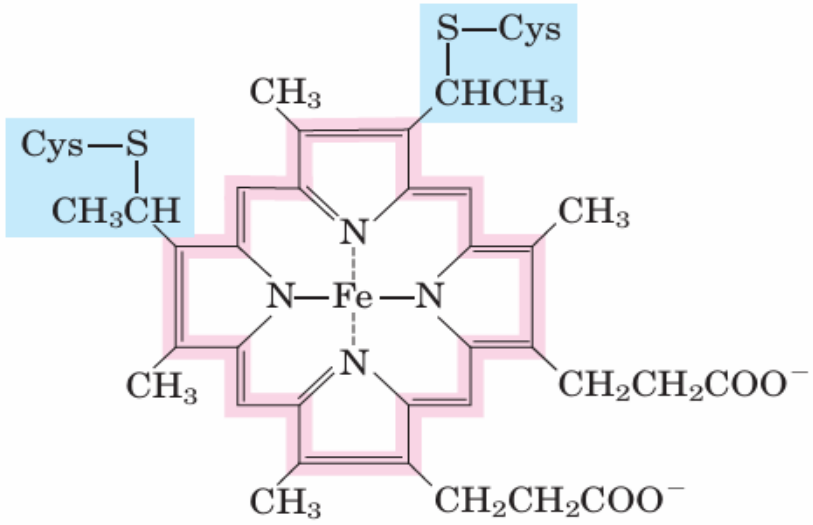
Absorption spectra of cytochrome c (cyt c) in its oxidized (red) and reduced (blue) forms.

cytochrome c of mitochondria is a soluble protein that associates through electrostatic interactions with the outer surface of the inner membrane

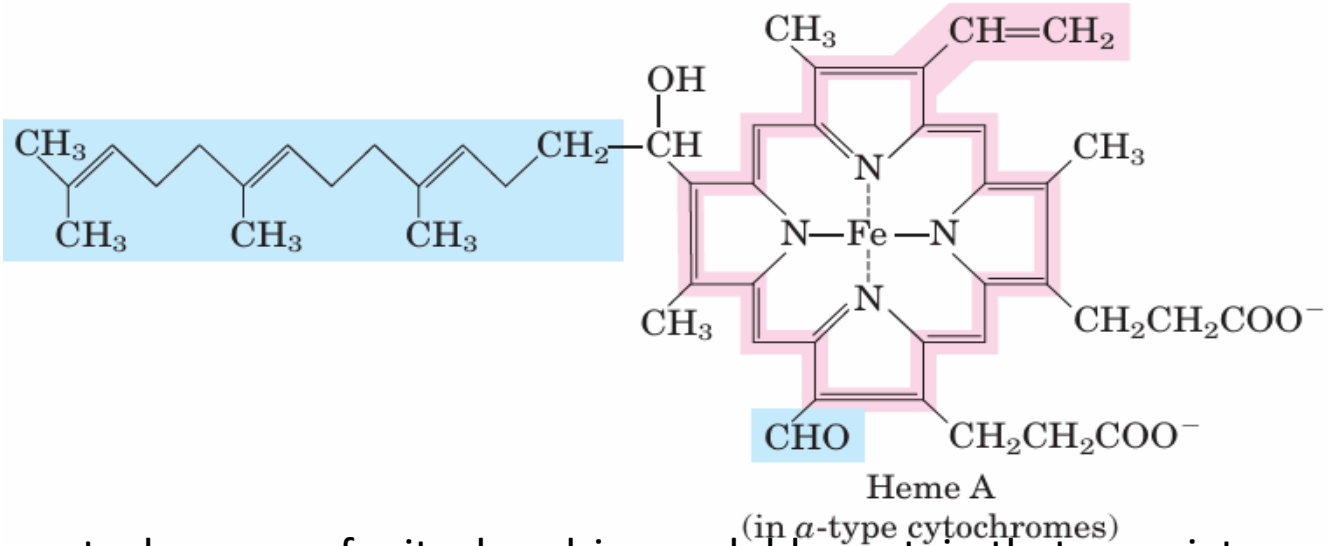
Cytochromes



Iron protoporphyrin IX
(in *b*-type cytochromes)



Heme C
(in *c*-type cytochromes)



Heme A
(in *a*-type cytochromes)

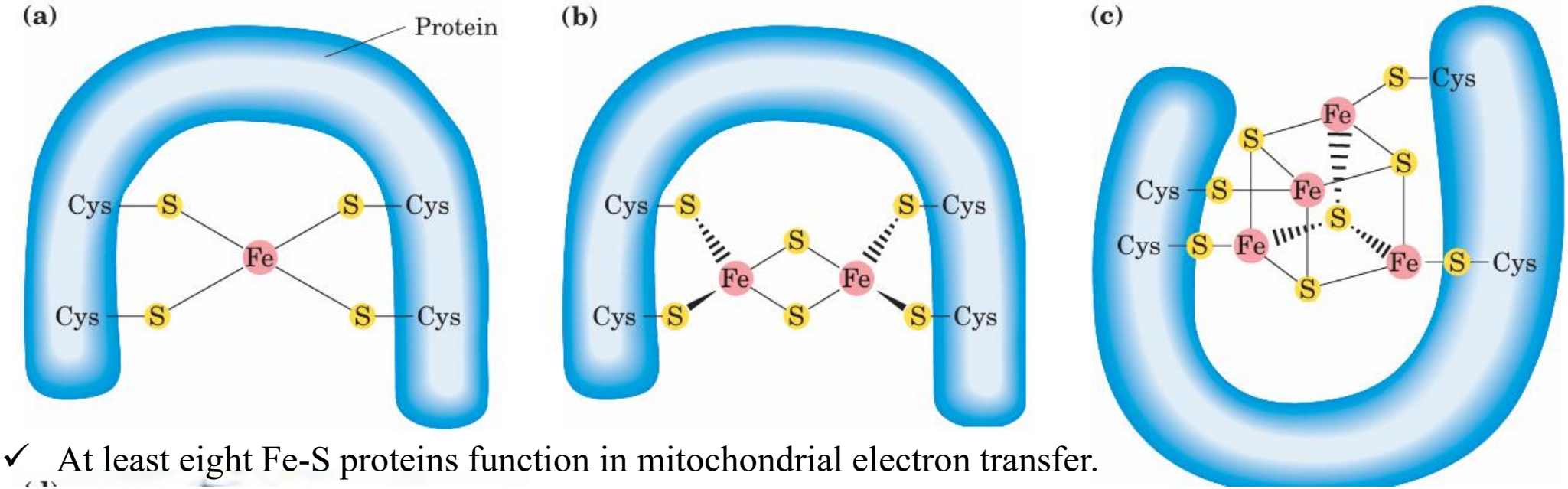
Each group consists of four five-membered, nitrogen-containing rings in a cyclic structure called a porphyrin.

- Heme a, found in the a-type cytochromes, has a long isoprenoid tail attached to one of the five-membered rings
- Iron protoporphyrin IX is found in b-type cytochromes and in hemoglobin and myoglobin.
- Heme c is covalently bound to the protein of cytochrome c through thioether bonds to two Cys residues.

cytochrome c of mitochondria, a soluble protein that associates through electrostatic interactions with the outer surface of the inner membrane

Iron-sulfur proteins

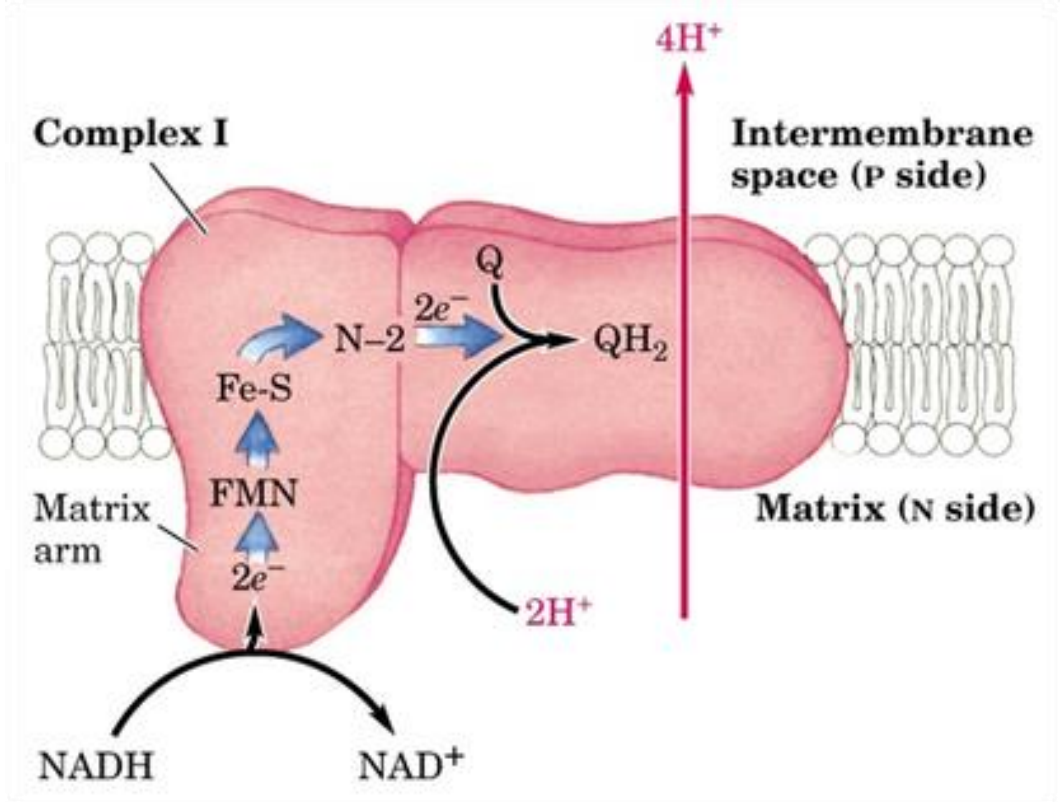
- ✓ Here, iron is present in association with inorganic sulfur atoms or with the sulfur atoms of Cys residues in the protein, or both.
- ✓ Iron sulfur proteins can be of simple structures with a single Fe atom coordinated to four Cys-SH groups to more complex Fe-S centers with two or four Fe atoms
- ✓ Rieske iron-sulfur proteins are a variation on this theme, in which one Fe atom is coordinated to two His residues rather than two Cys residues.
- ✓ Participate in one-electron transfers in which one iron atom of the iron-sulfur cluster is oxidized or reduced.



- ✓ At least eight Fe-S proteins function in mitochondrial electron transfer.

