

METABOLISM

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



MID – Lecture 8

Oxidative Phosphorylation (Pt.2)

وَإِن تَتَوَلَّوْا يَسْتَبَدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَلَكُمْ

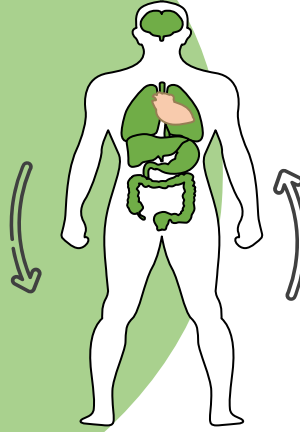
اللهم استعملنا ولا تستبدلنا

Written by:

- Basil Alakhras
- Muthanna Khalil

Reviewed by:

- Mohammad Mahasneh

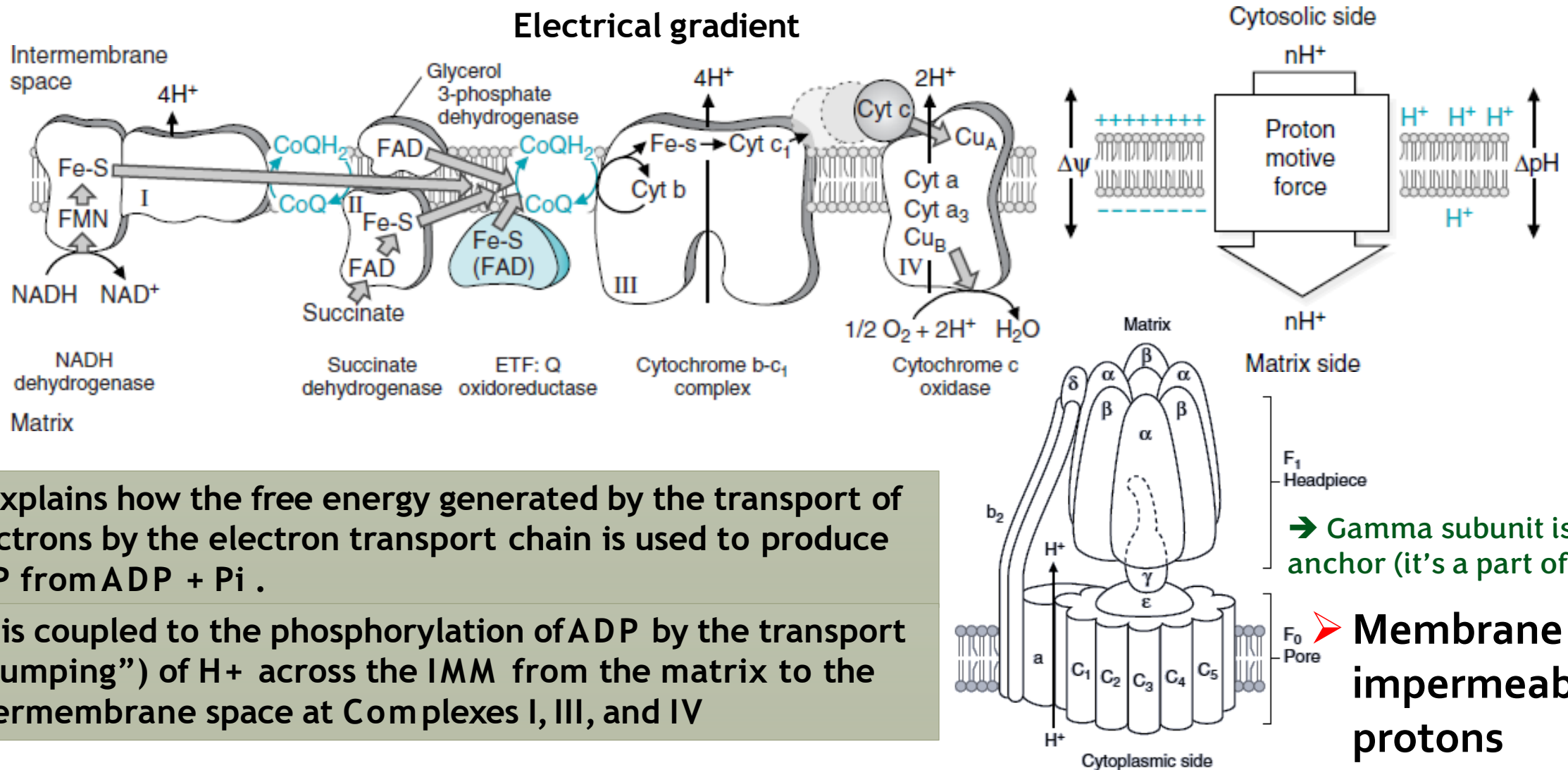


A quick revision regarding the previous lecture

- In complex I, electrons move this way: NADH gives its electrons to FMN (which becomes FMNH₂) in the form of hydride ion. FMN then gives its electrons to the iron sulfur center (Fe-S) which finally gives its electrons to CoQ.
- Complex II is an enzyme complex (succinate dehydrogenase, the same enzyme used in the 6th step of the Krebs cycle) that receives electrons from FADH₂ in the form of hydrogen. It contains iron-sulfur (Fe-S) clusters.
- At Complex II, the electrons move in the following path: from FADH₂ to Fe (in Fe-S centers) then to coenzyme Q.
- CoQ will give the electrons to cytochromes b-c₁ (complex III), then electrons will go from complex III to cytochrome C, then from cytochrome C to Cytochrome a + a₃ which is also called cytochrome C oxidase (complex IV).
- Complex IV is bound to oxygen which is the last acceptor that accepts the electrons to form H₂O.
- The enzyme complex ATP synthase (Complex V) synthesizes ATP by using the energy of the proton's gradient generated by the electron transport chain.
- Complex V is not involved in the electron transport process, it only synthesizes ATP.
- Coenzyme Q is a lipophilic molecule, cytochrome C is a protein, both are mobile carriers.

ET to O₂, How does this occur?

“the Chemiosmotic (Mitchell) hypothesis”



✓ It explains how the free energy generated by the transport of electrons by the electron transport chain is used to produce ATP from ADP + Pi .

✓ ET is coupled to the phosphorylation of ADP by the transport (“pumping”) of H⁺ across the IMM from the matrix to the intermembrane space at Complexes I, III, and IV

→ Gamma subunit is the anchor (it's a part of F₁).

