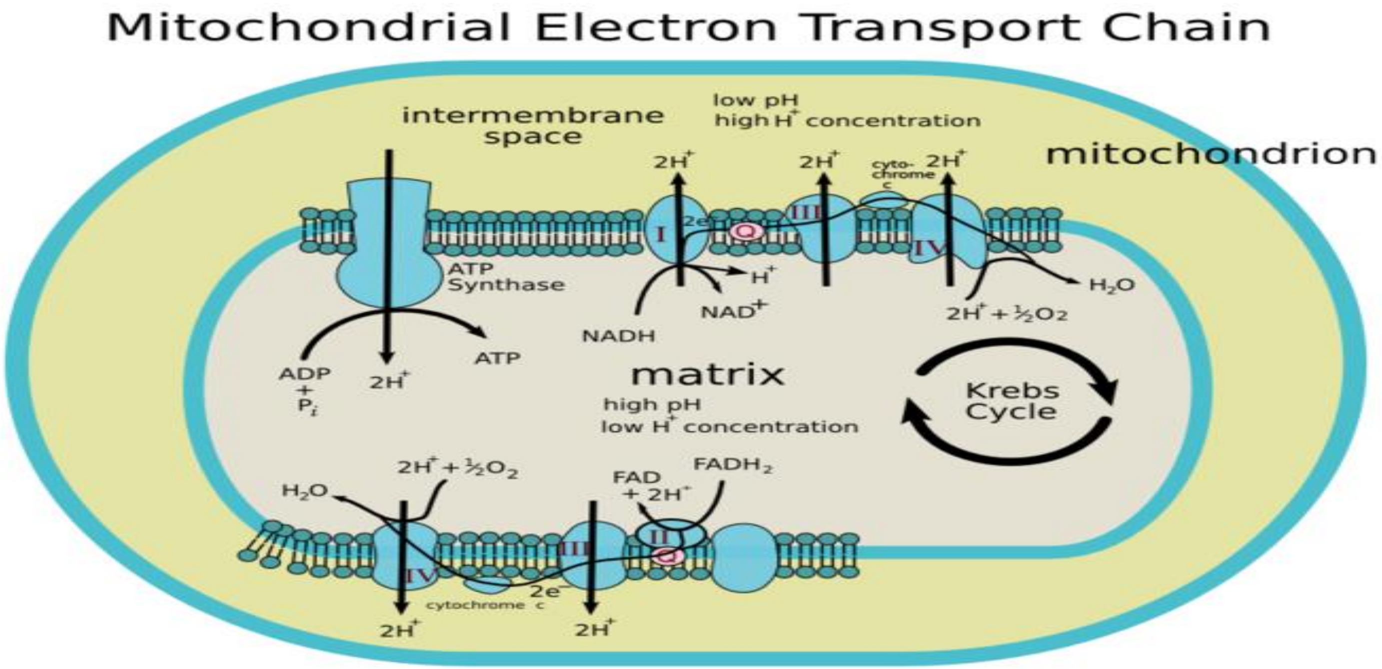
# Mitochondrial E.T.C. and Oxidative Phosphorylation

**CC-12**
**UNIT-4**

DR. ARINDAM MANDAL
**Assistant Professor**
Bejoy Narayan Mahavidyalaya
Itachuna, Hooghly
West Bengal

# Introduction

1. Electrons from Krebs cycle are passed to electron transport chain by NADH & $FADH_2$
2. At end of the chain an enzyme combines electrons from the electron chain with $H^+$ ions and oxygen to form water
3. Each time 2 high-energy electrons transport down the electron chain, their energy is used to transport $H^+$ ions across the membrane.

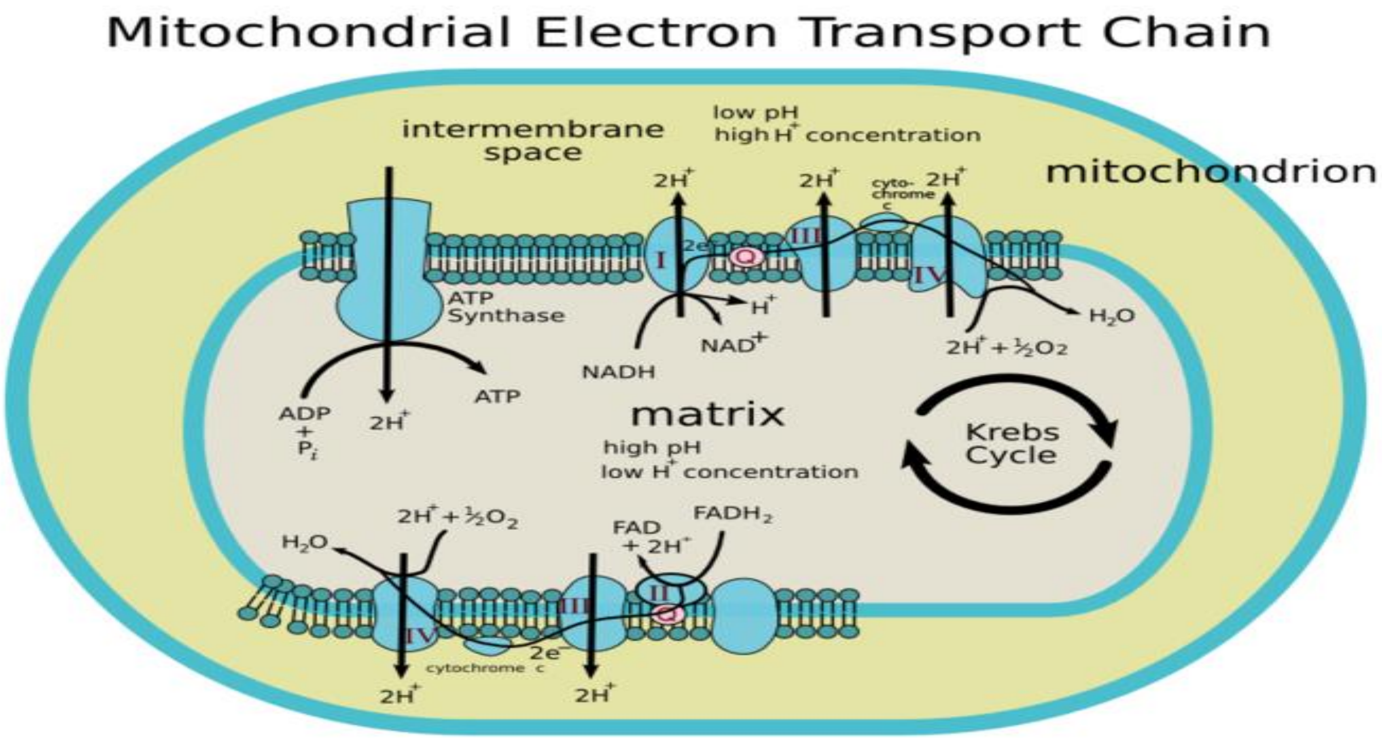


# Introduction

4. $H^+$ ions build up in intermembrane space it is now positively charged, other side of membrane negatively charged (DISPLAY)