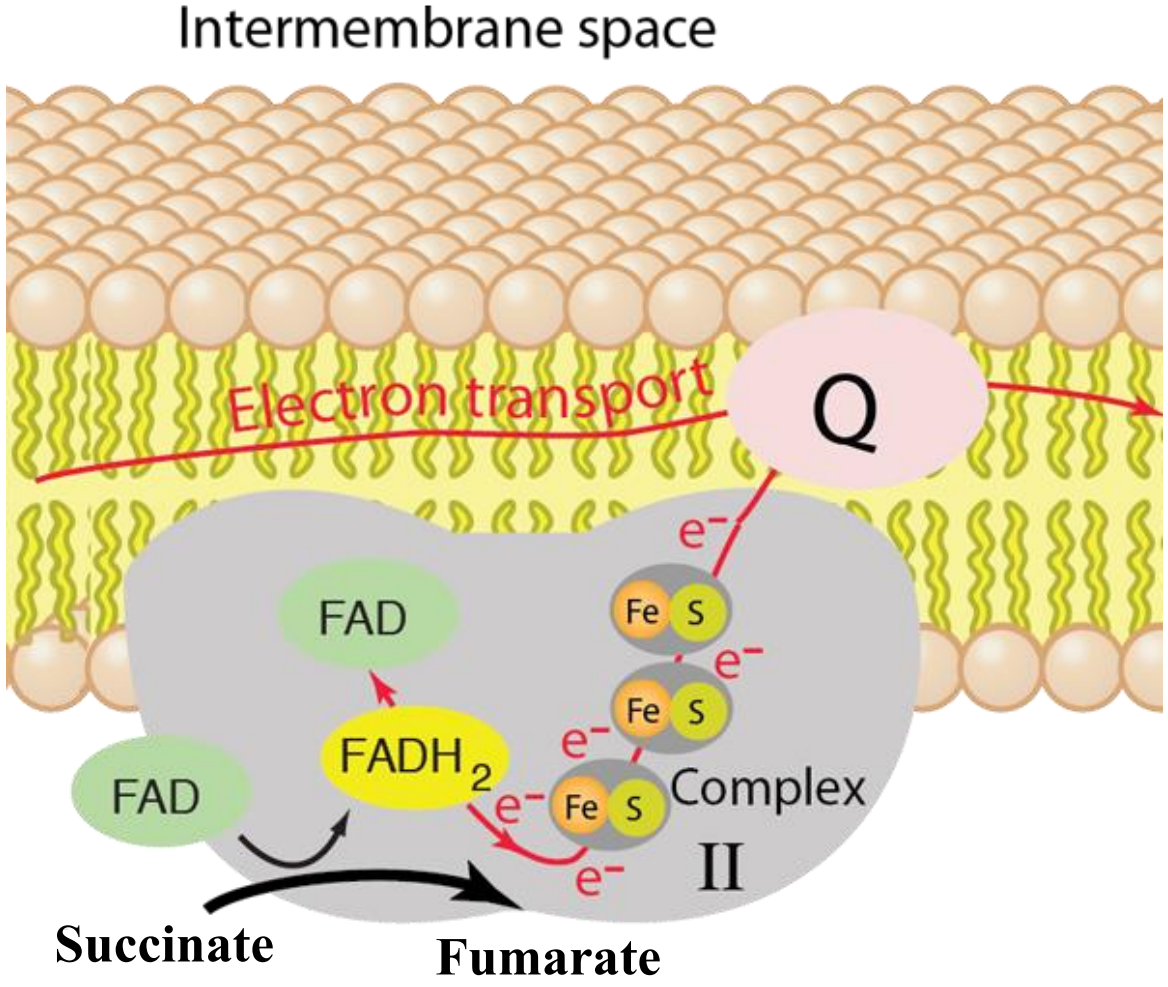
Complex I and Complex II of ETC

NADH:ubiquinone oxidoreductase (Complex I)



$$NADH + 5H^+_N + Q \longrightarrow NAD^+ + QH_2 + 4H^+_P$$

Succinate-Q oxidoreductase (Complex II)



It is the only membrane-bound enzyme in the citric acid cycle

Complex I

Complex I catalyzes the oxidation of NADH, with the reduction of coenzymes Q. Also known as NADH dehydrogenase, contains 46 proteins along with prosthetic groups FMN and various Fe-S centers. It pumps four protons across the mitochondria.

The initial electron transfer involves the following reaction

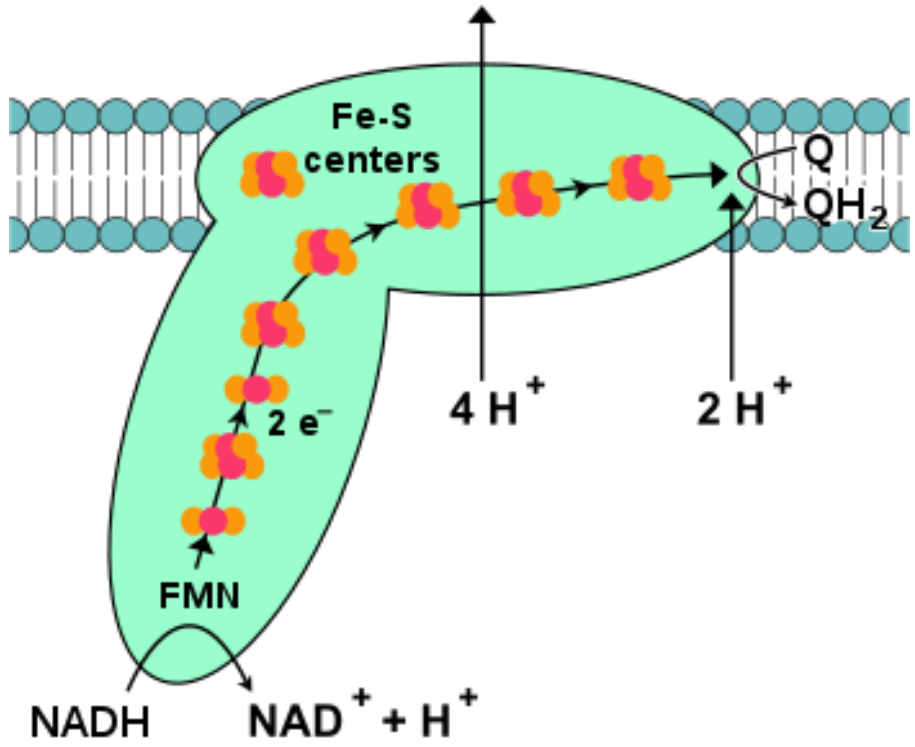
$$NADH + H^+ + FMN \rightarrow NAD^+ + FMNH_2$$

$$FMNH_2 + (Fe\text{-}S)_{ox} \rightarrow FMNH\cdot + (Fe\text{-}S)_{red} + H^+$$

Once Fe-S is re-oxidized by transfer of the electron to the next Fe-S center in the pathway:

$$FMNH\cdot + (Fe\text{-}S)_{ox} \rightarrow FMN + (Fe\text{-}S)_{red} + H^+$$

$$NADH + 5H^+_N + Q \longrightarrow NAD^+ + QH_2 + 4H^+_P$$

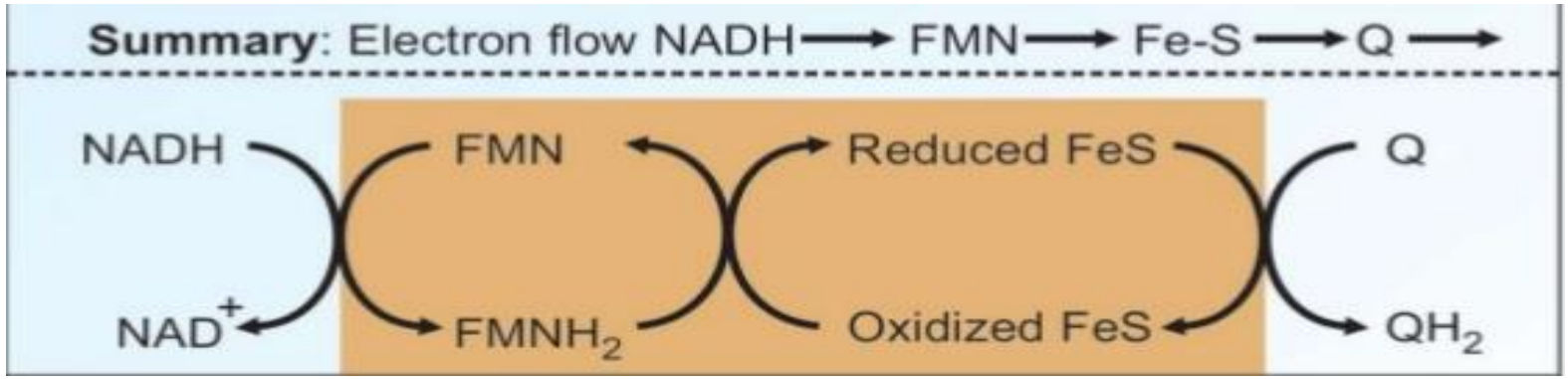


- Eventually 2 electrons are transferred to Ubiquinone (UQ). Electron movement accompanied by a net movement of protons from the matrix to the intermembrane space. During oxidation of 1 NADH and transfer of 2 electrons to UQ by complex I, 4 protons are also pumped across the mitochondria membrane.

- Ubiquinone (also known as Coenzyme Q) is a benzoquinone linked to a number of isoprene units. It exist in 3 redox states: 1. Fully oxidized ubiquinone Q

  2. Partially oxidized Semiquinone

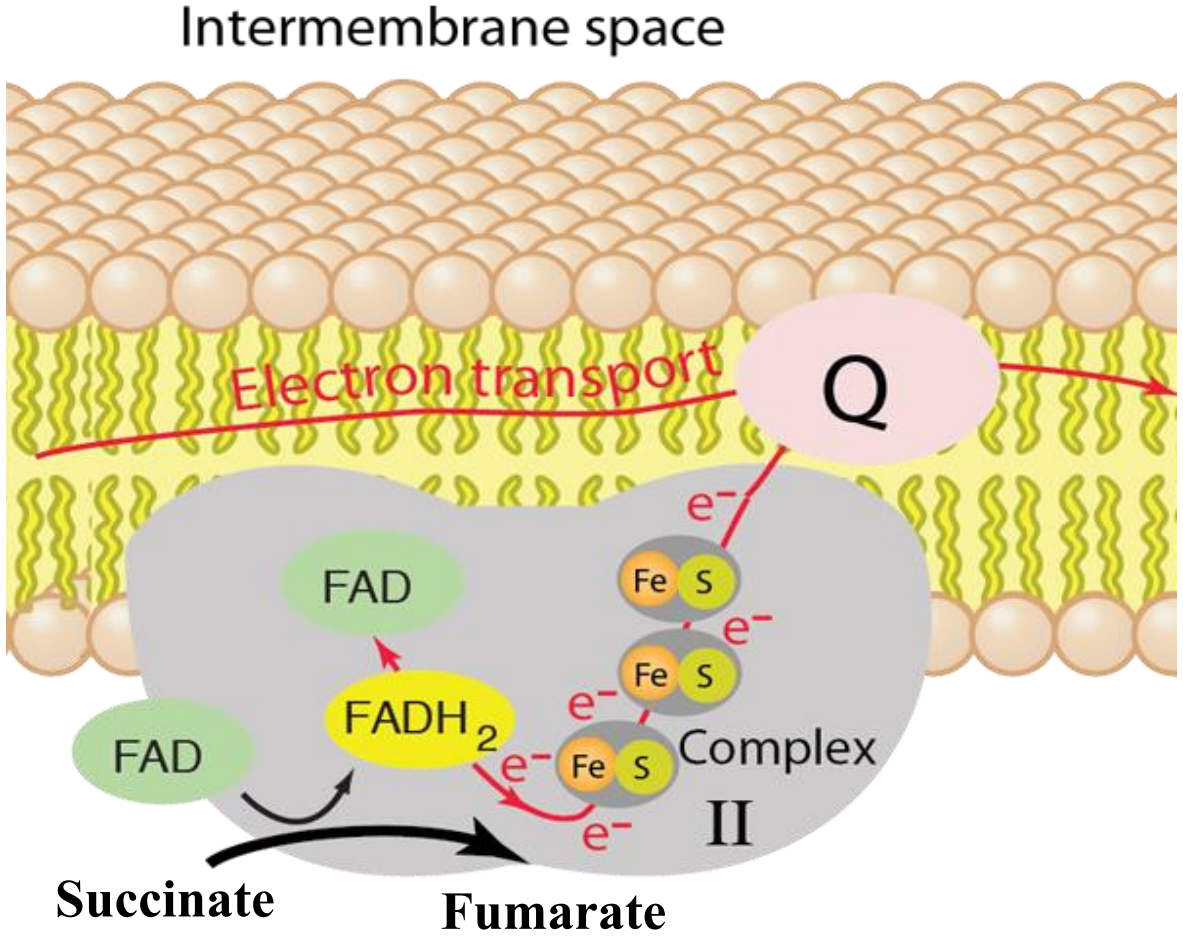
  3. Fully reduced ubiquione



Succinate-Q oxidoreductase (Complex II)

- **Complex II** (also known as Succinate- UQ reductase / Succinate dehydrogenase**)** contains four polypeptide chain, the 1st two constitute the SDH; a Krebs cycle enzyme that catalyzes:

Succinate + FAD ⟶ Fumarate + FADH2



- FADH2 is then re-oxidized by transfer of electrons through a series of 3 Fe-S centers to CoQ, yielding QH2 . It does not pump any proton during transport of electron across the inner mitochondrial membrane.