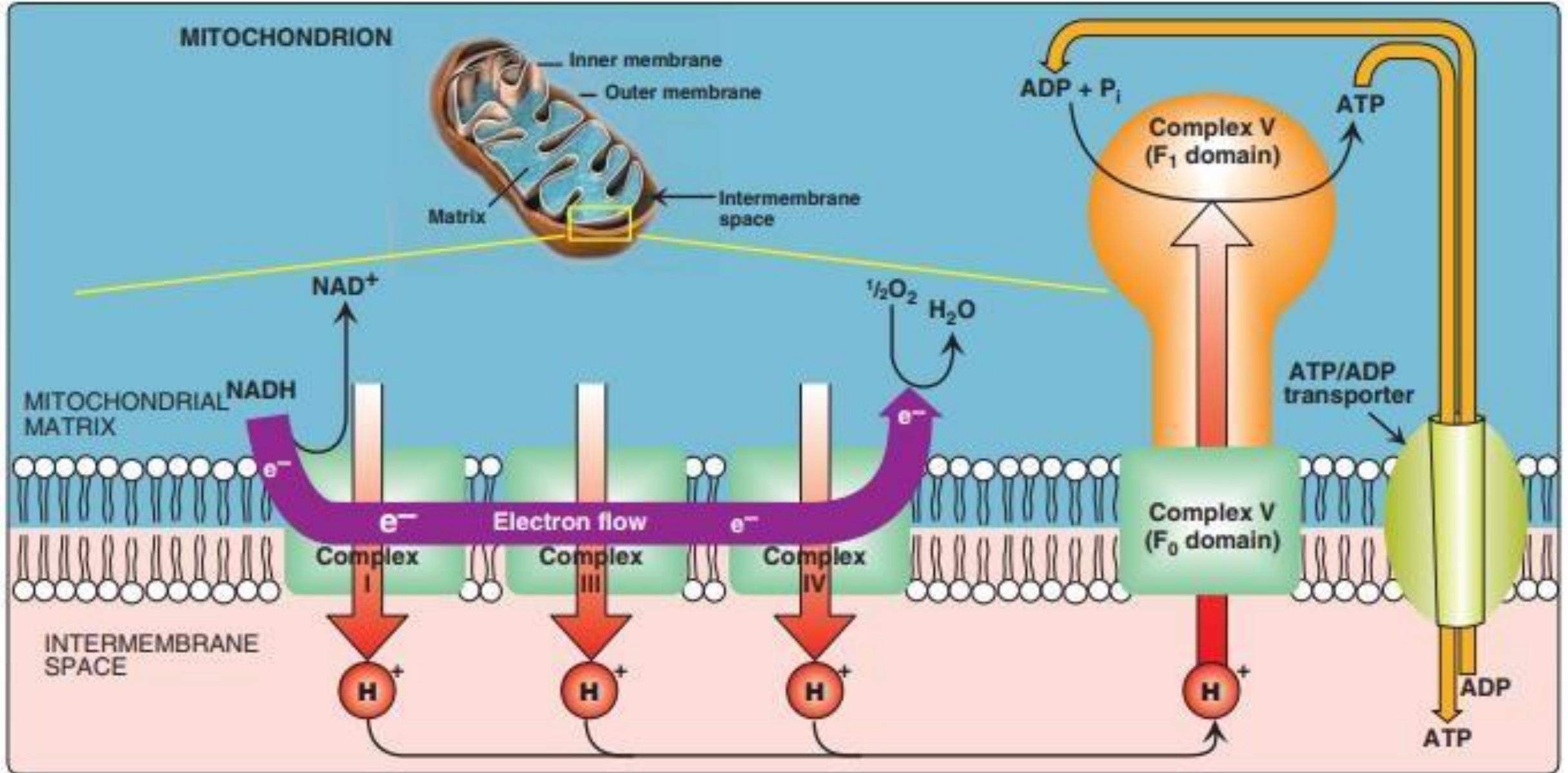
➤ Membrane is impermeable to protons

How does the proton gradient occur?

- The movement of electrons causes the protons to be casted through some complexes (I, III, IV) from the matrix to the inter membranous space.
- Complex II isn't a protein that passes via the two leaflets of the inner mitochondrial membrane completely, making it unable to transport protons to the intermembrane space.
- This movement of protons generates a chemical and electrical gradient for the protons with a higher concentration in the intermembrane space; this gradient generates a force that favors the movement of the protons from the intermembrane space to the matrix.
- We use this energy of protons to drive the synthesis of ATP by returning the protons to the matrix through complex V (ATP synthase).
- *ATP synthase mechanism of work will be discussed in slide 15.*
- Finally, energy is stored in ATP in the form of Potential energy in the bonds between phosphates.

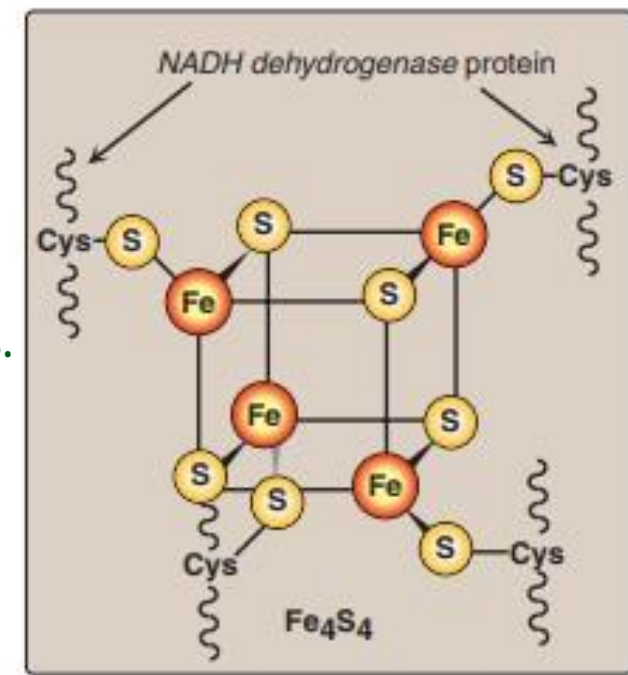
- ADP and inorganic phosphate are needed for this reaction
- F_0 : intermembrane domain of complex V
- F_1 : matrix domain of complex V

Electron transport chain coupled to the transport of H⁺



Redox Components of the ETC Complex I (NADH dehydrogenase)