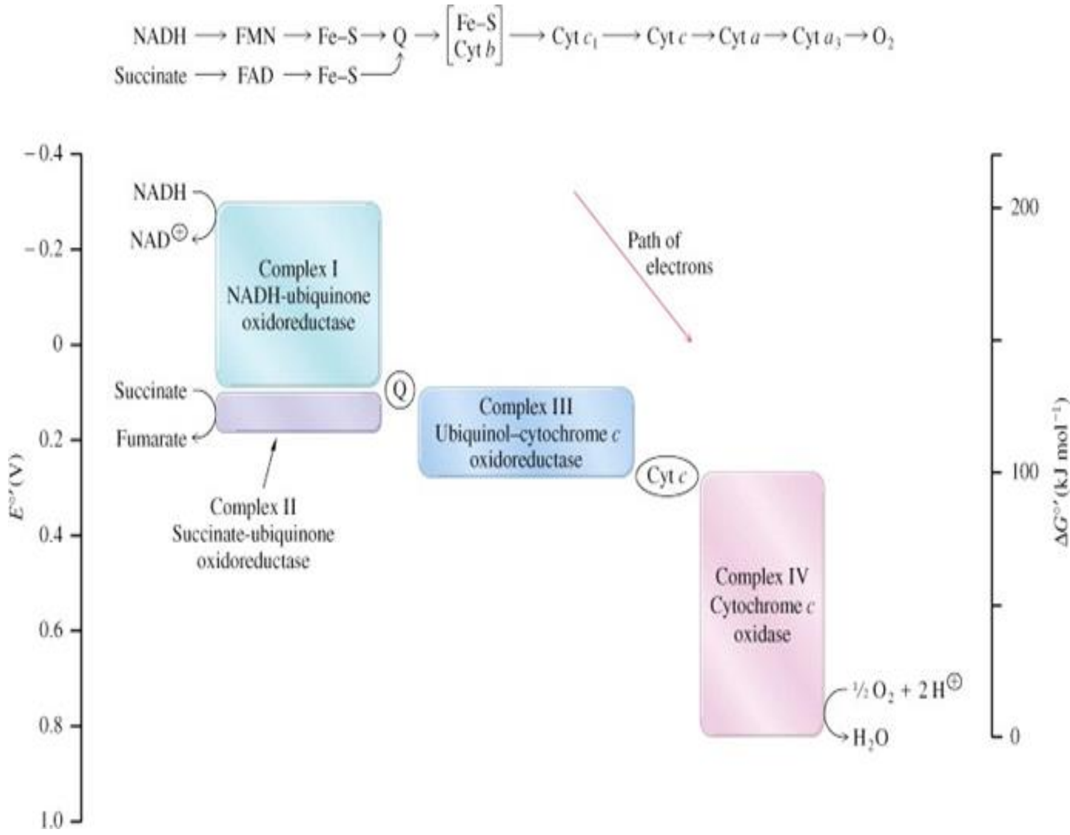
5. Electrochemical gradient (chemiosmotic gradient) created for ATP synthase to work.

6. ATP synthase converts ADP into ATP.



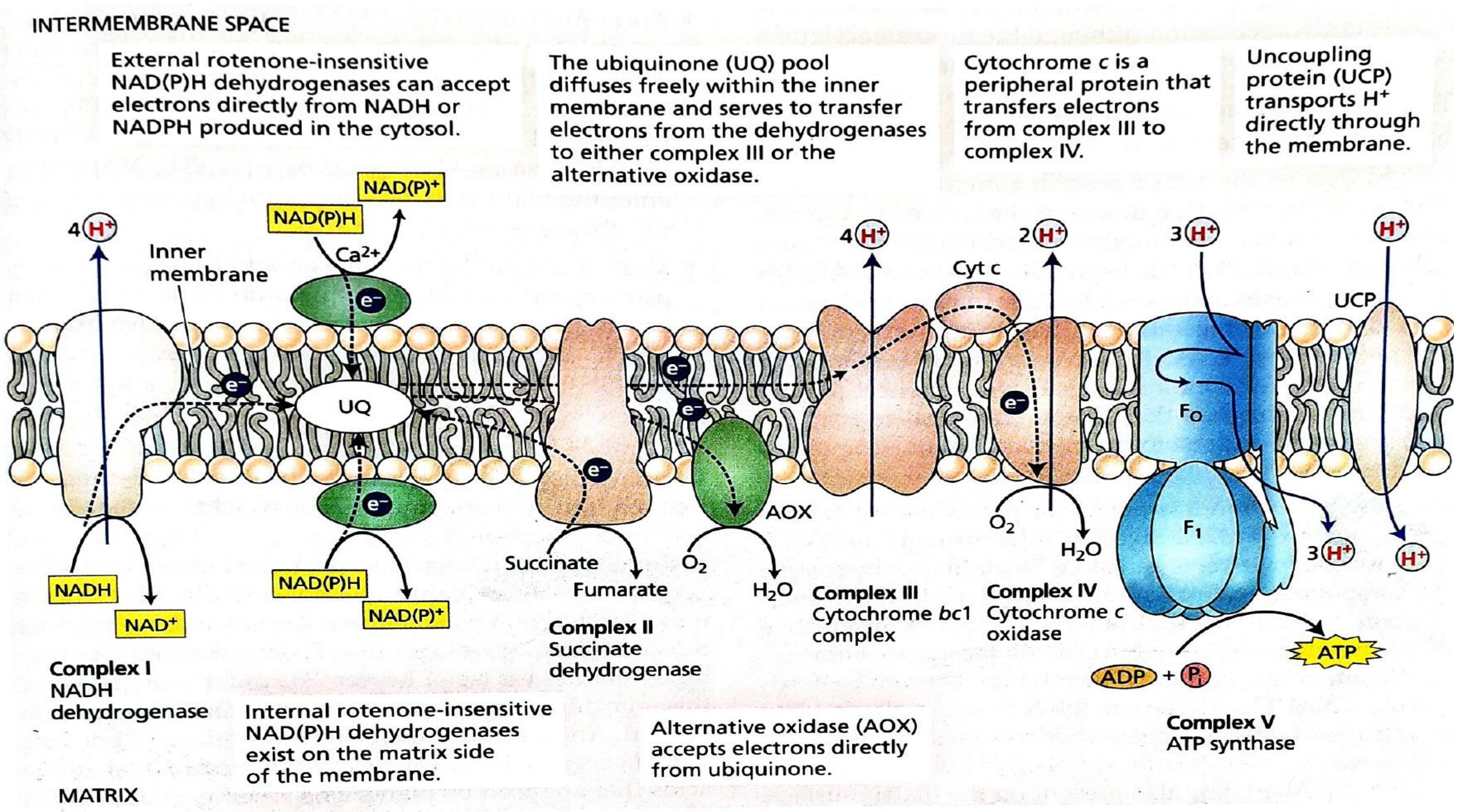
# Mitochondrial Electron Transport Chain

- Electron transport is a series of redox reactions, to the endpoint of the chain where the electrons reduce molecular oxygen, producing water.
- There are four complexes composed of proteins, labeled I through IV, and the aggregation of these four complexes, together with associated mobile, accessory electron carriers, is called the electron transport chain.



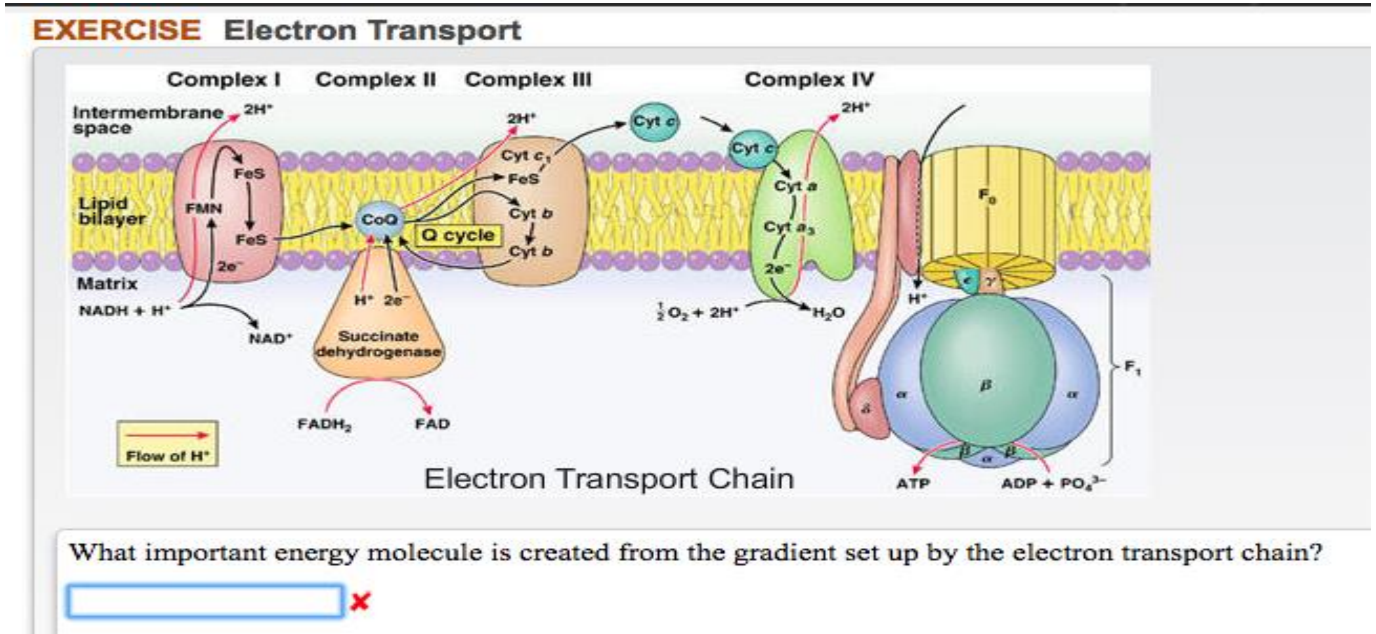
# Mitochondrial Electron Transport Chain