It is the only membrane-bound enzyme in the citric acid cycle

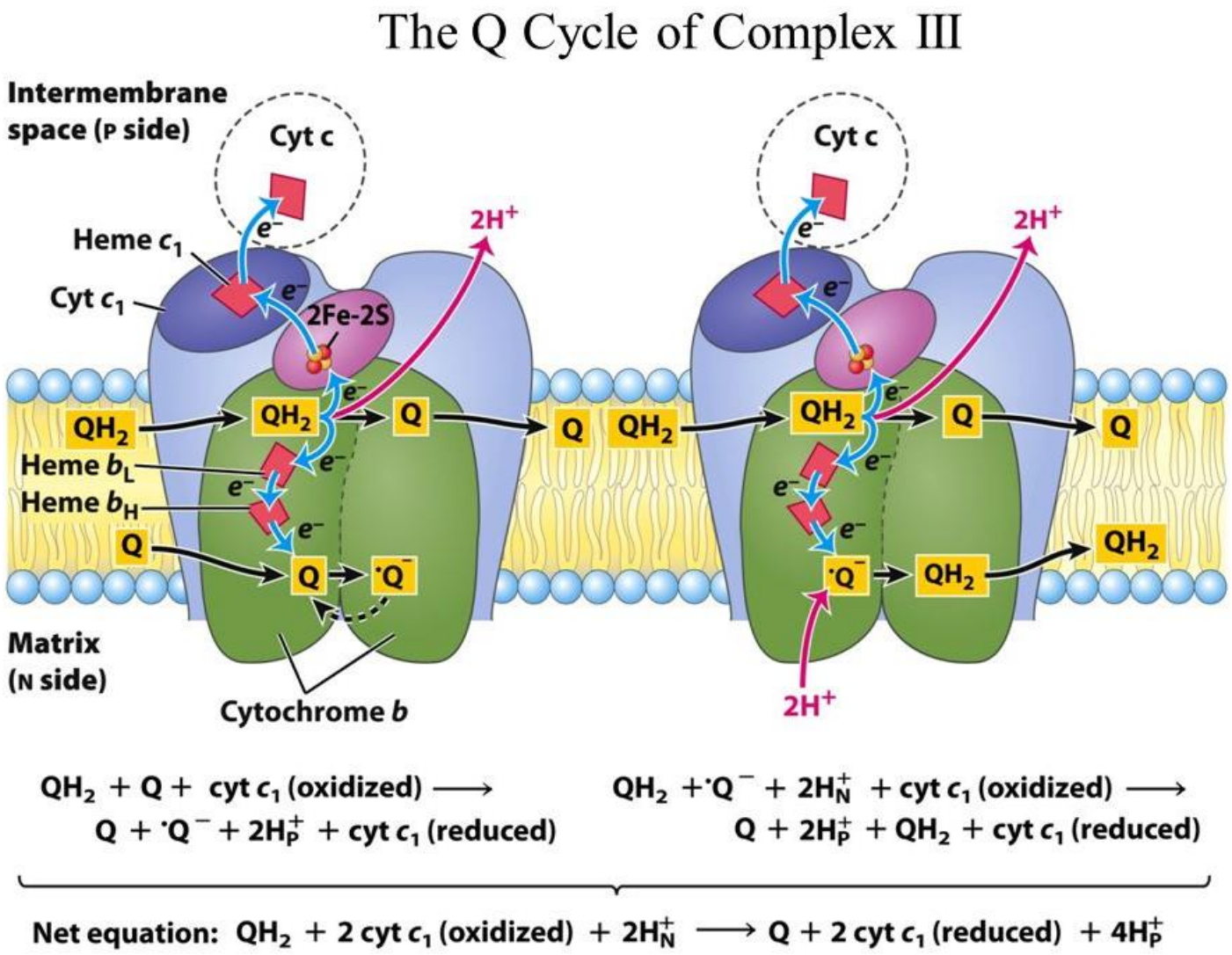
Complex III: Ubiquinone to Cytochrome c or Cytochrome c reductase

The transfer of electrons from ubiquinol (QH2) to cytochrome c take place by the process known as Q-cycle.

Q-cycle consist of two half cycles as shown in the Figure.

Overall, in one Q cycle:

- ✓ Two QH2 are oxidized into Q, releasing 4 H+
- ✓ One Q is reduced into QH2 (recycling step)
- ✓ Two cytochrome c molecules are oxidized



Complex III: Ubiquinone to Cytochrome c or Cytochrome c reductase

- **Complex III** (also known as cytochrome reductase or cytochrome b-c1 complex) accepts electrons from coenzyme QH2 that is generated by electron transfer in complexes I and II.

- Collaterally, it releases two protons into transmembrane space. Within the complex III, the released electrons are transferred to an Fe-S center and later on to two b-type cytochrome or cytochrome C_1

- Finally, the two electrons are transferred to two molecules of the oxidized form of cytochrome c and two additional protons are translocated from mitochondrial matrix across the intermembrane space. This transfer of protons involve the proton motive Q cycle.

Complex IV: Cytochrome c to O_2 / Cytochrome c oxidase

Complex IV, also called cytochrome oxidase, carries electrons from cytochrome c to molecular oxygen, reducing it to H_2O.

- ✓ Two heme groups (Heme a and Heme a3)
- ✓ Three Cu atoms (CuA/CuA) and CuB

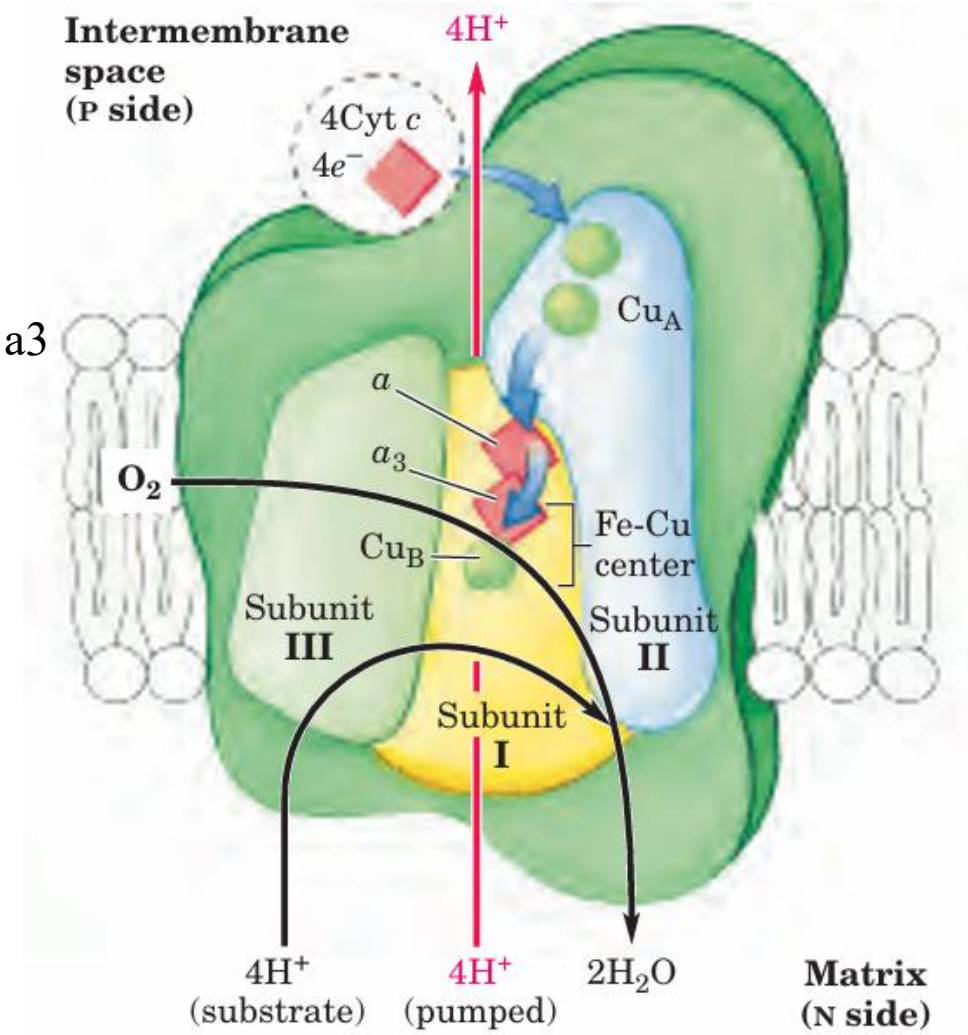
Step1: 2 reduced Cyt C gives 2 e-, one stops at CuB and other stops at Heme a3

Step 2: Once CuB and Heme a3 are in reduced form, the O2 molecule can abstract the electron to form a peroxide bridge

Step 3: Further two more CytC give 2 e-, and 2 H+ is taken from matrix to break the peroxide bridge to form CuB-OH and Heme a3-OH

Step 4: Again, two more H+ ions are taken from matrix to oxidize CuB-OH and Heme a3-OH to form CuB and Heme a3 along with 2 molecules of water