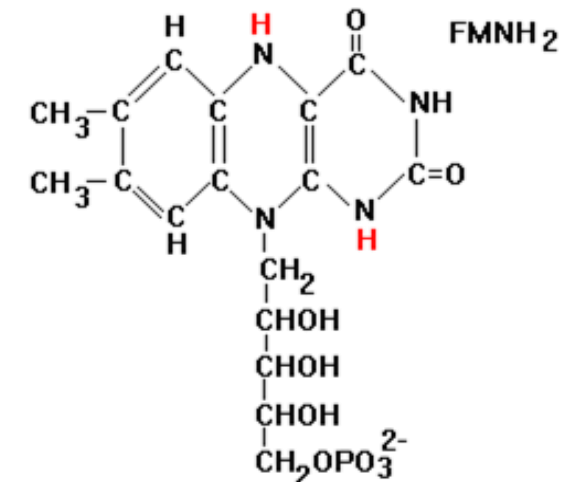
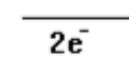
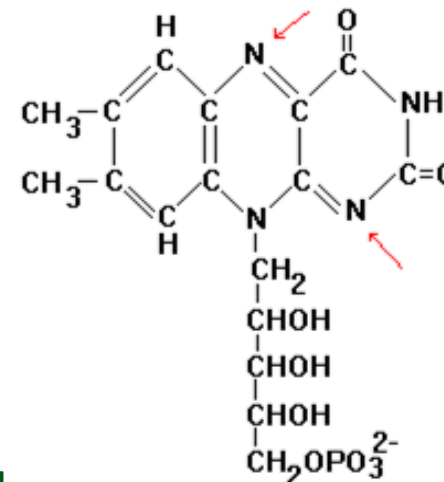
- **NADH-Q oxidoreductase** Has both an enzymatic and proton transporting abilities.
- More than 25 polypeptide chain
- A huge flavoprotein membrane-spanning complex
- The FMN is tightly bound
- Seven Fe-S centers of at least two different types
- Fe-S centers, transfer of the hydrogen atoms to coenzyme Q
- Binds NADH & CoQ
- **4 H⁺** are transported to the intermembrane space.



The ETC and oxidative phosphorylation (by ATP synthase) are coupled (dependent on each other).

Transporting electrons should (normally) be followed by ATP synthesis, and ATP synthesis needs the ETC to function properly.

FMN



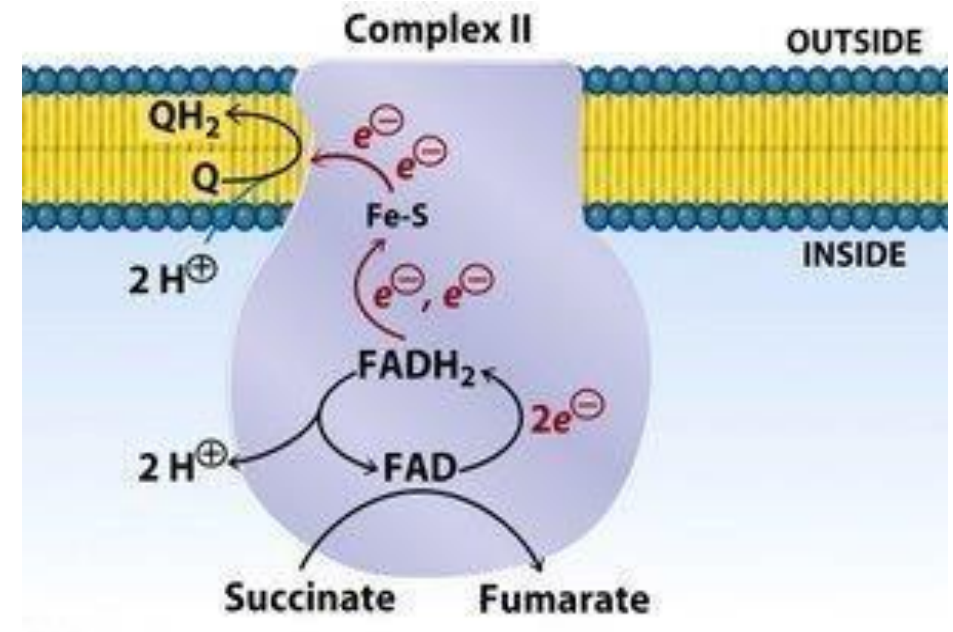
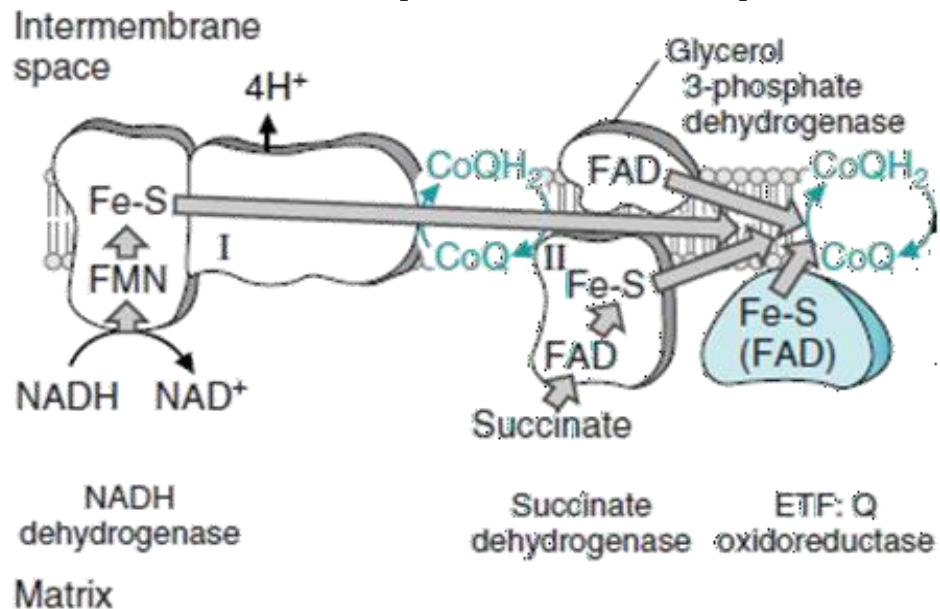
Redox Components of the ETC

Complex II (Succinate dehydrogenase)

- **Flavoprotein, iron sulfur centers**
- **TCA cycle** (Succinate dehydrogenase, the same enzyme used in the 6th step of the Krebs cycle)
- ✓ **Electron Transfer Flavoproteins, ETF-CoQ oxidoreductase** (ex. fatty acid oxidation)

Recall: $\text{FADH}_2 \rightarrow \text{Fe}$ (in Fe-S centers) \rightarrow coenzyme Q (gets reduced).