- The electron transport chain is present in multiple copies in the inner mitochondrial membrane of eukaryotes and the plasma membrane of prokaryotes.
- The important feature of all electron transport chains is the presence of a proton pump to create a proton gradient across a membrane.



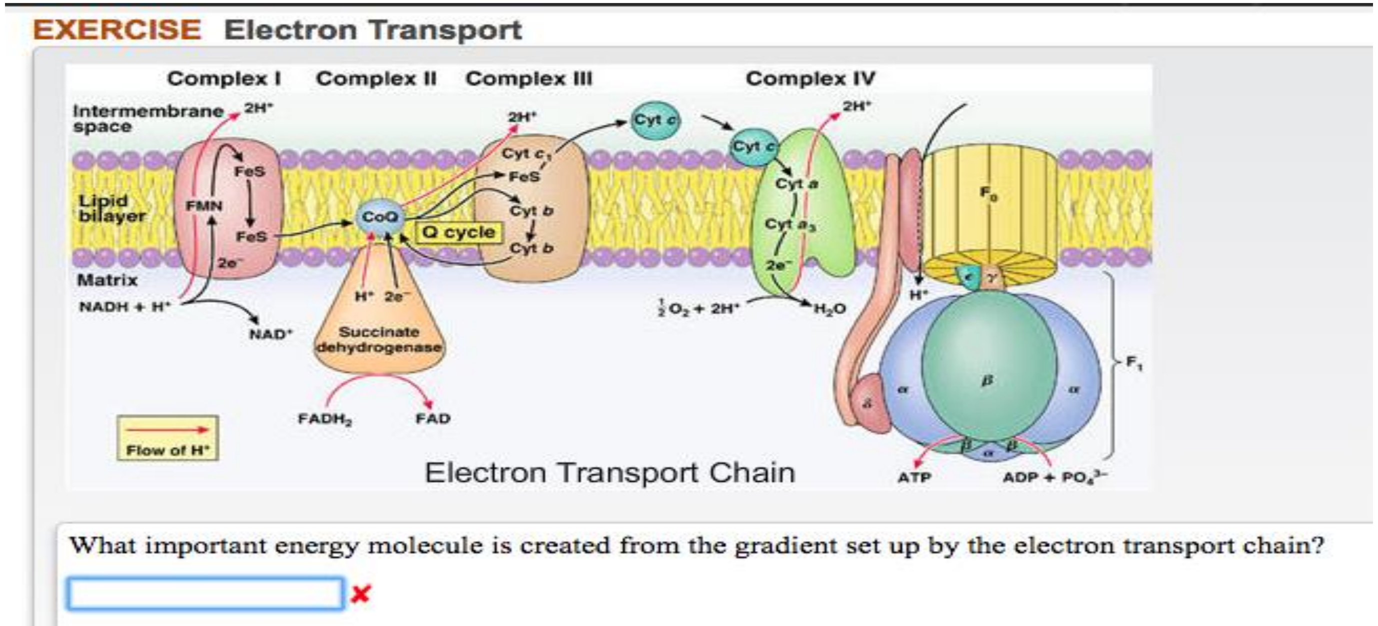
# Complex-I

- This complex, labeled I, is composed of flavin mononucleotide (FMN) and an iron-sulfur (Fe-S)-containing protein.
- FMN, which is derived from vitamin $B_2$, also called riboflavin, is one of several prosthetic groups or co-factors in the electron transport chain.
- The enzyme in complex I is NADH dehydrogenase and is a very large protein, containing 45 amino acid chains.
- The reduced coenzyme NADH binds to Complex I and accomplishes the reduction of Coenzyme Q10. Electrons are transferred through Complex I using FMN (flavin mononucleotide) and a series of Fe-S clusters. The process accomplishes the pumping of four protons across the inner mitochondrial membrane to the intermembrane space.