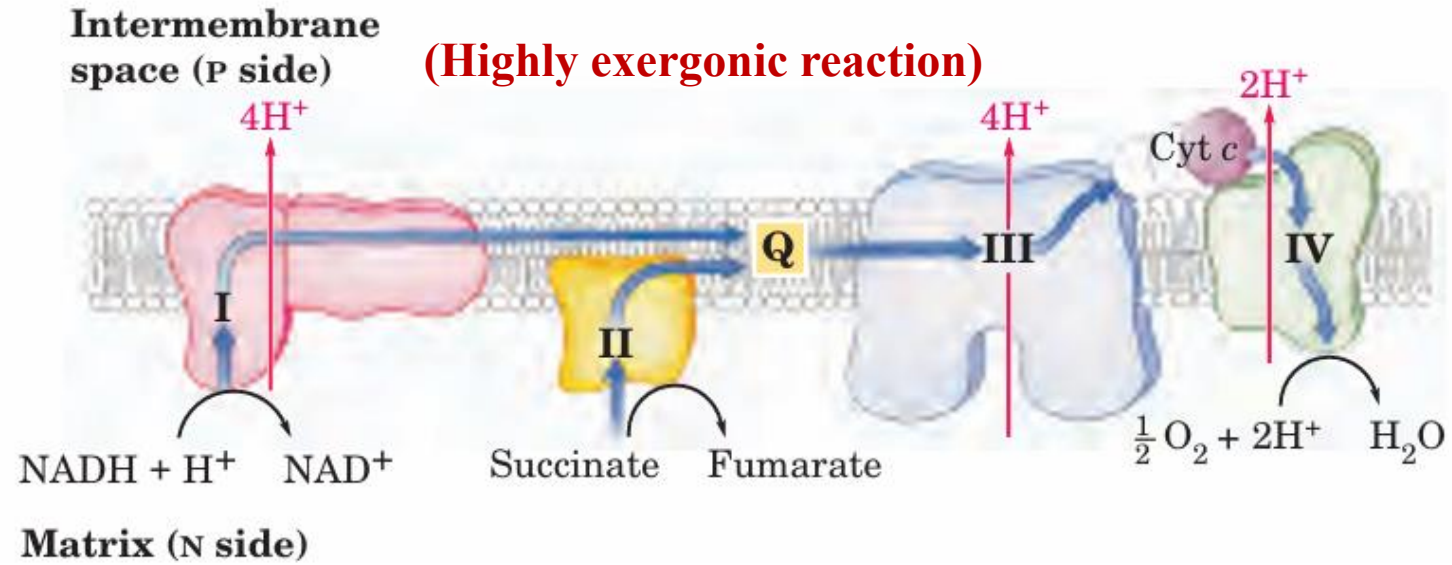
$$4\ \text{Cyt}\ c\ (\text{reduced}) + 8\text{H}^+_\text{N} + \text{O}_2 \longrightarrow 4\ \text{cyt}\ c\ (\text{oxidized}) + 4\text{H}^+_\text{P} + 2\text{H}_2\text{O}$$



Summary of ETC and Proton Motive Force

The transfer of two electrons from NADH through the respiratory chain to molecular oxygen can be written as:

$$NADH + H^+ + \tfrac{1}{2}O_2 \longrightarrow NAD^+ + H_2O$$

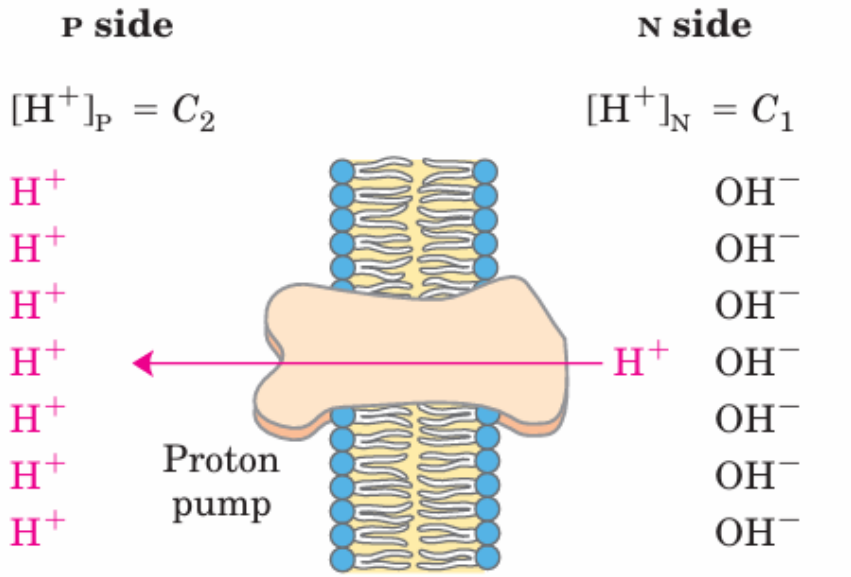


E'^{0} for NAD+/NADH is – 0.320 and O_2/H_2O is 0.816

The delta E'^{0} for this reaction becomes 1.14V, therefore the standard free energy can be calculated by:

$$\begin{aligned}\Delta G'^\circ &= -n\mathcal{F}\Delta E'^\circ \\ &= -2(96.5\ \text{kJ/V}\cdot\text{mol})(1.14\ \text{V}) \\ &= -220\ \text{kJ/mol (of NADH)}\end{aligned}$$

Proton motive force



Difference in chemical concentration (ΔpH) and charge distribution (Δψ) creates the PMF

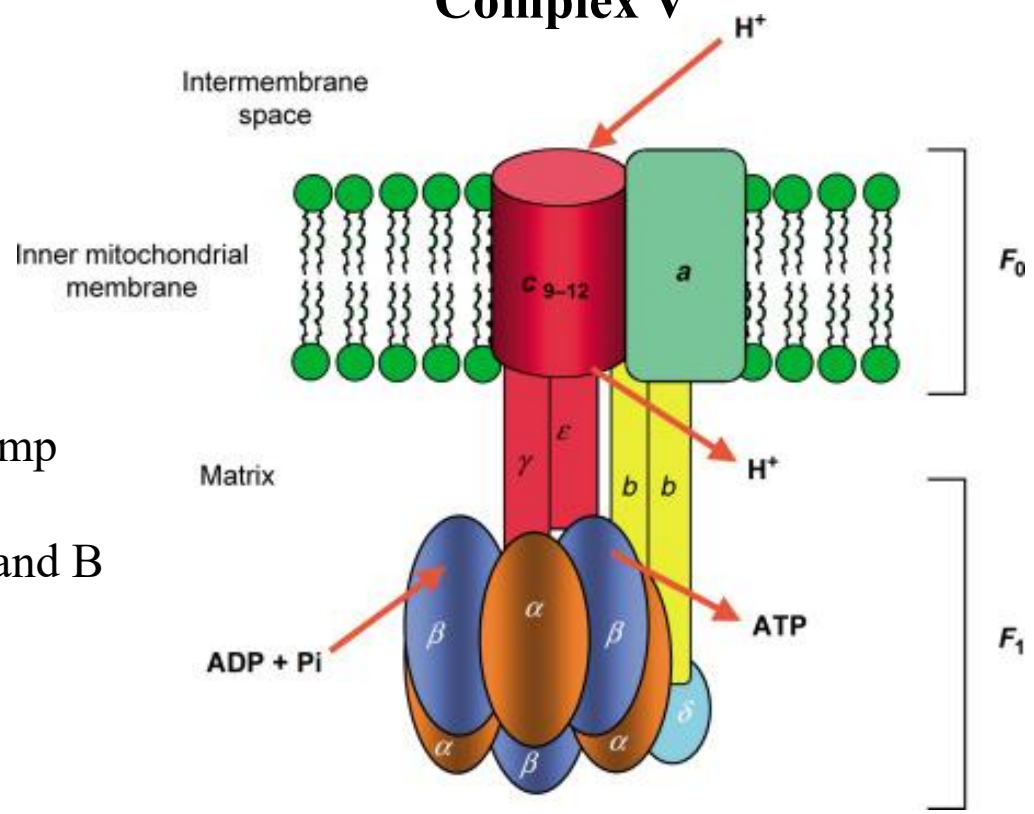
How is a concentration gradient of protons transformed into ATP??

ATP Synthesis: Chemiosmotic Model by ATP synthase (Complex V)

Chemiosmotic model was proposed by Peter Mitchell. It explains the

- ✓ dependence of electron transfer on ATP synthesis in mitochondria
- ✓ PMF drives the passive flow back of proton through a proton channel in ATP synthase.

Complex V



- ❖ F0 unit of ATP synthase is the hydrophobic region, catalyzes Proton Pump (Rotational Catalysis)
- ✓ Oligomycin sensitive and composed of two A unit (01), C unit (10-14), and B unit

- ❖ F1 is the hydrophilic region, **catalytic unit for ATP synthesis** (Binding Mechanism)
- ✓ Composed of 3α and 3β units forming a hexamer ring
- ✓ Have one γ, ε, and δ unit.
- ✓ γ and ε forms the central stalk through which protons are funneled to the F1 unit
- ✓ δ unit hold the hexamer rings and prevents from rotating

$$ADP + P_i + nH^+_P \longrightarrow ATP + H_2O + nH^+_N$$

The function of ATP synthase/ Complex V is to actually use the PMF, the proton electrochemical gradient developed in ETC to synthesize high energy molecule ATP.

F1 subunit : Each β- Subunit of ATP Synthase Can Assume Three Different Conformations

Protons pass from the intermembrane space to the matrix through F_o, which transfers the energy created by the proton electrochemical gradient to F_1, where ADP is phosphorylated to ATP.

- ✓ The isotopic-exchange experiments revealed that enzyme-bound ATP forms readily on the surface of F1 domain even in the absence of a proton-motive force.
- ✓ Although, ATP does not leave the catalytic site unless protons flow through the enzyme. Thus, the role of the proton gradient is not to form ATP but to release it from synthase.
- ✓ For the continuous synthesis of ATP, the enzyme must cycle between a form that binds ATP very tightly and a form that release ATP.

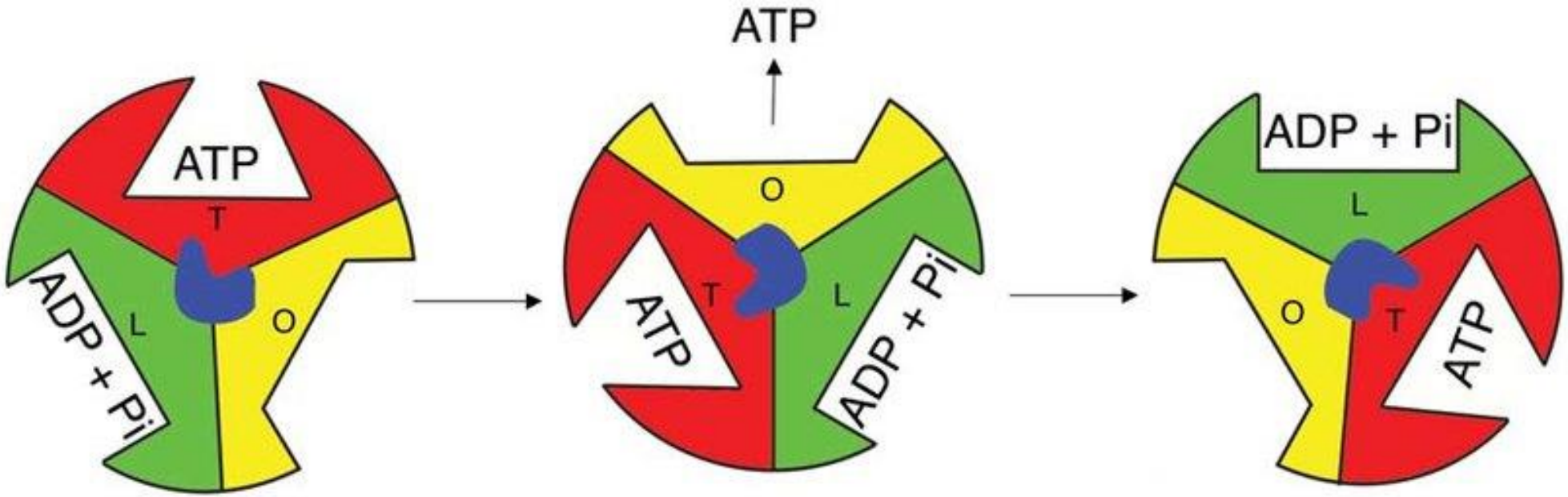
$$\text{enz:ADP} + \text{Pi} \longrightarrow \text{enz:ATP}$$