- ✓ ≈ 0 kcal, no proton transport



Sources of FADH₂ other than Krebs cycle:

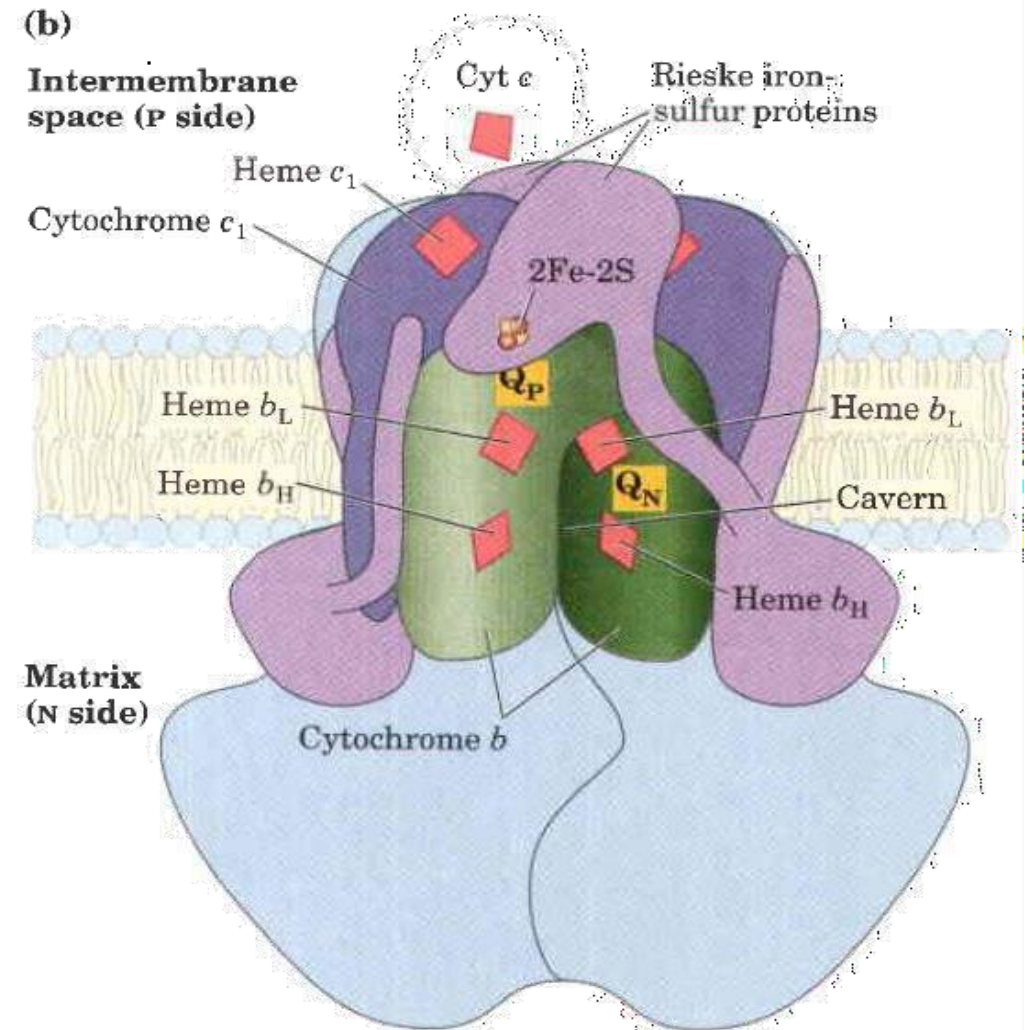
- ✓ Substrates oxidized by FAD-linked enzymes bypass complex-I
- ✓ Three major enzyme systems:
 - ✓ Succinate dehydrogenase
 - ✓ Fatty acylCoA dehydrogenase (Involved in fatty acids metabolism)
 - ✓ Mitochondrial glycerol phosphate dehydrogenase

Redox Components of the ETC

Complex III (Cytochrome bc₁)

Links between coQ
& cytochrome c.

- Also called: Q-cytochrome c Oxidoreductase
- Catalyzes the transfer of electrons from QH₂ to cytochrome c
- 11 subunits including two cytochrome subunits
- Contains iron sulfur center
- Contain three heme groups in two cytochrome subunits
- The cytochrome *b* subunit has two *b*-type hemes (*b_L* and *b_H*), the cytochrome *c* subunit has one *c*-type heme (*c₁*)
- *b_L* and *b_H* in cytochrome *b*; *c* type in cytochrome *c*₁
- Contain two CoQ binding sites
- 4H⁺ are transported to inter membranous space.



Redox Components of the ETC

Complex IV (Cytochrome c oxidase)

- Passes electrons from Cytochrome c to O₂
- Contains cytochrome a and a₃
- Contains two copper sites
- Contains oxygen binding sites
- O₂ must accept 4 electrons to be

Fully reduced to 2 H₂O (2H⁺/2e⁻)

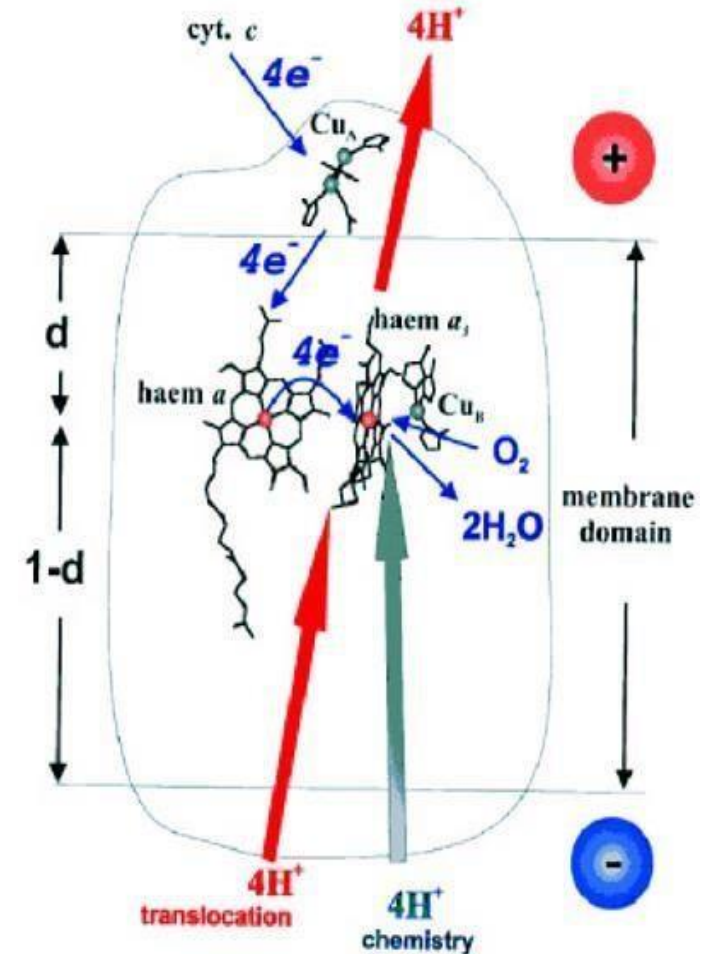


- Cytochrome oxidase has a much lower K_m for O₂ than myoglobin and hemoglobin

Has high affinity to bind to oxygen, good for achieving full reduction of oxygen and the production of high amount of energy by ETC.