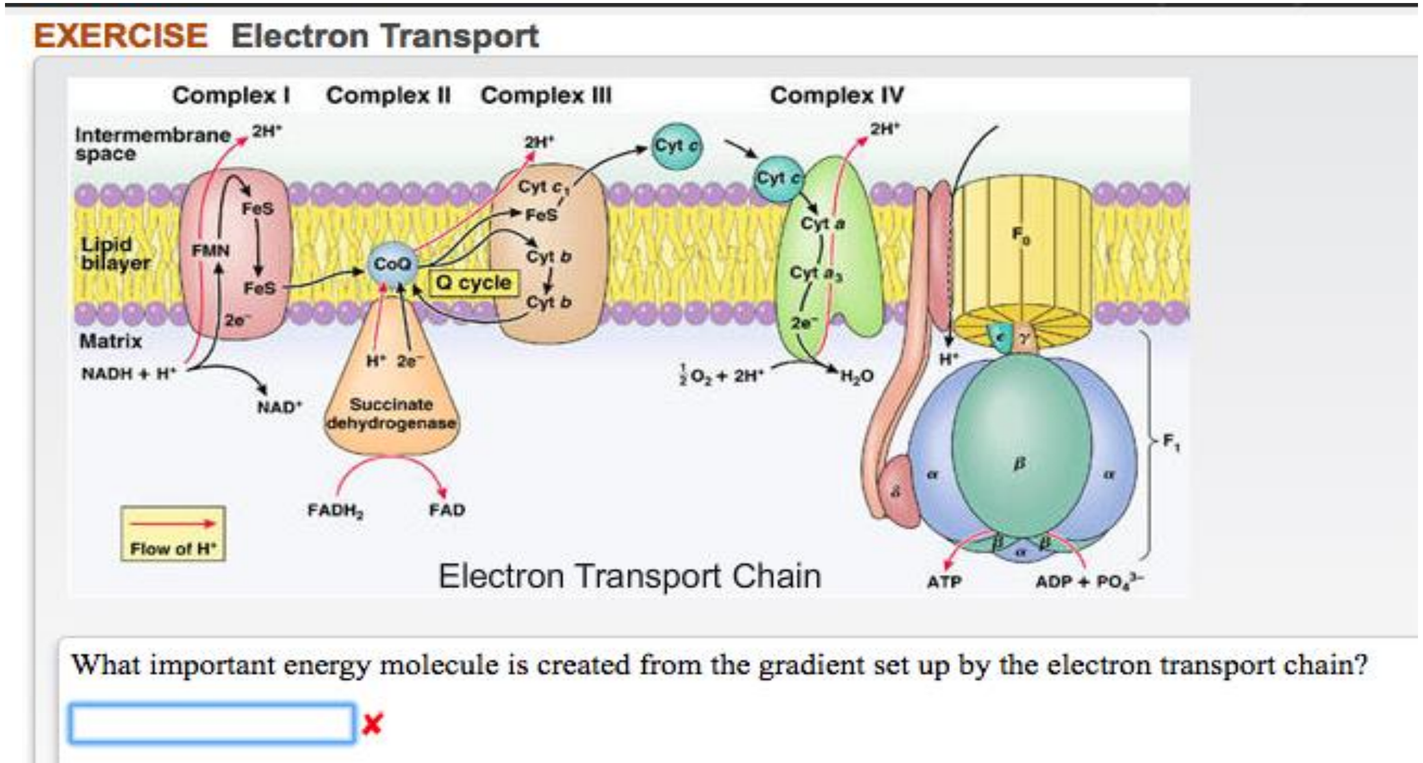
# Complex-II

- Complex II directly receives $FADH_2$, which does not pass through complex I.
- This enzyme and $FADH_2$ form a small complex that delivers electrons directly to the electron transport chain, bypassing the first complex.
- This complex forms a second entry point into the electron transport chain using the succinate product of the TCA cycle.
- Since these electrons bypass and thus do not energize the proton pump in the first complex, fewer ATP molecules are made from the $FADH_2$ electrons.
- The number of ATP molecules ultimately obtained is directly proportional to the number of protons pumped across the inner mitochondrial membrane.



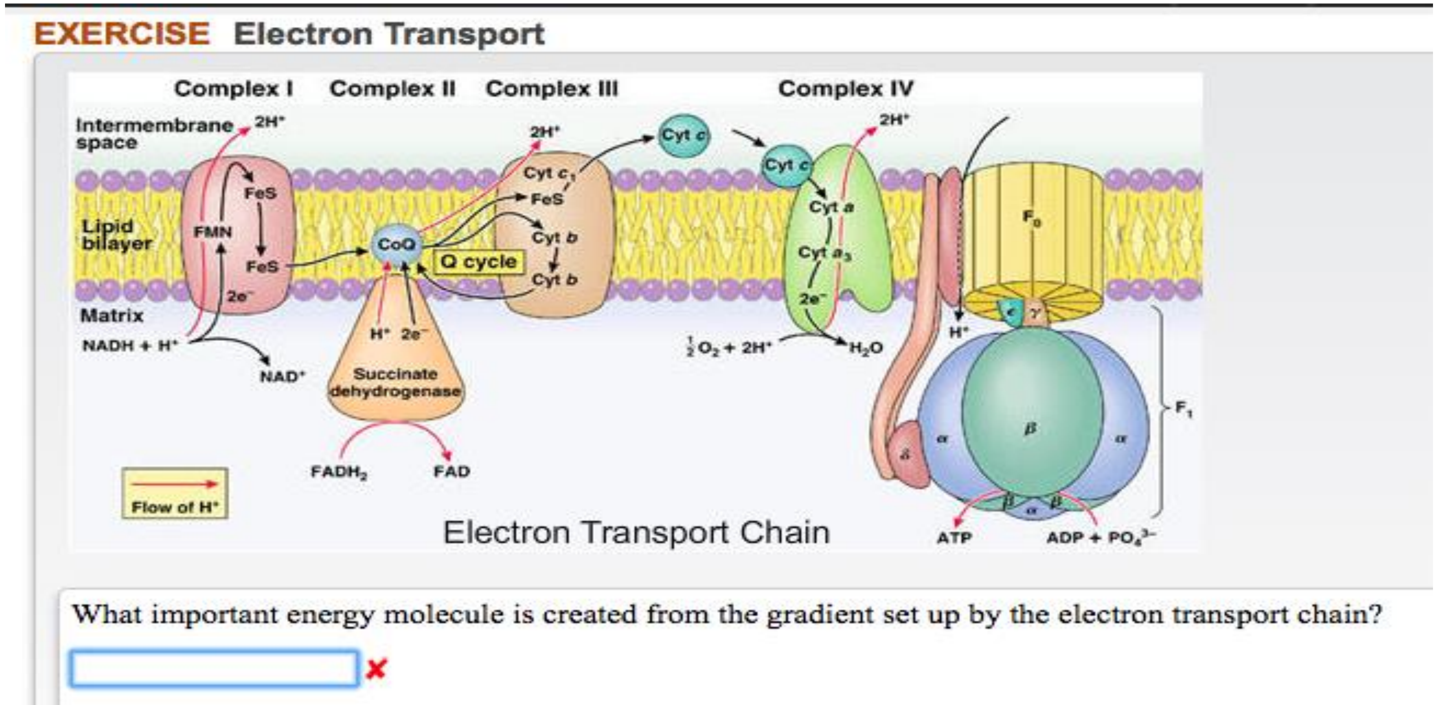
# Complex III

- The third complex is composed of cytochrome b, another Fe-S protein, Rieske center (2Fe-2S center), and cytochrome c proteins.
- This complex is also called cytochrome oxidoreductase.
- Cytochrome proteins have a prosthetic group of heme.
- This complex accomplishes the oxidation of ubiquinol and the reduction of two molecules of cytochrome-c. Four hydrogens are pumped across the membrane to the intermembrane space.



# Complex IV

- The fourth complex is composed of cytochrome proteins c, a, and $a_3$.
- This complex contains two heme groups (one in each of the two cytochromes, a, and $a_3$) and three copper ions (a pair of $Cu_A$ and one $Cu_B$ in cytochrome $a_3$).
- The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced.
- The reduced oxygen then picks up two hydrogen ions from the surrounding medium to make water ($H_2O$).
- At the end of the pathway, the electrons are used to reduce an oxygen molecule to oxygen ions. The extra electrons on the oxygen attract hydrogen ions (protons) from the surrounding medium, and water is formed.



# Major protein complexes

**TABLE 14.1** Characteristics of protein complexes of the mitochondrial respiratory electron-transport chain in bovine heart

| Complex | Subunits | Molecular weight | Oxidation-reduction components |
|---|---|---|---|
| I. NADH-ubiquinone oxidoreductase | 42 or 43 | > 900 000 | 1 FMN<br>22–24 Fe–S in 7 or 8 clusters |
| II. Succinate-ubiquinone oxidoreductase | 4 | 125 000 | 1 FAD<br>3 Fe–S clusters<br>Cytochrome $b_{560}$ |
| III. Ubiquinol–cytochrome *c* oxidoreductase | 2 | ~250 000 (dimer of 11-chain subunits) | 1 Fe–S cluster<br>Cytochrome *b*<br>Cytochrome $c_1$ |
| IV. Cytochrome *c* oxidase | 2 | 420 000 (dimer of 13-chain subunits) | Cytochrome *a*<br>Cytochrome $a_3$<br>2 Copper ions |

# Mobile electron carriers

## Mobile electron carriers – serve as links between ETC complexes

### 1. Ubiquinone (Q)

- Also called coenzyme Q
- A membrane-soluble low molecular weight compound
- Long hydrophobic tail keeps Q anchored in the mitochondrial inner membrane
- Q is a lipid soluble molecule that diffuses within the lipid bilayer, and shuttles electrons from Complexes I and II and pass them to III
- Not a part of any complex