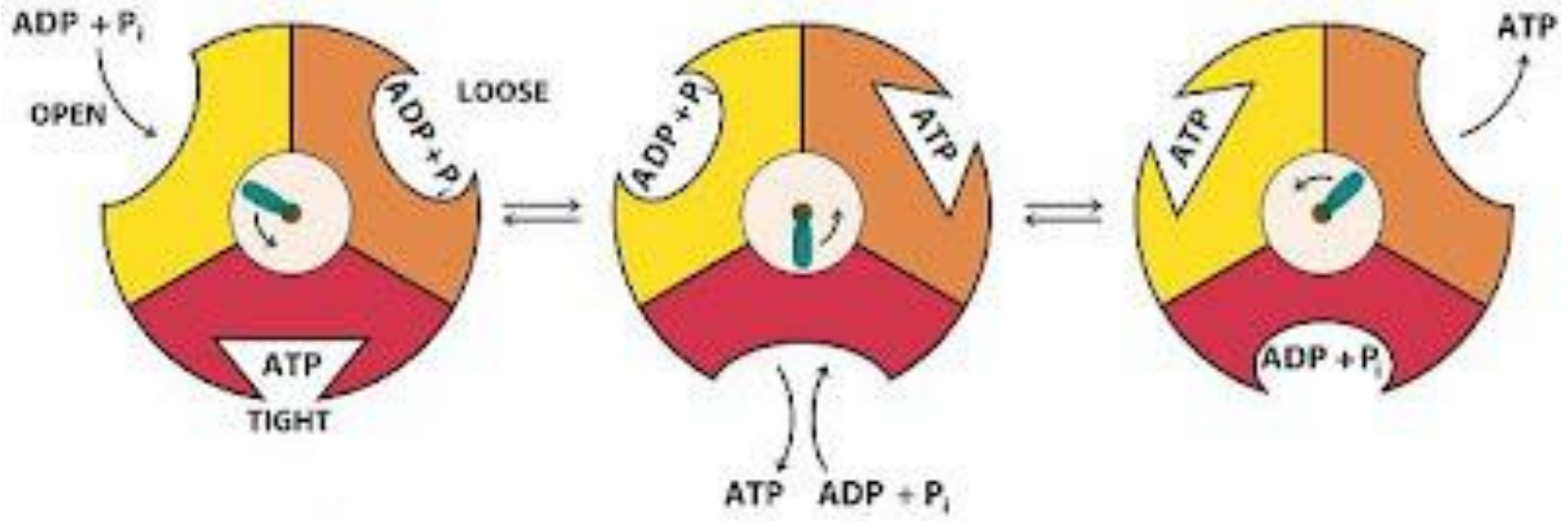
Binding mechanism of ATP synthesis

- ✓ Paul boyer proposed the rotational catalysis mechanism in which the 3 active sites (β) of F_1 take turns catalyzing ATP synthesis.
- ✓ Passage of the protons through the F_o "pore" causes the cylinder of C subunits & the attached γ subunits to rotate about the long axis of γ which is perpendicular to the plane of the membrane.
- ✓ With each 120◦ rotation, β adopts an another conformation:

β – empty	β – ADP	β - ATP
(Open or O)	(Loose or L)	(Tight or T)

- ✓ In L conformation β binds to ADP & Pi
- ✓ In T conformation there is synthesis of ATP from ADP & Pi
- ✓ In O conformation there is release of ATP


ATP
T
ADP + Pi
L
O
ATP
O
T
L
ATP
ADP + Pi
ADP + Pi
L
O
T
ATP

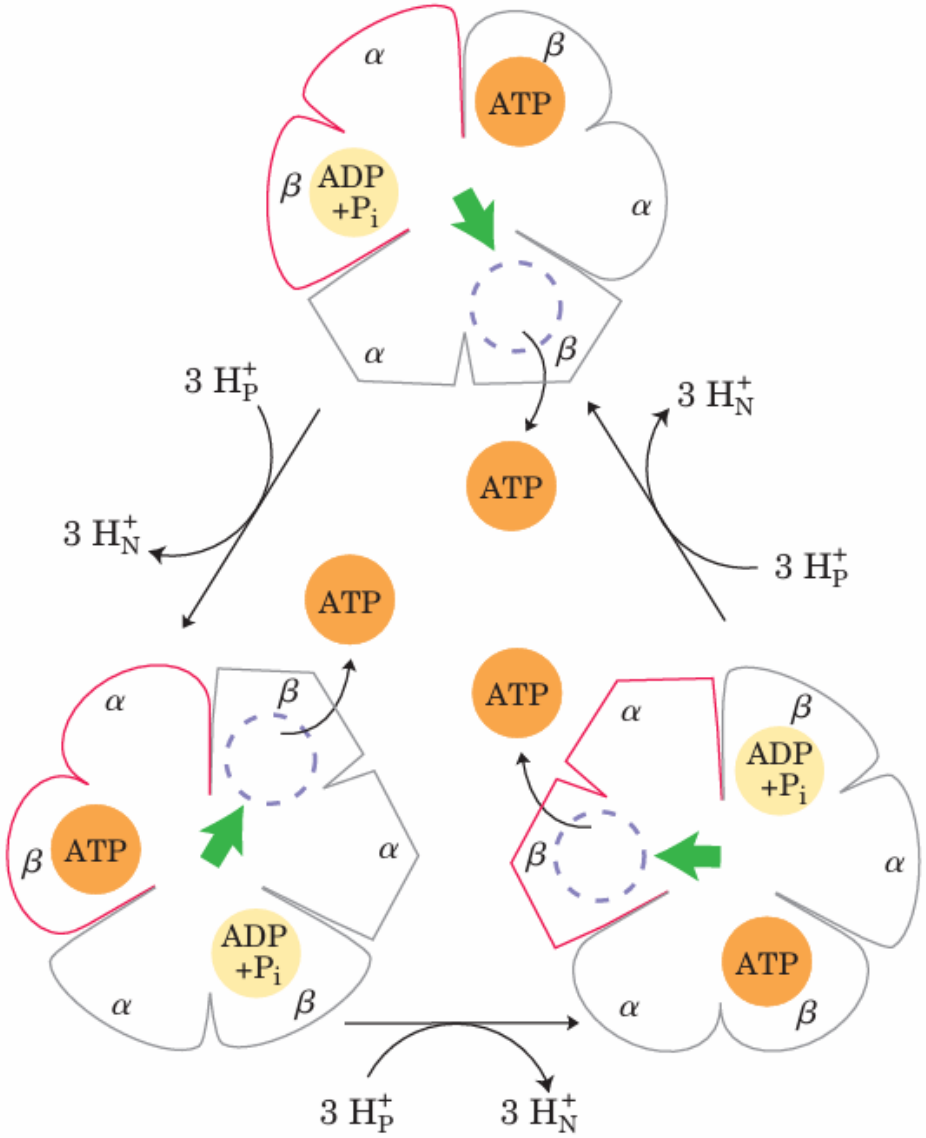

ADP + Pi
OPEN
LOOSE
ADP + Pi
ATP
TIGHT
ADP + Pi
ATP
ATP
ADP + Pi
ATP
ATP
ADP + Pi

Rotational Catalysis

- ✓ At any given moment, one of these sites is in the β – ATP conformation, a second is in the β – ADP conformation, and a third is in the β – empty conformation.

- ✓ The proton motive force causes rotation of the central shaft - the subunit, showed as a green arrowhead – which comes into contact with each subunit pair in succession.

- ✓ This produces a cooperative conformational changes in which the β – ADP site is converted to β – ATP conformation, which promotes condensation of bound ADP – Pi to form ATP; and the β – empty site becomes a β – ADP sites, which loosely binds ADP – Pi entering from the solvent



ATP synthesis and net calculation

ATP synthase works like a **molecular turbine** powered by the **proton gradient** across the **inner mitochondrial membrane**.

ATP synthase has two main parts:

- **F_0 unit**: Embedded in the membrane, rotates as protons flow through it.
- **F_1 unit**: In the matrix, synthesizes ATP as F_0 rotates.

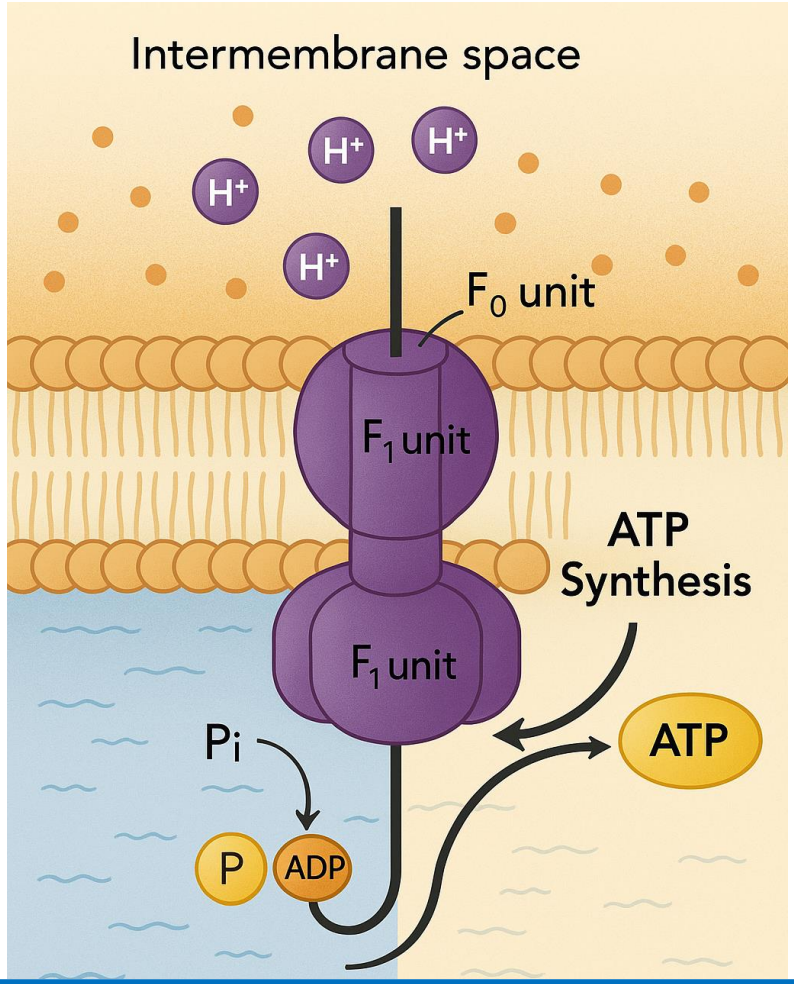
Proton for Transport (Phosphate Shuttle)

1 additional H^+ is used by the **phosphate translocase and ATP/ADP antiporter** to import **inorganic phosphate (Pi)** into the matrix and exchange **ATP for ADP** across the membrane.