✓ Partial reduction of O₂ is hazardous

Partial reduction results in ROS (such as H₂O₂) which have a very degradative effect to the cell, which may lead to cell death.



Further notes regarding the previous slides.

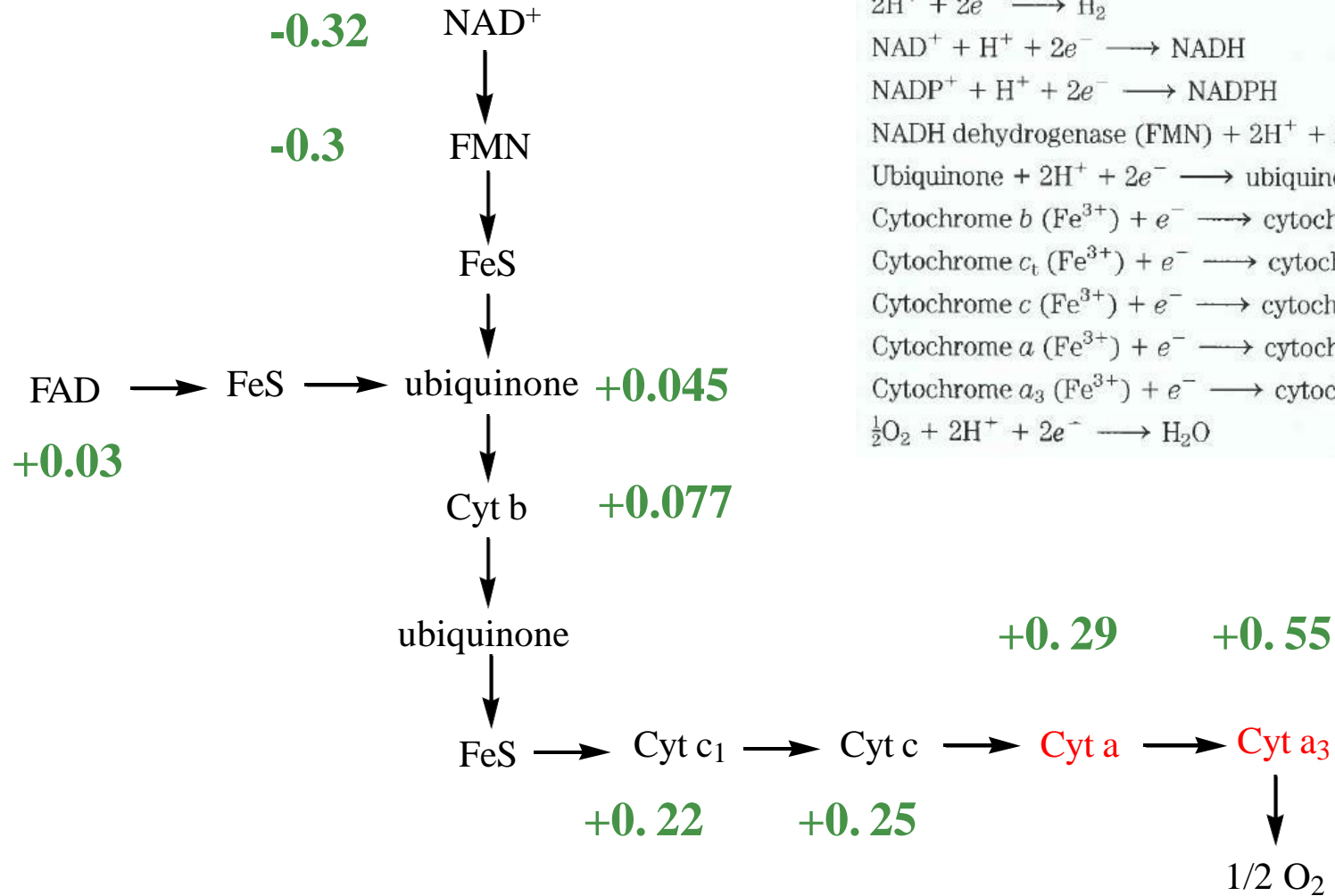
- NADH and FADH_2 are produced from different degradative pathways in the cytosol or in the mitochondria; they can interact with the ETC as Kreb's cycle isn't the only source of these molecules.
- Complex I is a flavoprotein as it is linked to FMN, complex II is also a flavoprotein as it is linked to FAD which gets reduced to FADH_2 is the sixth step in Kreb's cycle.
- Extremely important: For each two electrons, 10 protons are transported from the matrix to the intermembrane space.
- 4 protons from complex I
- 4 protons from complex III
- 2 protons from complex IV
- This is in case of NADH since it starts from complex I. However, for FADH_2 , only 6 protons are transported – 4 protons from complex III and 2 protons from complex IV – notice that FADH_2 contribution to the gradient is less than NADH; this explains why NADH leads to more ATP production than FADH_2 .

How can we prove the right arrangement of ET?

➤ 1. Measuring the standard reduction potentials

Redox reaction (half-reaction)	E'° (V)
$2\text{H}^+ + 2e^- \longrightarrow \text{H}_2$	-0.414
$\text{NAD}^+ + \text{H}^+ + 2e^- \longrightarrow \text{NADH}$	-0.320
$\text{NADP}^+ + \text{H}^+ + 2e^- \longrightarrow \text{NADPH}$	-0.324
$\text{NADH dehydrogenase (FMN)} + 2\text{H}^+ + 2e^- \longrightarrow \text{NADH dehydrogenase (FMNH}_2)$	-0.30
$\text{Ubiquinone} + 2\text{H}^+ + 2e^- \longrightarrow \text{ubiquinol}$	0.045
$\text{Cytochrome } b (\text{Fe}^{3+}) + e^- \longrightarrow \text{cytochrome } b (\text{Fe}^{2+})$	0.077
$\text{Cytochrome } c_t (\text{Fe}^{3+}) + e^- \longrightarrow \text{cytochrome } c_t (\text{Fe}^{2+})$	0.22
$\text{Cytochrome } c (\text{Fe}^{3+}) + e^- \longrightarrow \text{cytochrome } c (\text{Fe}^{2+})$	0.254
$\text{Cytochrome } a (\text{Fe}^{3+}) + e^- \longrightarrow \text{cytochrome } a (\text{Fe}^{2+})$	0.29
$\text{Cytochrome } a_3 (\text{Fe}^{3+}) + e^- \longrightarrow \text{cytochrome } a_3 (\text{Fe}^{2+})$	0.35
$\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- \longrightarrow \text{H}_2\text{O}$	0.8166

Increasing E°



“Walk” along the ETC components, E° increases as we move along the ETC, so each component is more likely to be reduced than the preceding one. We can also say that each component is a stronger oxidizing agent than the one before, so it oxidizes it and is itself reduced. → O_2 is the strongest oxidizing agent in the ETC.