# Mobile electron carriers

## 2. Cytochrome c

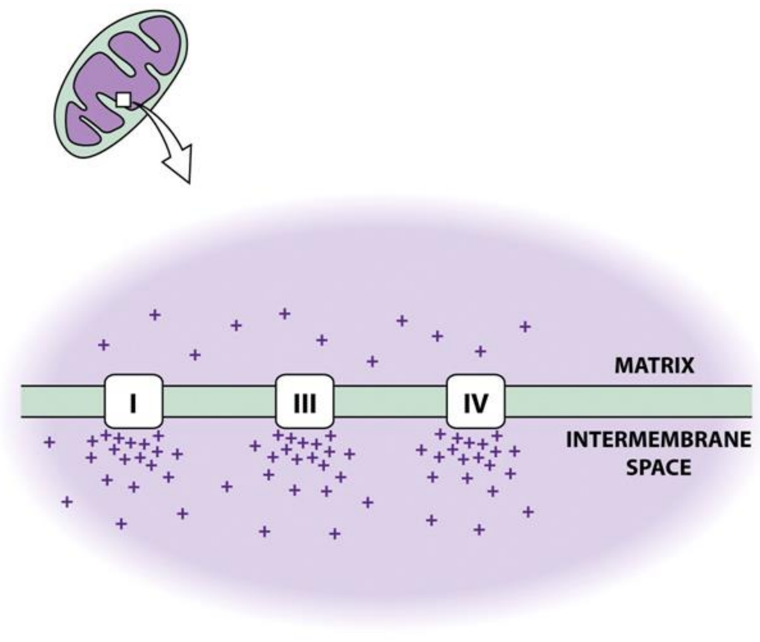
- A peripheral membrane protein associated with the outer face of the membrane, transports electrons from III to IV
- Cytochromes are heme-containing proteins – contains Fe
- Not a part of any complex
- Shuttles electrons and protons from Complex III to Complex IV

# Oxidative phosphorylation

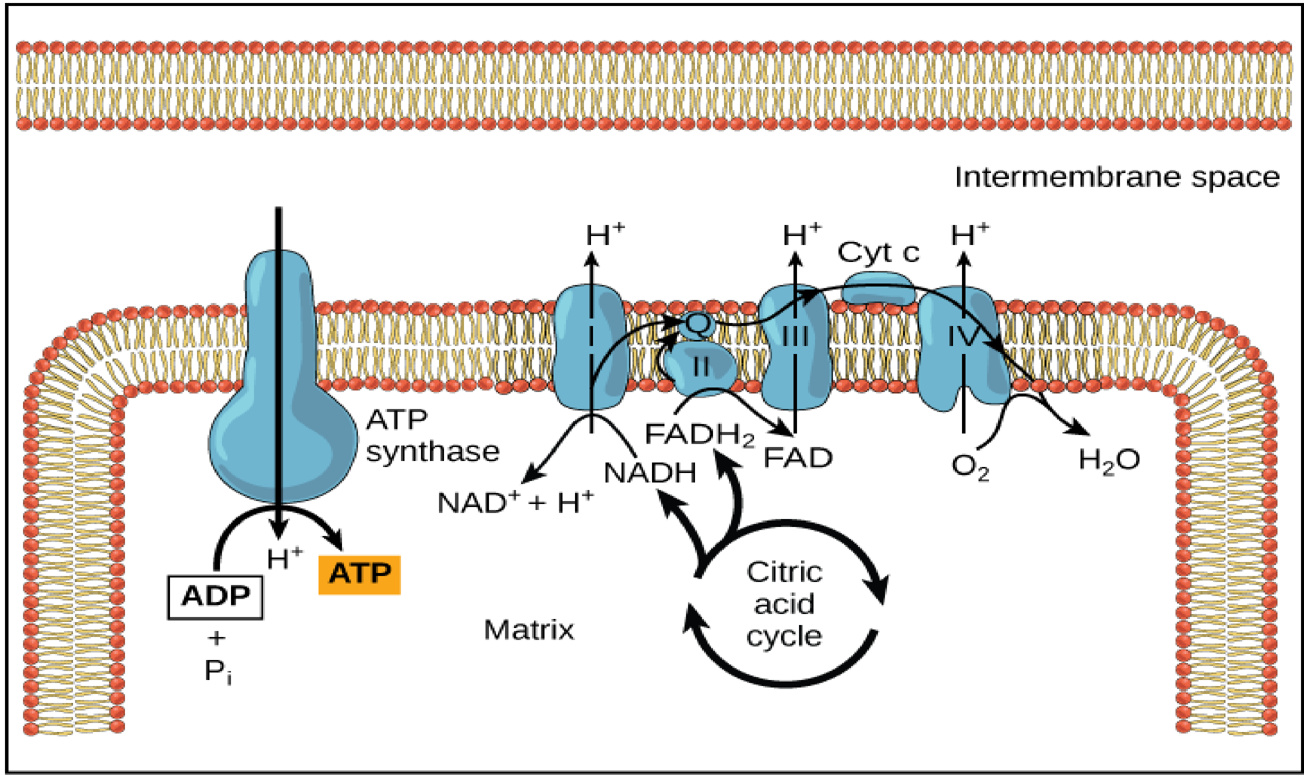
# Chemiosmosis

In chemiosmosis, the free energy from the series of redox reactions (Electron Transport Chain)is used to pump hydrogen ions (protons) across the membrane.

The uneven distribution of $H^+$ ions across the membrane establishes both concentration and electrical gradients (thus, an electrochemical gradient), owing to the hydrogen ions' positive charge and their aggregation on one side of the membrane.