Total: 3 H^+ for ATP synthase rotation + 1 H^+ for Pi transport = 4 H^+ per ATP

10 H^+ pumped per NADH → 10 ÷ 4 = 2.5 ATP
6 H^+ pumped per $FADH_2$ → 6 ÷ 4 = 1.5 ATP

Total: 10 H^+ pumped per NADH
6 H^+ per $FADH_2$



Photosynthesis

- ✓ Reaction sequence in which the flow of electrons is coupled to the synthesis of ATP and light-driven phosphorylation.

- ✓ Photosynthetic organisms trap solar energy and form ATP and NADPH, which they use as energy sources to make carbohydrates and other organic compounds from CO_2 and H_2O; simultaneously, they release O_2 into the atmosphere.

$$CO_2 + H_2O \rightarrow O_2 + (CH_2O)_n$$

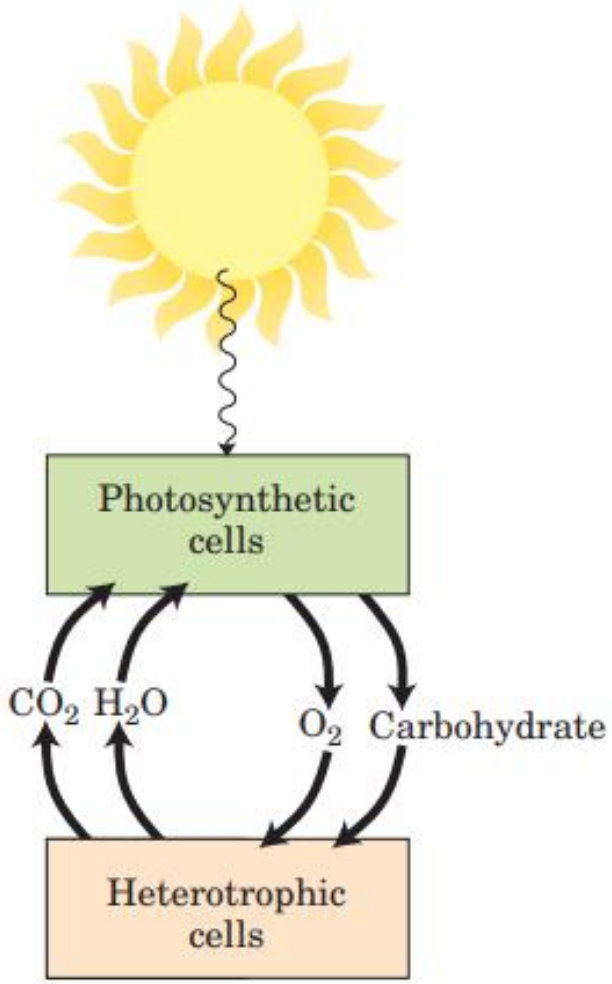


Figure: Solar energy as the ultimate source of all biological energy.
Photosynthetic organisms use the energy of sunlight to manufacture glucose and other organic products, which heterotrophic cells use as energy and carbon sources

Photosynthetic reactions

Photosynthesis encompasses two processes:

light-dependent reactions, or **light reactions,**

carbon-assimilation reactions (or **carbon-fixation reactions**)

- ✓ **Light reactions:** chlorophyll and other pigments of photosynthetic cells absorb light energy and conserve it as ATP and NADPH; simultaneously, O_2 is evolved.
- ✓ **Carbon-assimilation reactions:** ATP and NADPH are used to reduce CO_2 to form triose phosphates, starch, and sucrose, and other products derived from them.

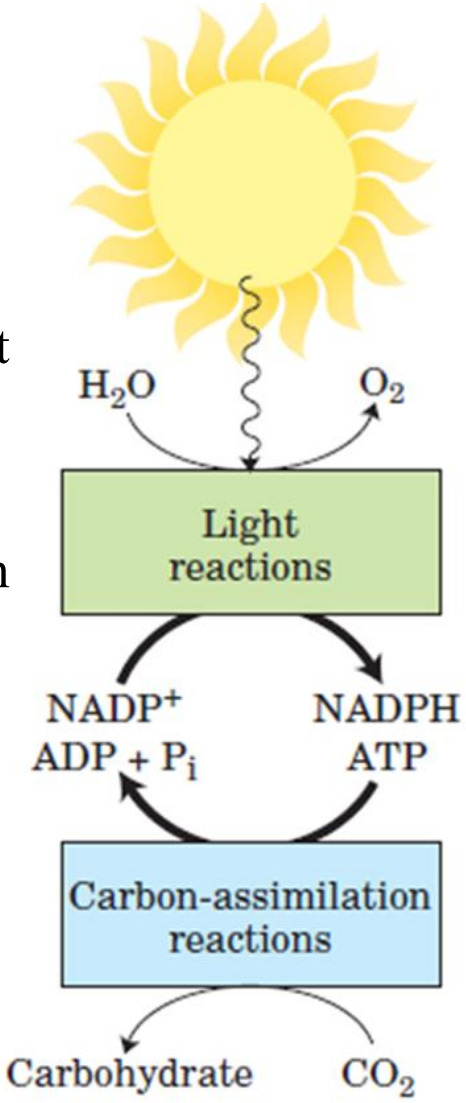


Figure: The light reactions of photosynthesis generate energy rich NADPH and ATP at the expense of solar energy.

These products are used in the carbon assimilation reactions, which occur in light or darkness, to reduce CO_2 to form trioses and more complex compounds (such as glucose)derived from trioses

Photosynthesis in Plants Takes Place in Chloroplasts

Chloroplast is an intracellular organelles that are surrounded by:

- ✓ an **outer membrane** that is permeable to small molecules and ions, and
- ✓ an **inner membrane** that encloses the internal compartment.

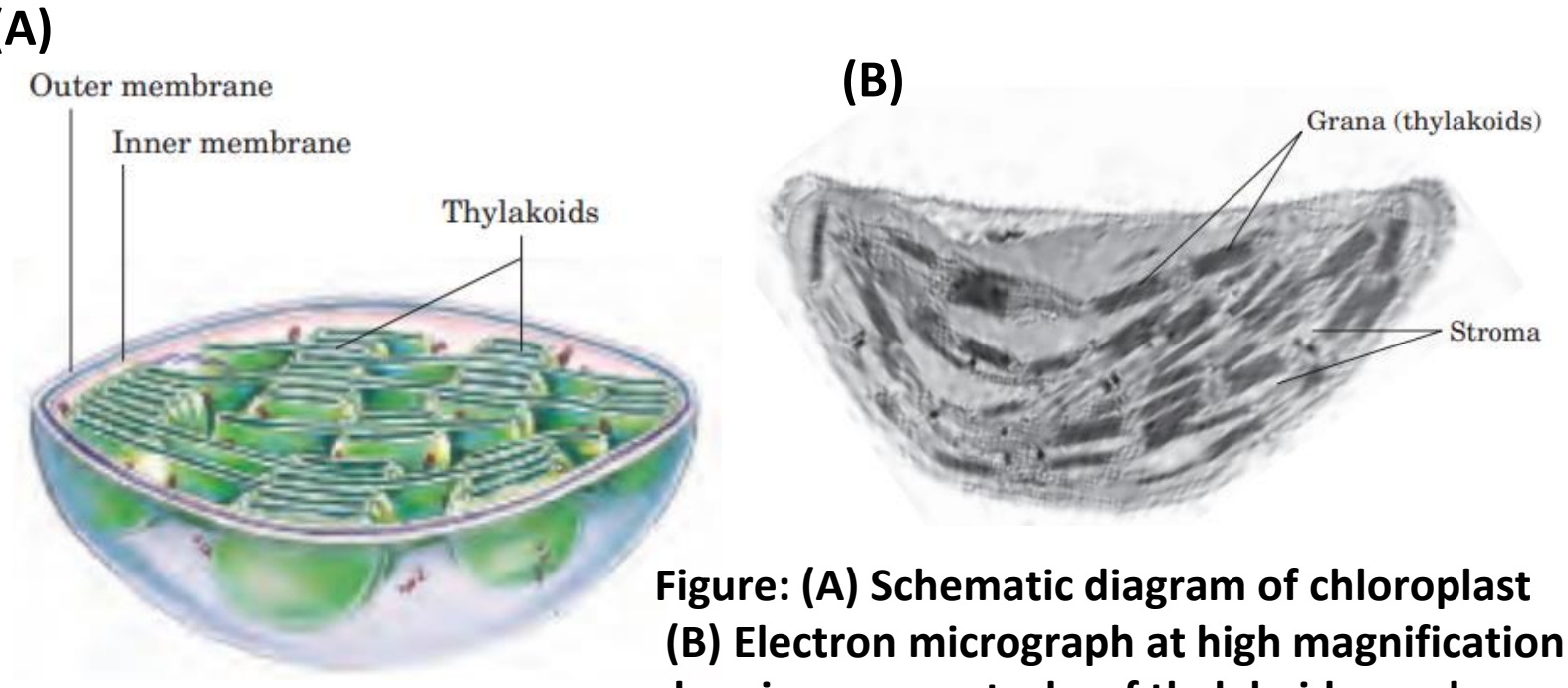
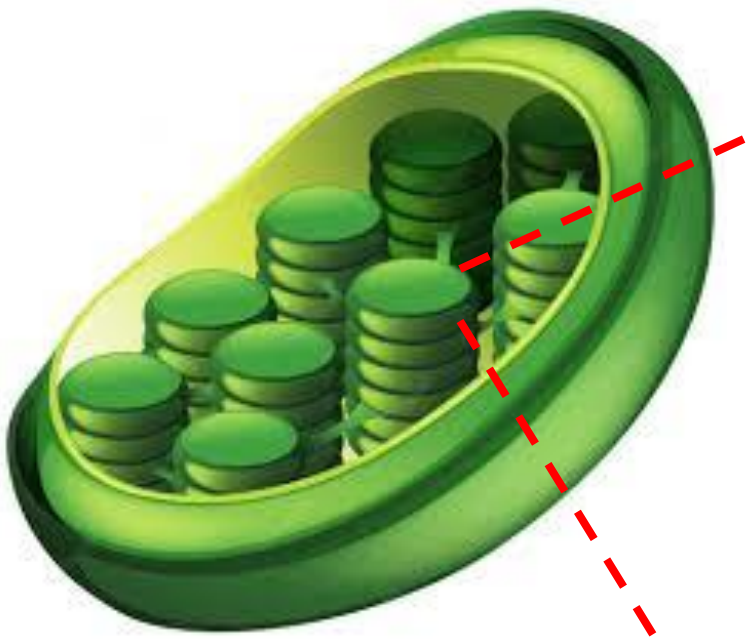
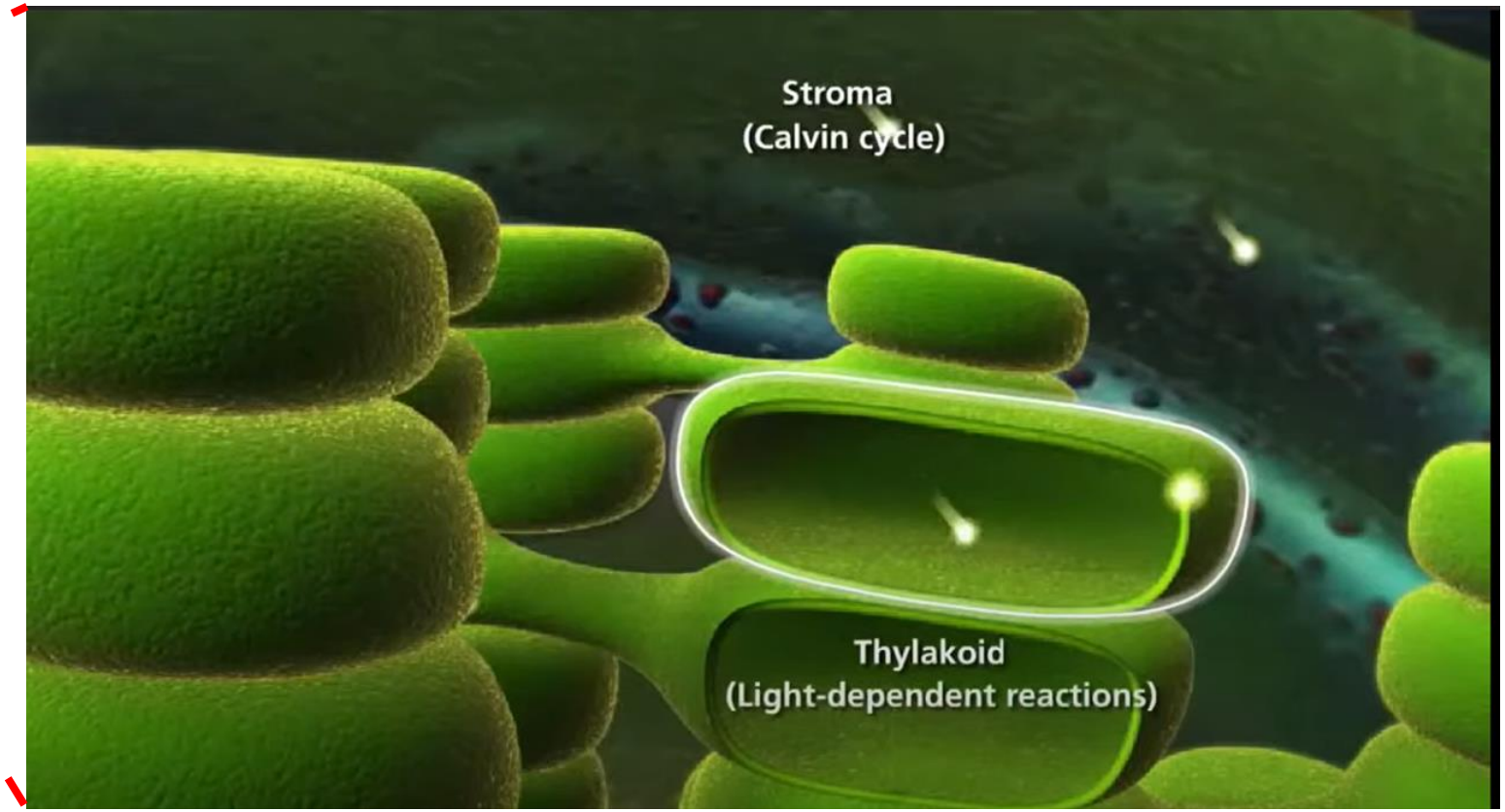