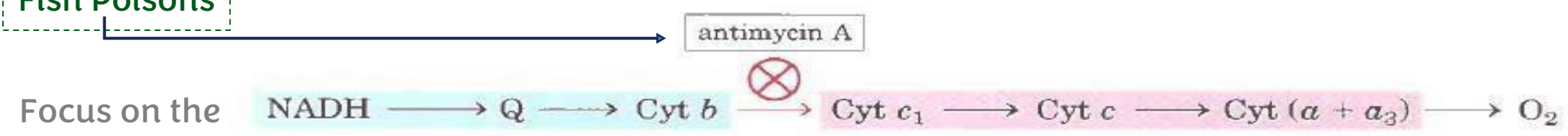
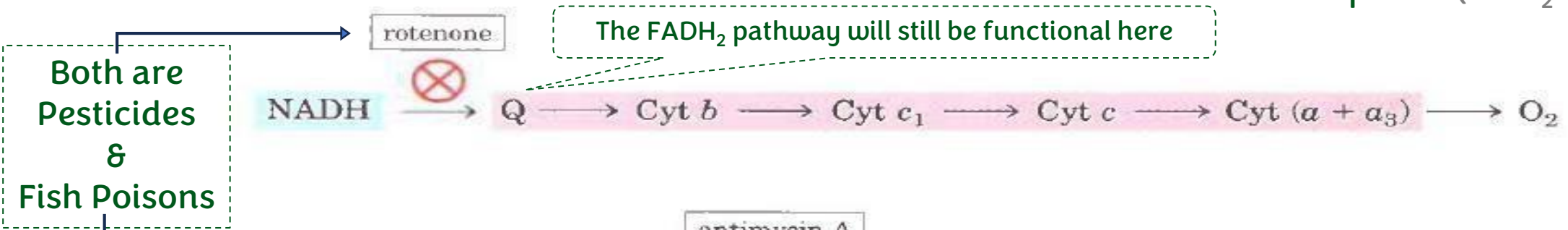
NADH → Q → cytochrome b → cytochrome c₁ → cytochrome c → cytochrome a → cytochrome a₃ → O₂

How can we prove the right arrangement of ETC?

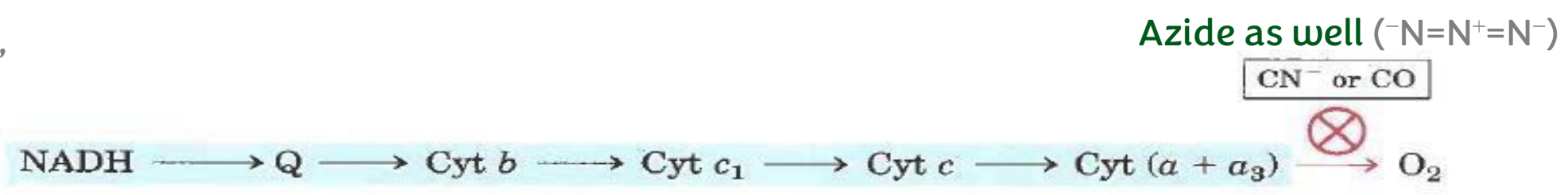
NADH → Q → cytochrome b → cytochrome c₁ → cytochrome c → cytochrome a → cytochrome a₃ → O₂

- **2. Reduction of the entire ETC with no O₂** → Doesn't usually happen in cells. It is done experimentally to make sure that the hypothesized order in the ETC is actual; this method will not make ATP since the chain is not completed (no O₂ reduction).
- **3. Addition of inhibitors**

Non-physiological inhibitors; also done experimentally

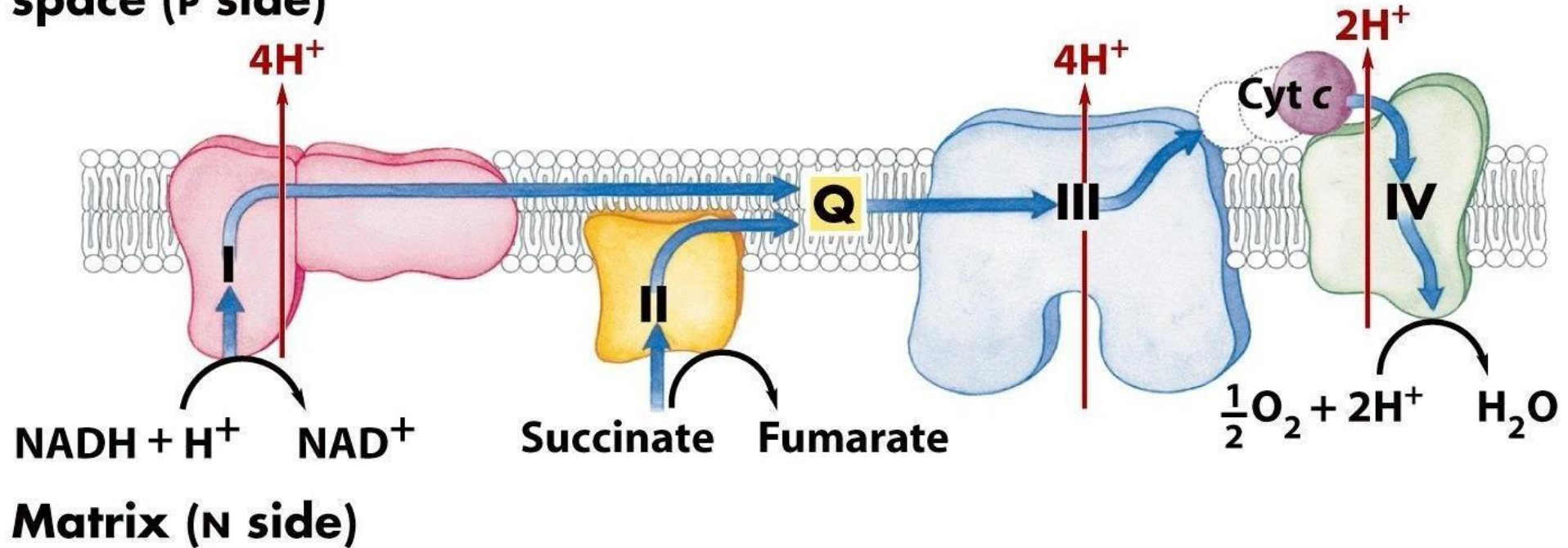


Focus on the step that each inhibitor acts on, and what components are affected.