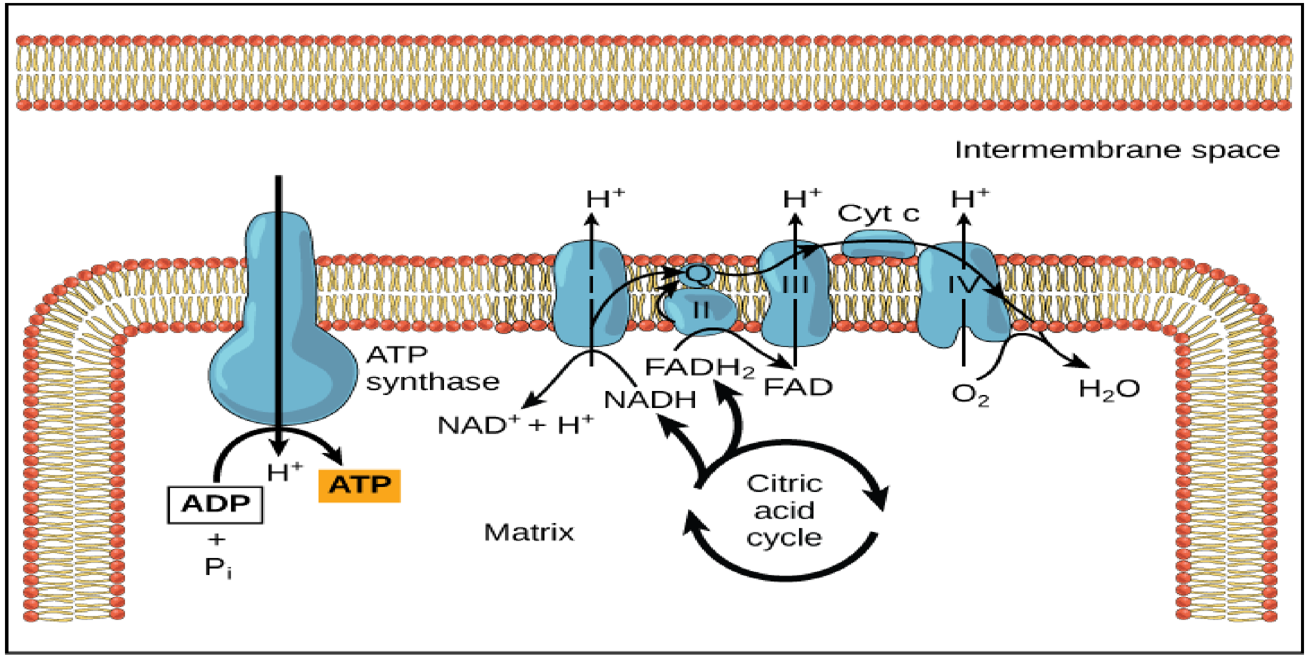




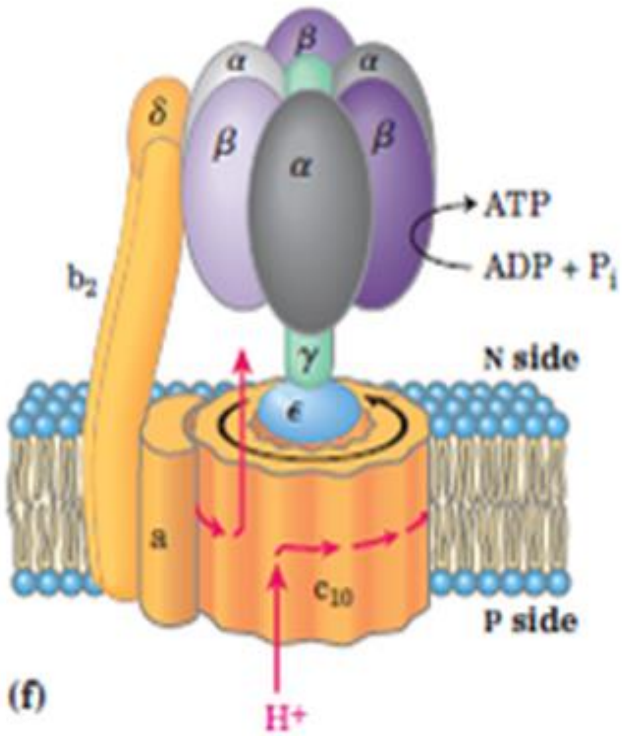
# Chemiosmosis

- If the membrane were open to diffusion by the hydrogen ions, the ions would tend to diffuse back across into the matrix, driven by their electrochemical gradient.
- Recall that many ions cannot diffuse through the nonpolar regions of phospholipid membranes without the aid of ion channels.
- Similarly, hydrogen ions in the Intermembrane space can only pass through the inner mitochondrial membrane through an integral membrane protein called ATP synthase.



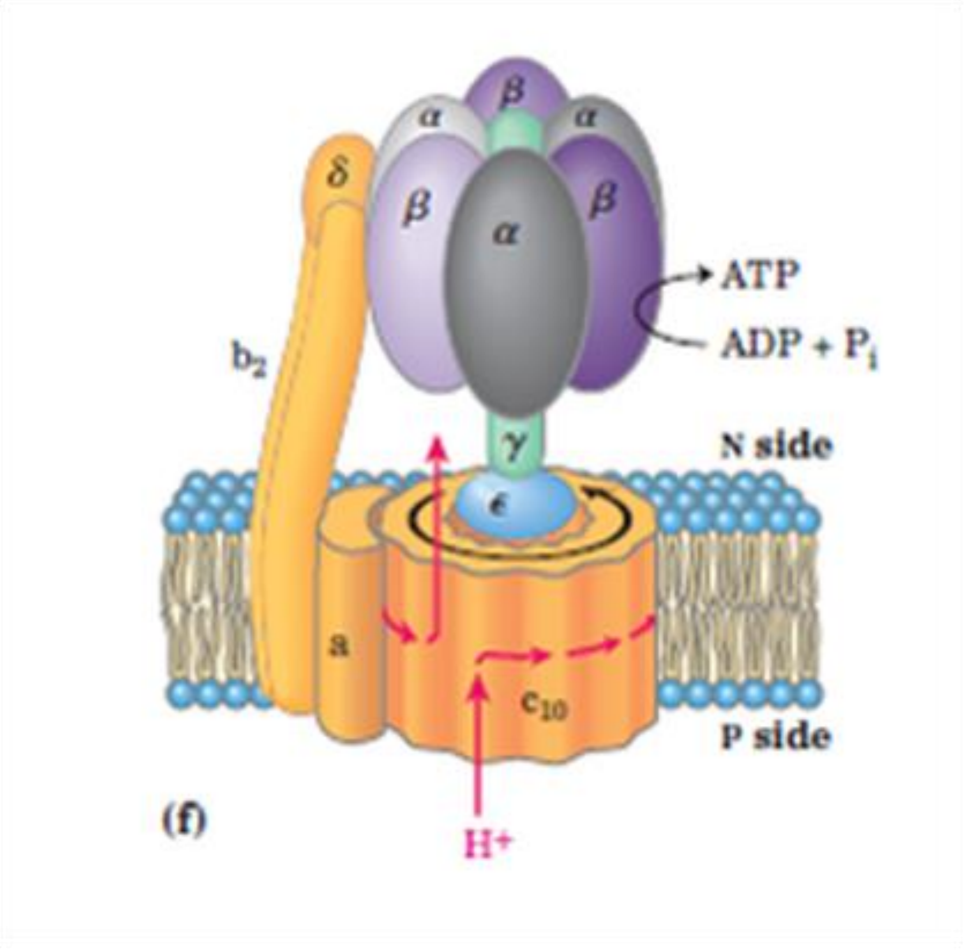
# ATP Synthase

- This large enzyme complex of the inner mitochondrial membrane catalyzes the formation of ATP from ADP and Pi, accompanied by the flow of protons from the P to the N side of the membrane
- ATP synthase, also called Complex V, has two distinct components:
- F1, a peripheral membrane protein, and
- Fo (o denoting oligomycin-sensitive), which is integral to the membrane.
- Mitochondrial F1 has nine subunits of five different types, with the composition α3ß3γδε.
- Each of the three ß subunits has one catalytic site for ATP synthesis.
- The knob like portion of F1 is a flattened sphere, 8 nm high and 10 nm across, consisting of alternating α and ß subunits arranged like the sections of an orange.



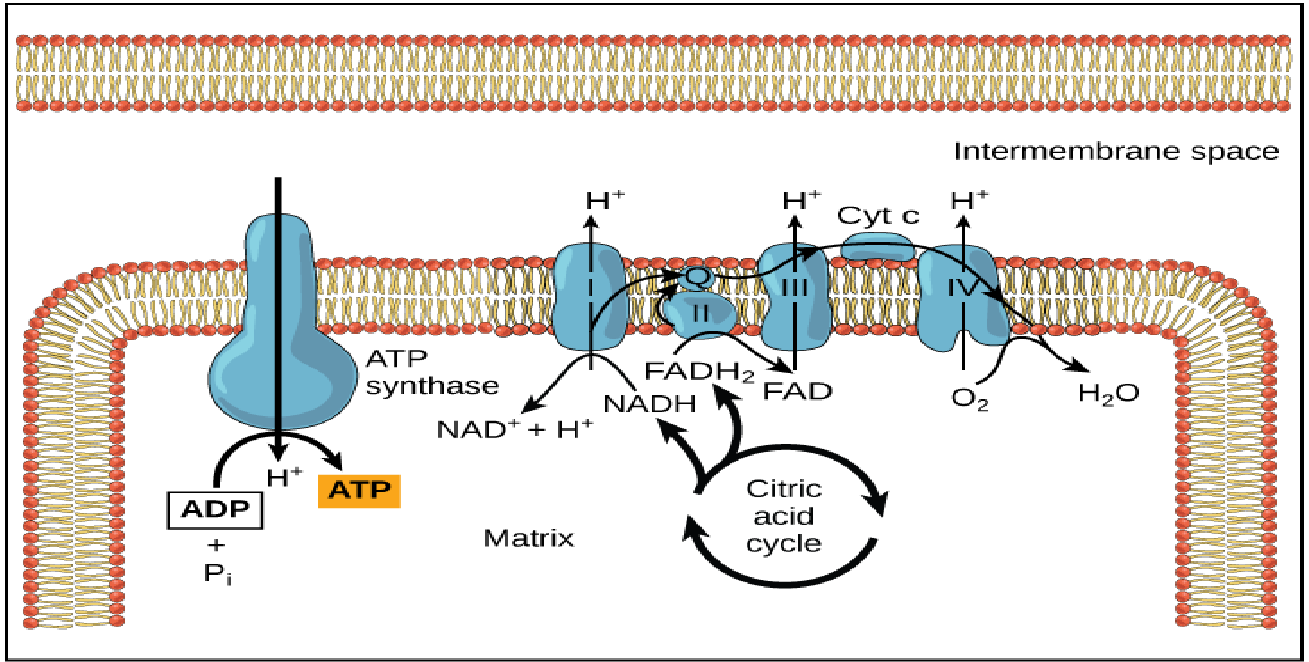
# ATP Synthase

- The Fo complex making up the proton pore is composed of three subunits, a, b, and c, in the proportion $ab_2c_{10-12}$.
- Subunit c is a small (Mr 8,000), very hydrophobic polypeptide, consisting almost entirely of two trans-membrane helices, with a small loop extending from the matrix side of the membrane.
- The ε and γ and  subunits of F1 form a leg-and-foot that projects from the bottom (membrane) side of F1 and stands firmly on the ring of c subunits



# Chemiosmosis

- ATP synthase acts as a tiny generator, turned by the force of the hydrogen ions diffusing through it, down their electrochemical gradient.
- The turning of parts of this molecular machine facilitates the addition of a phosphate to ADP, forming ATP, using the potential energy of the hydrogen ion gradient.
- The production of ATP using the process of chemiosmosis in mitochondria is called oxidative phosphorylation.