Figure: (A) Schematic diagram of chloroplast (B) Electron micrograph at high magnification showing grana, stacks of thylakoid membranes.

- ✓ The compartment consists several flattened, membrane-surrounded vesicles or sacs, the **thylakoids**, usually arranged in stacks called **grana**.
- ✓ Embedded in the **thylakoid membrane/lamellae** are the photosynthetic pigments and the enzyme complexes that carry out the light reactions and ATP synthesis.
- ✓ The aqueous phase enclosed by the inner membrane called **stroma** contains enzyme required for carbon-assimilation reactions.

Chloroplast





Photosystems

- ✓ Chlorophyll funnels the absorbed energy to reaction centers by exciton transfer.
- ✓ The light-absorbing pigments of thylakoid or bacterial membranes are arranged in functional arrays called photosystems.

Photosystems are tightly packed in the thylakoid membrane, with several hundred antenna chlorophylls and accessory pigments surrounding a photoreaction center.

For example, in spinach chloroplasts, each photosystem contains about 200 chlorophyll and 50 carotenoid molecules.

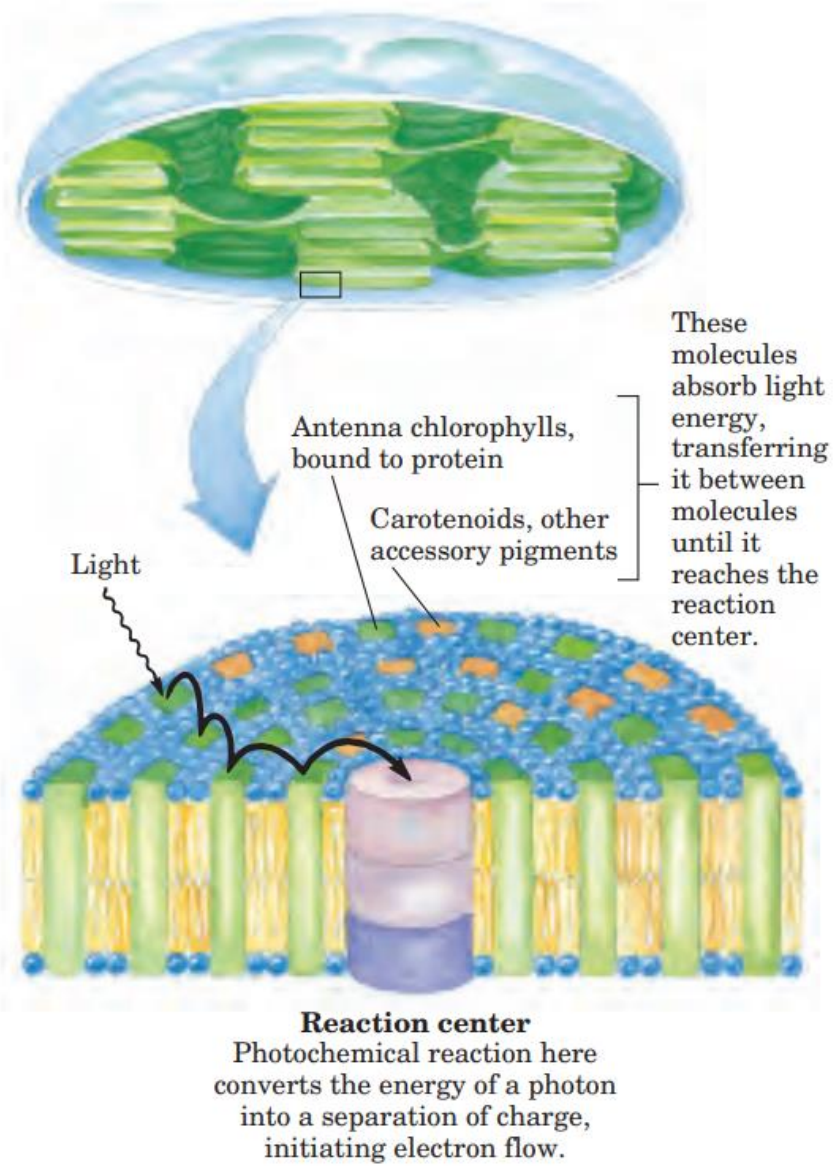
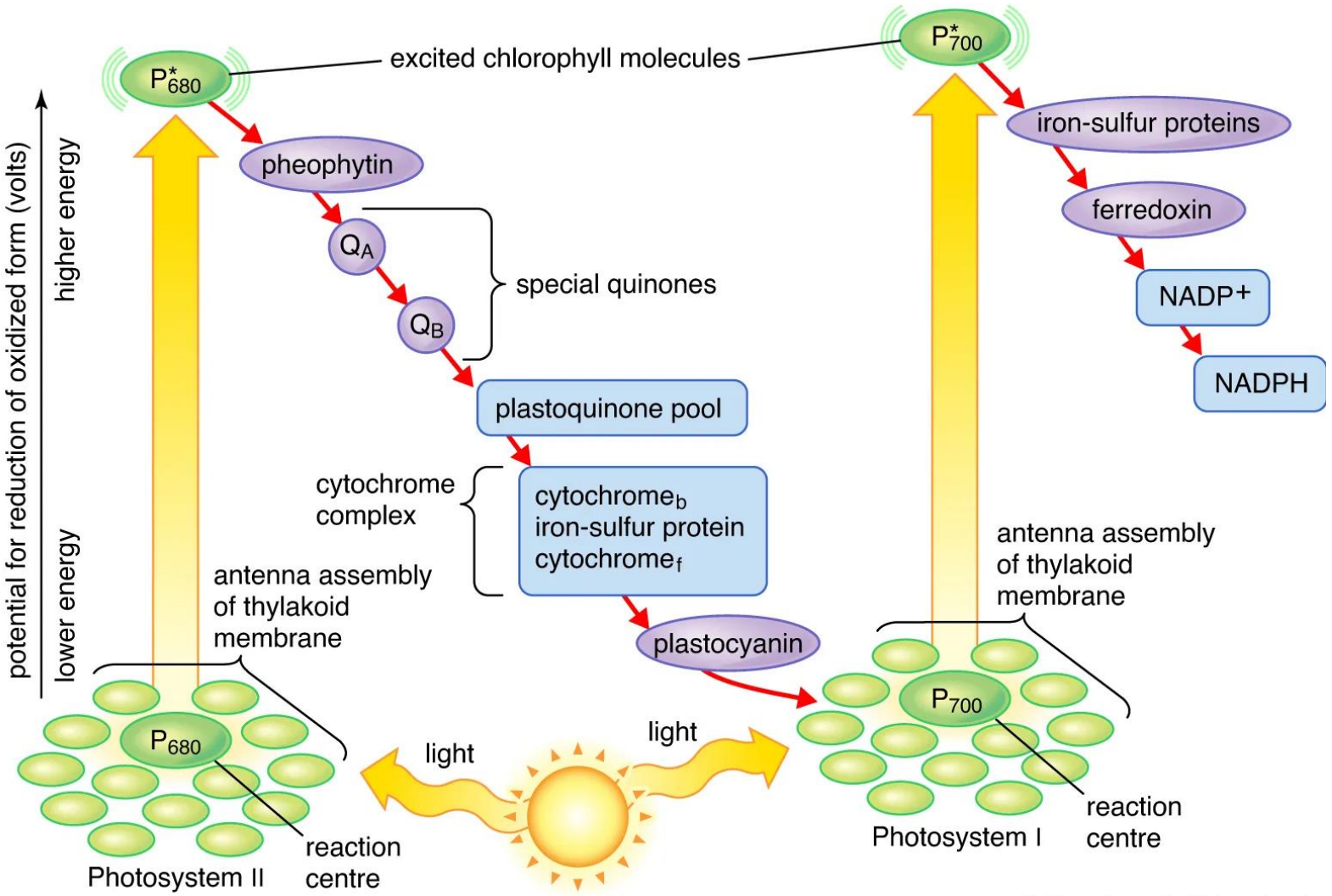


Figure: Organization of photosystems in the thylakoid membrane. Absorption of a photon by any of the antenna chlorophylls leads to excitation of the reaction center by exciton transfer (black arrow).