Pumping of Protons

Intermembrane
space (P side)



➤ For every 2 electrons passing:

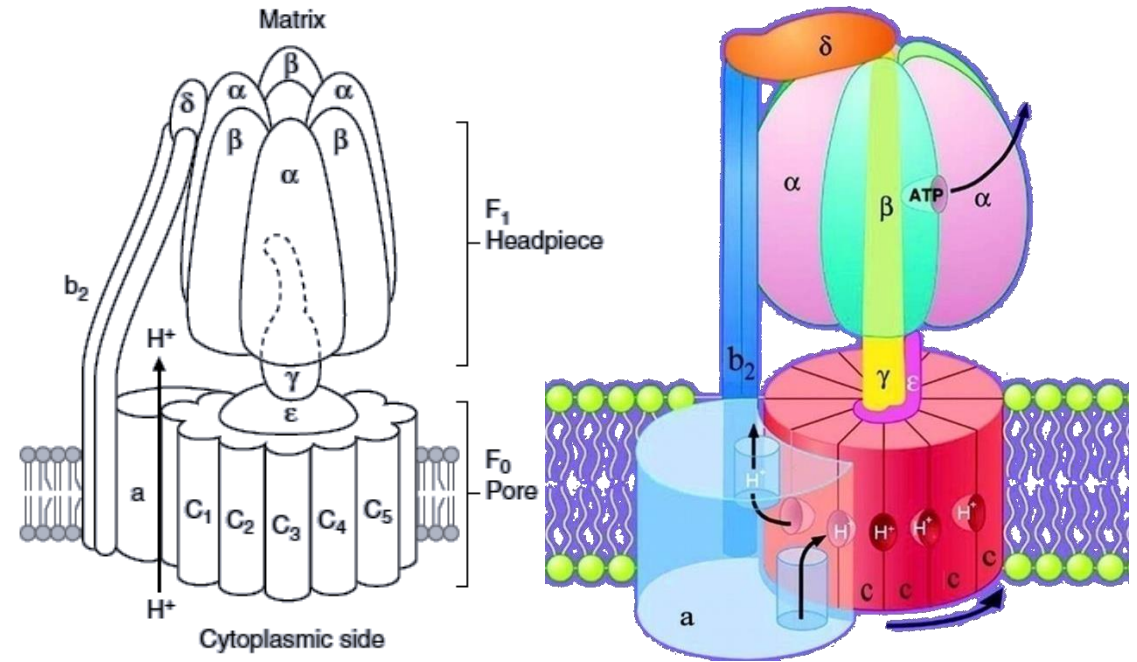
➤ 4H^+ (complex I); 0H^+ (complex II); 4H^+ (complex III), 2H^+ (complex IV)

TOTAL = 10H^+ (for each 2e^-), given that all 3 complexes (I, III, and IV) have participated ⇔ in the case of NADH.

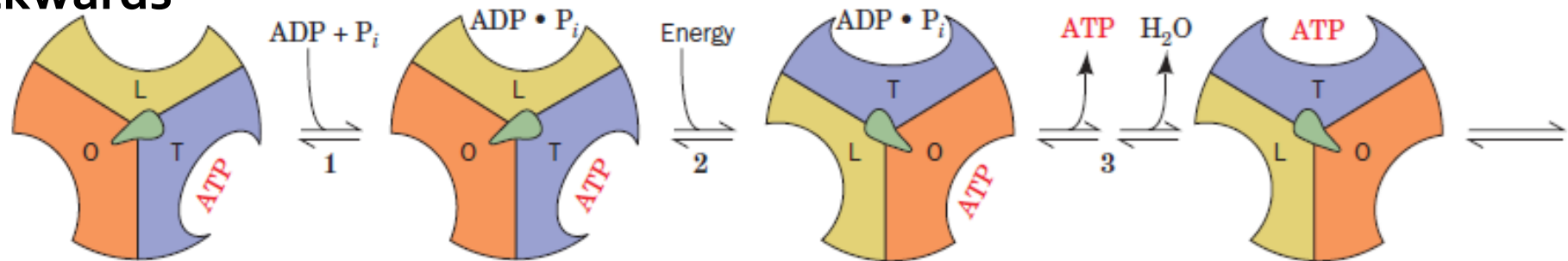
➔ In the case of FADH_2 , only 6H^+ (for each 2e^-) because complex I is skipped.

ATP Synthase

- **F₁ (enzyme that synthesizes ATP):**
 - "γ" subunit: rotates
 - "β" subunit: binds
 - "α" subunit: structural
 - 3 conformations: tight (T), loose (L), open (O)
- **F₀ (transmembrane domain):**
 - "a" subunit: point of entry & exit of H⁺
 - "c" subunit rotates
 - 4H⁺/ATP
- Can run backwards



Proton passage drives the rotation of F₀ dissipating the pH and electrical gradients. F₀ rotation causes conformational changes in the extra-membranous F₁ domain that allow it to bind ADP + P_i, phosphorylate ADP to ATP, and release ATP.