# Rotational Catalysis : Mechanism of ATP synthesis

- Paul Boyer proposed a rotational catalysis mechanism in which the three active sites of F1 take turns catalyzing ATP synthesis
- Three ß subunits assume 3 different conformations
  1.ß-ADP
  2.ß-ATP and
  3.ß-empty
  A given subunit starts in the ß-ADP conformation, which binds ADP and Pi from the surrounding medium.
- The subunit now changes conformation, assuming the ß-ATP form that tightly binds and stabilizes ATP, bringing about the ready equilibration of ADP+Pi with ATP on the enzyme surface.
- Finally, the subunit changes to the ß-empty conformation, which has very low affinity for ATP, and the newly synthesized ATP leaves the enzyme surface.

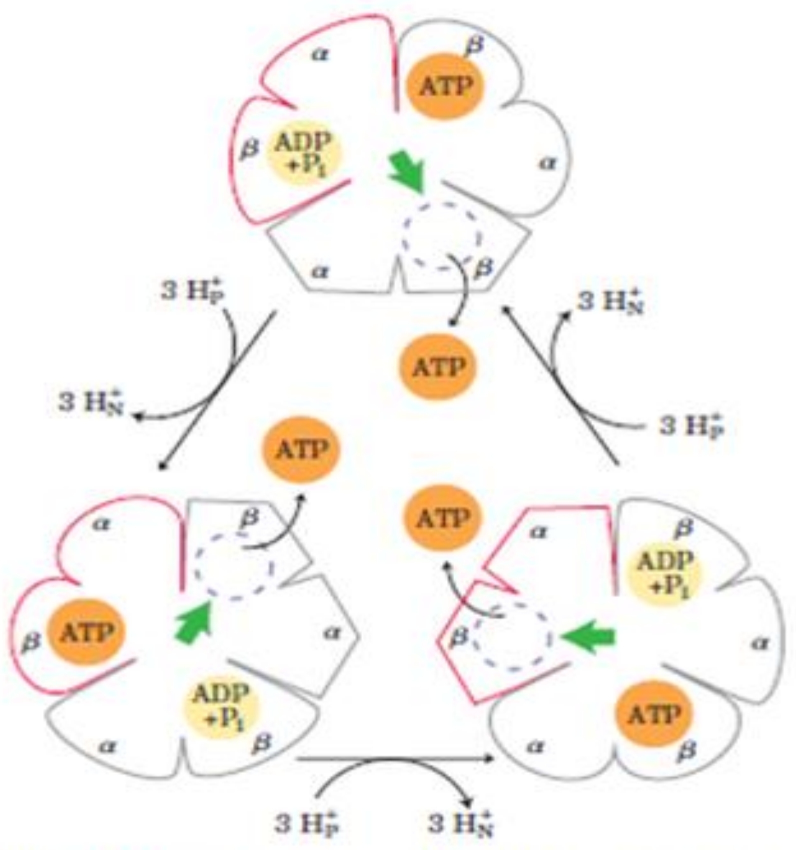



**FIGURE 19–24** Binding-change model for ATP synthase. The F$_1$ com-

# Rotational Catalysis : Mechanism of ATP synthesis

- Another round of catalysis begins when this subunit again assumes the ß-ADP form and binds ADP and Pi
- The conformational changes central to this mechanism are driven by the passage of protons through the Fo portion of ATP synthase.

Thus one complete rotation of the γ subunit causes each ß subunit to cycle through all three of its possible conformations, and for each rotation, three ATP are synthesized and released from the enzyme surface.