Light Drives Electron Flow in Chloroplasts



✓ Light drives the electron flow from water through PSII, plastoquinone ($P\bar{Q}$), cytochrome b_{6f}, plastocyanin (PC), and PSI to ferredoxin and ultimately to $NADP^+$, producing NADPH.

Type of photosystems

Two types of photosystems – **PS I** and **PS II** are present in the chloroplasts

- ✓ The photosystems generates a proton gradient and NADPH for mediating electron transfer during photosynthesis.
- ✓ The two photosystems mediate electron transport via **cyclic or non-cyclic (Z scheme)** electron transport chain

Photosystem II (PSII)	Photosystem I (PSI)
• pheophytin-quinone type of system (like the single photosystem of purple bacteria) • Containing roughly equal amounts of chlorophylls a and b. • Excitation of its reaction center P680 drives electrons through the cytochrome b6 f complex with concomitant movement of protons across the thylakoid membrane.	• structurally and functionally related to the type I reaction center of green sulfur bacteria. • It has a reaction center designated P700 and a high ratio of chlorophyll a to chlorophyll b. • Excited P700 passes electrons to the Fe-S protein ferredoxin, then to $NADP^+$, producing NADPH.

Photophosphorylation

- ✓ The combined activities of the two plant photosystems move electrons from water to NADP, conserving some of the energy of absorbed light as NADPH.
- ✓ Simultaneously, protons are pumped across the thylakoid membrane and energy is conserved as an electrochemical potential which is then transformed into phosphate bond energy bond ATP.
- ✓ This process is called photophosphorylation (synthesis of ATP through proton gradient generated during electron transfer in photosystems).

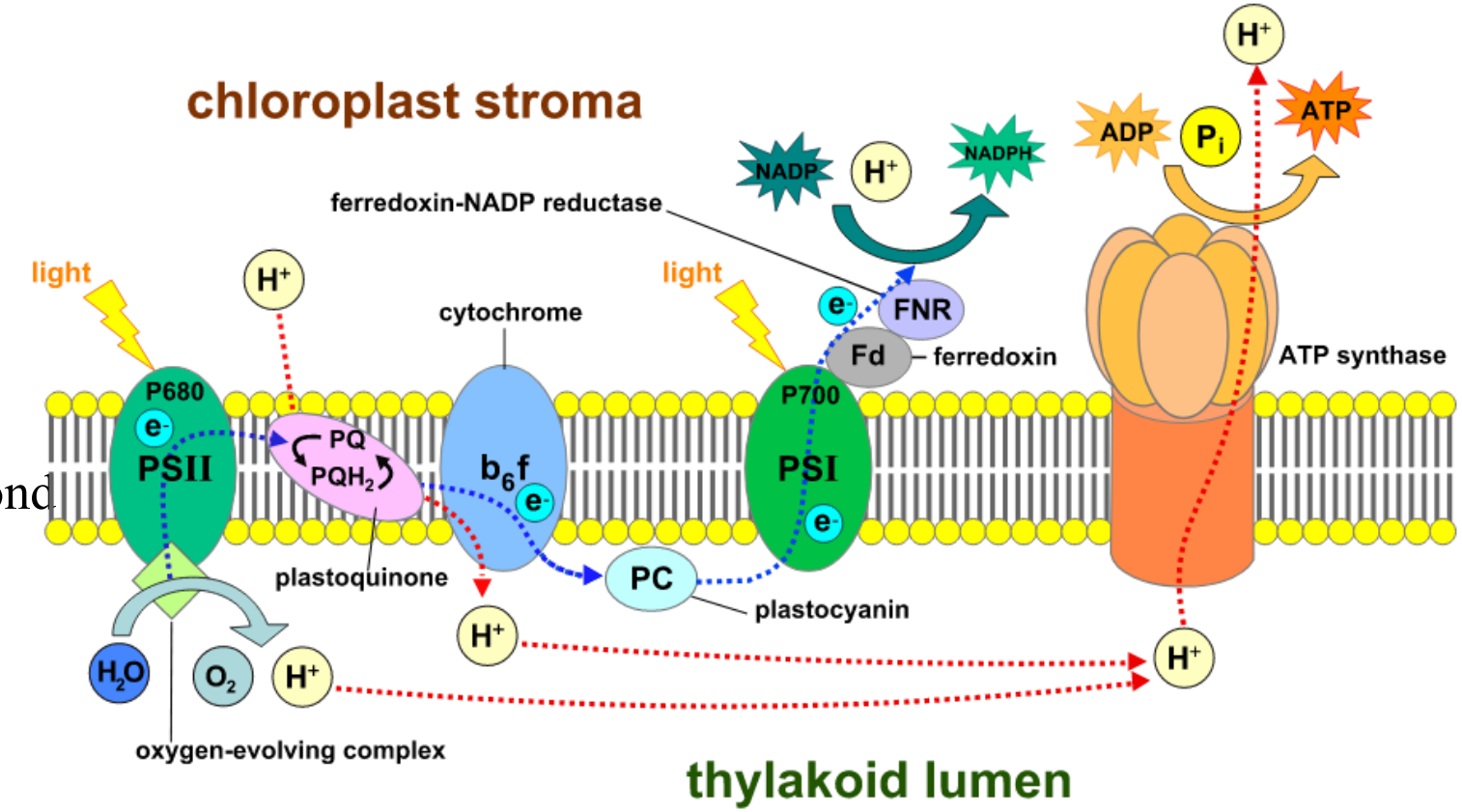


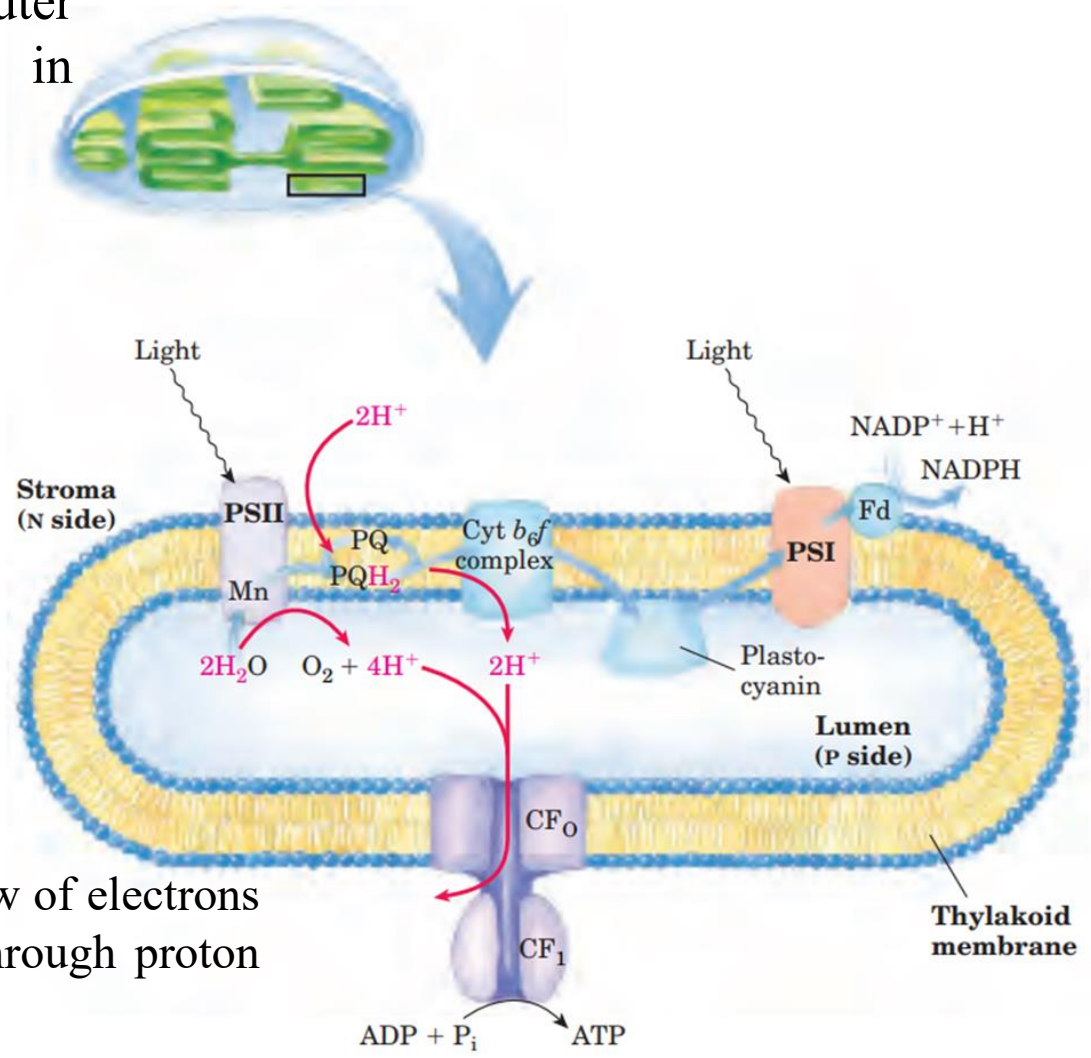
Figure: Overview of photophosphorylation

Proton gradient across thylakoid membrane

- ✓ ATP synthesis is catalyzed by F_oF_1 complexes, located on the outer surface of the thylakoid membranes, that are very similar in structure and function to the F_oF_1 complexes of mitochondria.

- ✓ Electron-transferring molecules in the chain of carriers connecting PSII and PSI are oriented asymmetrically in the thylakoid membrane, so photoinduced electron flow results in the net movement of protons across the membrane, from the stromal side to the thylakoid lumen

  - ✓ Electrons (blue arrows) move from H_2O through PSII, through the intermediate chain of carriers, through PSI, and finally to $NADP^+$.



- ✓ Protons (pink arrows) are pumped into the thylakoid lumen by the flow of electrons through the carriers linking PSII and PSI, and re-enter the stroma through proton channels formed by the F_o (designated CF_o) of ATP synthase.

- ✓ The F1 subunit (CF_1) catalyzes synthesis of ATP.

Proton gradient across thylakoid membrane

The transfer of electrons generates a gradient of proton movement across thylakoid membrane from stroma to thylakoid lumen

A total of six protons are generated during the electron transport between two photosystems:

- ✓ Four protons are generation by photooxidation of two water molecule
- ✓ Two protons are generated by oxidation of plastoquinones (PQ)

- The protons generated by oxidation of PQs are pumped into the lumen via cytochrome b_0f complex.
- Once the gradient is set, the CF_0 of the membrane embedded ATP synthase behaves as a proton channel and pumps the proton back into the stroma.
- The energy released by such movement is utilized by the CF_1 subunit of ATP synthase for generation of ATP molecules.
- One cycle of electron transport chain produces three ATPs using 8 photons.

$2H_2O + 2NADP^+ + 8$ photons $+ \sim 3ADP + \sim 3P_i \rightarrow O_2 + 2NADPH + \sim 3ATP$