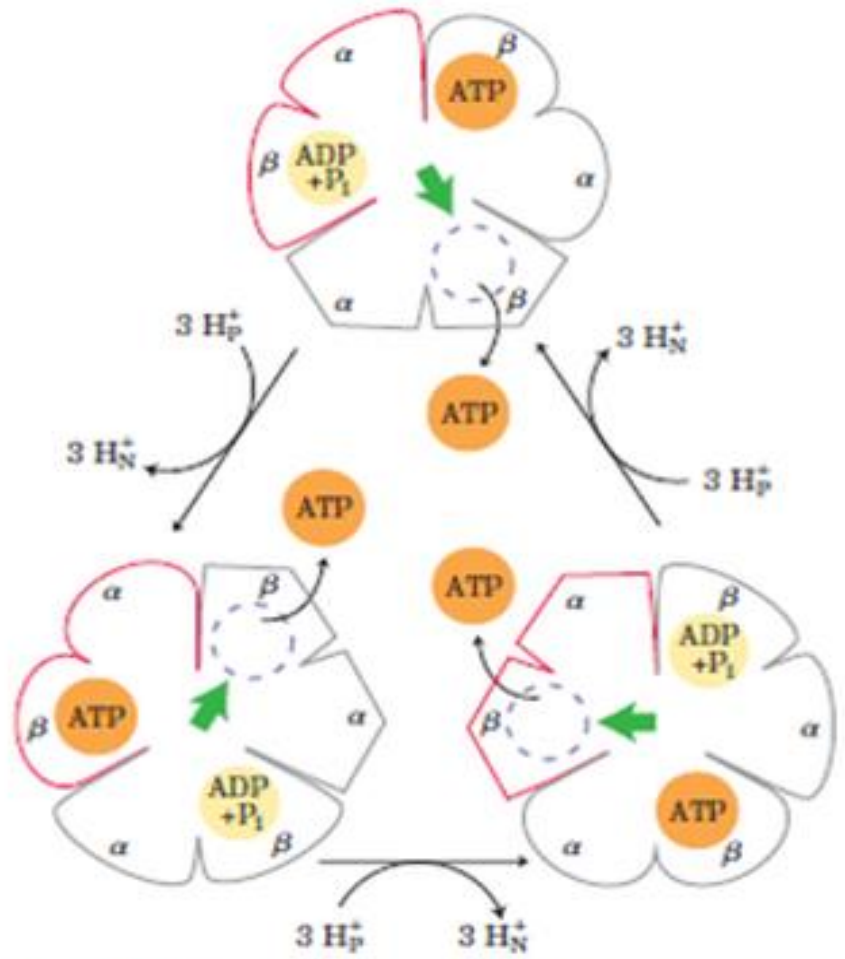
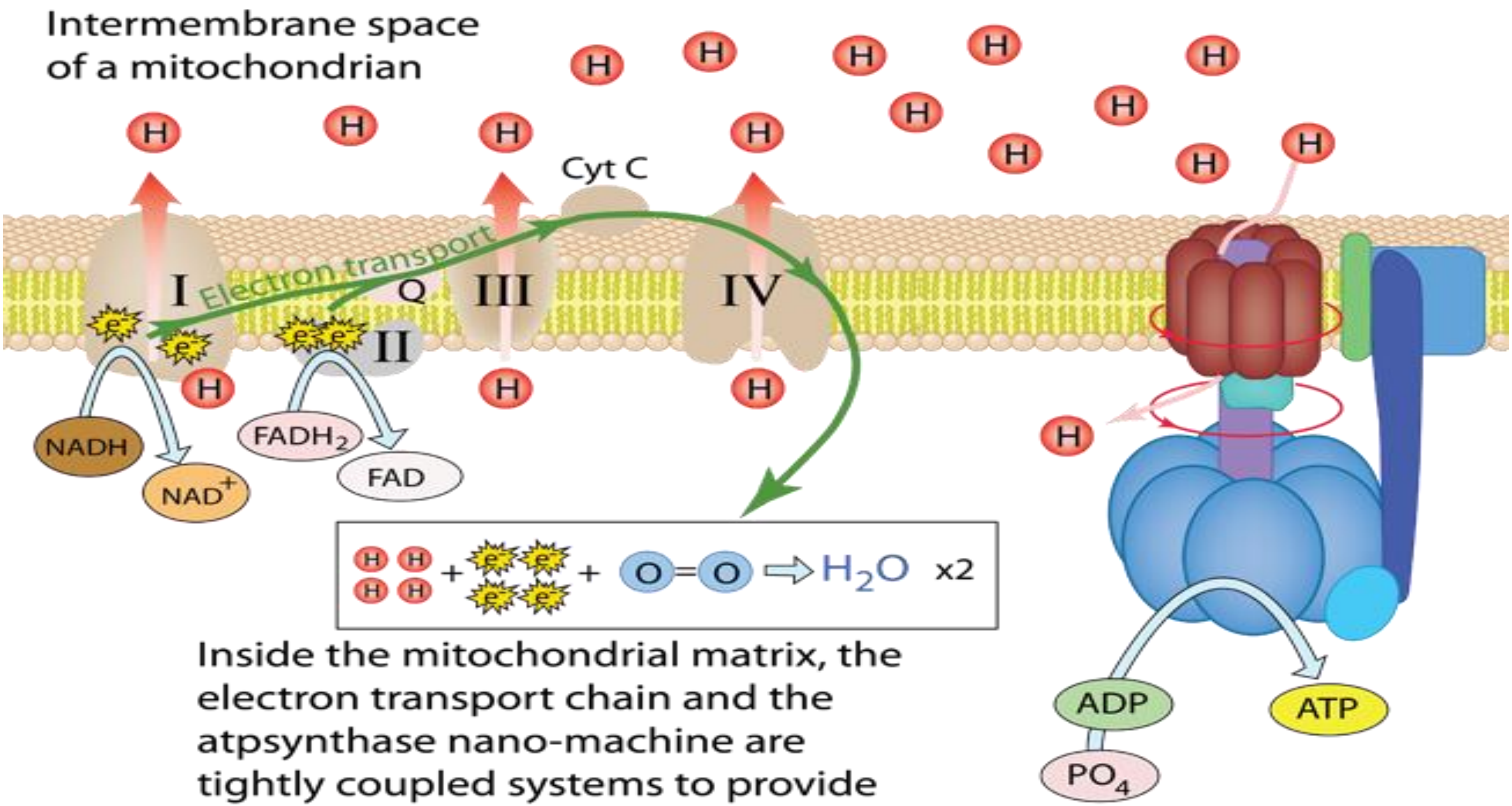
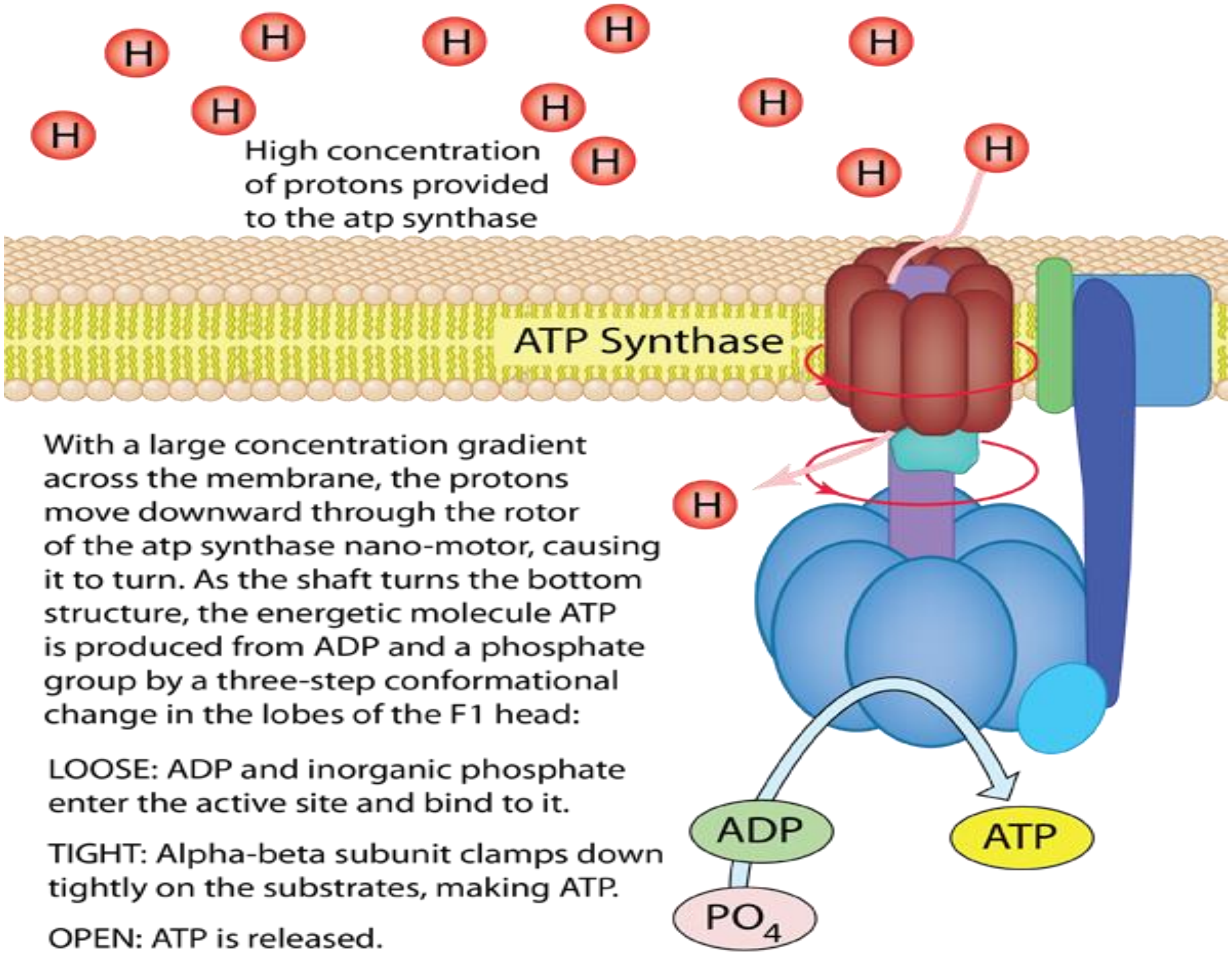



FIGURE 19-24 Binding-change model for ATP synthase. The $F_1$ com-




Inside the mitochondrial matrix, the electron transport chain and the atpsynthase nano-machine are tightly coupled systems to provide energy for metabolism.



H
High concentration of protons provided to the atp synthase
ATP Synthase
With a large concentration gradient across the membrane, the protons move downward through the rotor of the atp synthase nano-motor, causing it to turn. As the shaft turns the bottom structure, the energetic molecule ATP is produced from ADP and a phosphate group by a three-step conformational change in the lobes of the F1 head:
LOOSE: ADP and inorganic phosphate enter the active site and bind to it.
TIGHT: Alpha-beta subunit clamps down tightly on the substrates, making ATP.
OPEN: ATP is released.
ADP
$PO_4$
ATP