***See next slide for explanation

ATP Synthase Mechanism of Work

1. The “a” subunit undergoes a conformational change when H^+ passes through it, which causes a change in the adjacent subunits (c_1, c_2, c_3, \dots) and thus causing the rotation of the whole F_0 domain.
2. The rotatory motion is transmitted from F_0 to F_1 by the γ (gamma) subunit, resulting in the rotation of α and β and the phosphorylation of ADP to ATP.
 - ATP is not directly released after it is formed.
 - “ α and β ” subunits are present in 3 conformations:
 - Tight (T) \Leftrightarrow is the site of ATP directly after phosphorylation from ADP.
 - Loose (L) \Leftrightarrow is the site of binding ADP and P_i before phosphorylation to ATP.
 - Open (O) \Leftrightarrow is a state which allows ATP to be released.
1. When ATP is at “T”, it cannot escape.
2. ADP and P_i bind to an adjacent “L”.
3. Using energy for the conformational changes, the “L” becomes “T”, and the “T” becomes “O”, causing the ATP (was at “T” and now “O”) to be released into the matrix.
4. ADP will have been phosphorylated and **ATP** is now residing in “T” (back to step 1).
 - The cycle repeats over and over.
 - *SEE THE FIGURE AT THE BOTTOM OF THE PREVIOUS SLIDE FOR STEPS 1 \rightarrow 4.*

Energy yield of the ETC

NADH and FADH₂ oxidation supplies energy, but not all energy is used to make ATP. *YOU can do the math... Ok we'll do it for you!*

- NADH, -53 kcal, ATP? 53 available - 7.3 * 2.5 used for ATP = **34.75 kcal**
- FADH₂, -41 kcal, ATP? 41 available - 7.3 * 1.5 used for ATP = **30.05 kcal**
- ΔG° for the phosphorylation of ADP to ATP is +7.3 kcal/mol
- $\Delta G^\circ'$ is so negative, never reversible
- Electron transport chain is our major source of heat

Used for thermogenesis and other ancillary functions (cellular functions such as transport of molecules).



shutterstock.com · 481856635

[Electron Transport Chain Animation \(youtube.com\)](https://www.youtube.com/watch?v=QCctQRoOB4M)

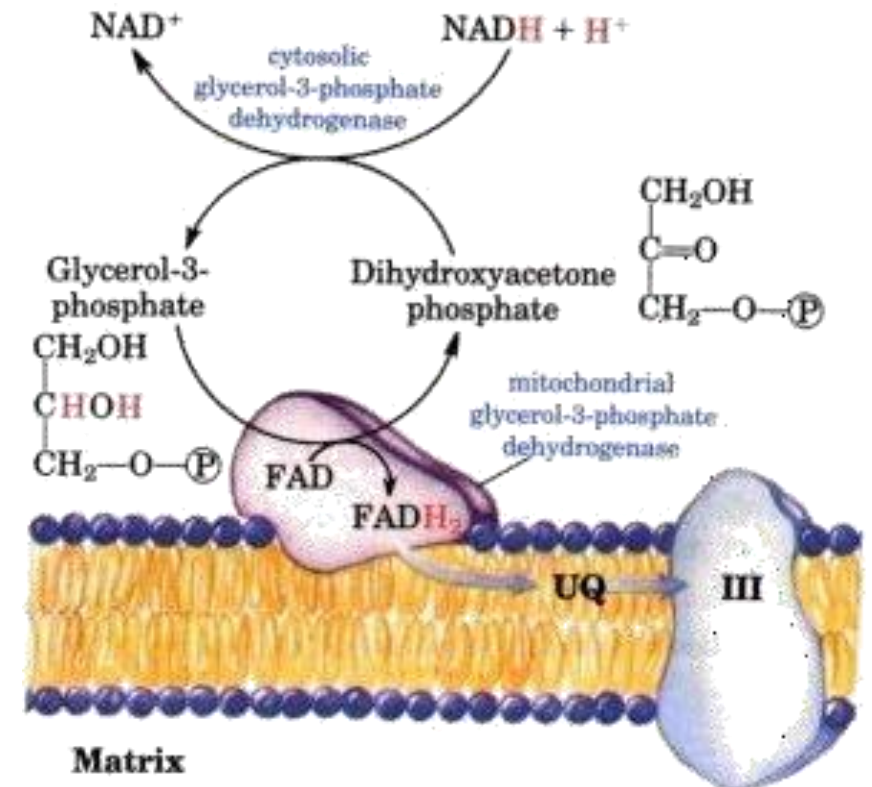
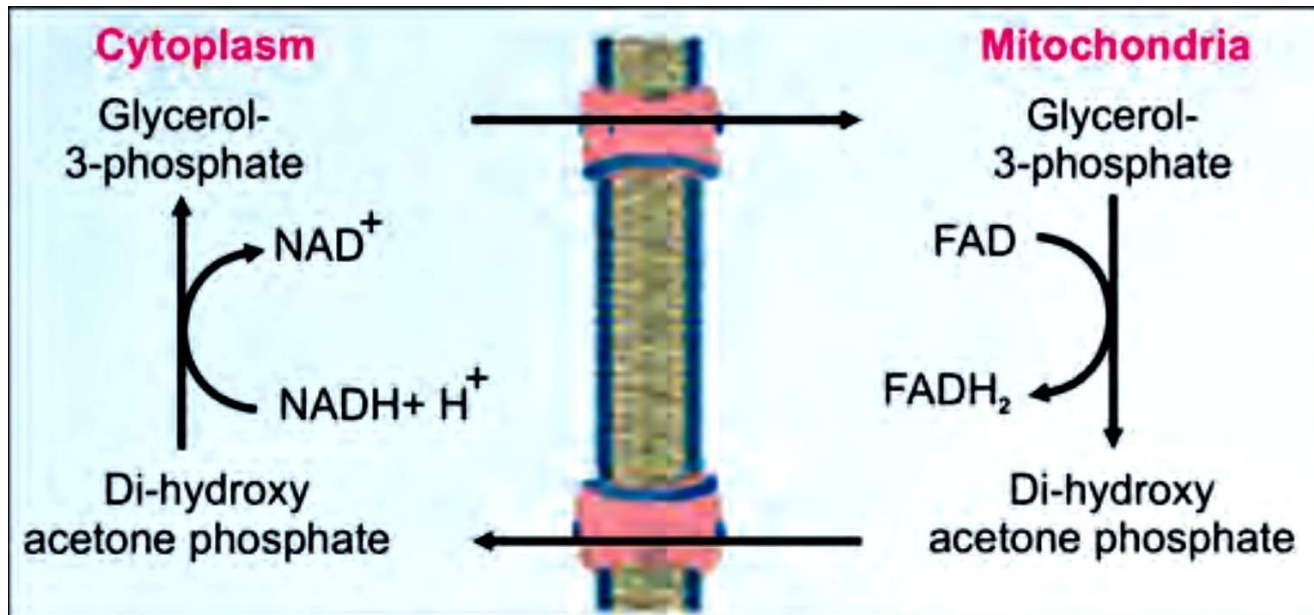
<https://youtu.be/QCctQRoOB4M?si=4LI3YJGSAQxngPdX>

Mitochondrial Shuttling Systems for cytosolic NADH

- 1. Glycerol 3-phosphate shuttle by glycerophosphate dehydrogenase
- In skeletal muscle and brain
- Glycolytic pathway as an example
- How NADH passes?
- ATP yield= 2ATP for each cytosolic NADH

We can use cytosolic NADH's energy in the ETC. HOW?

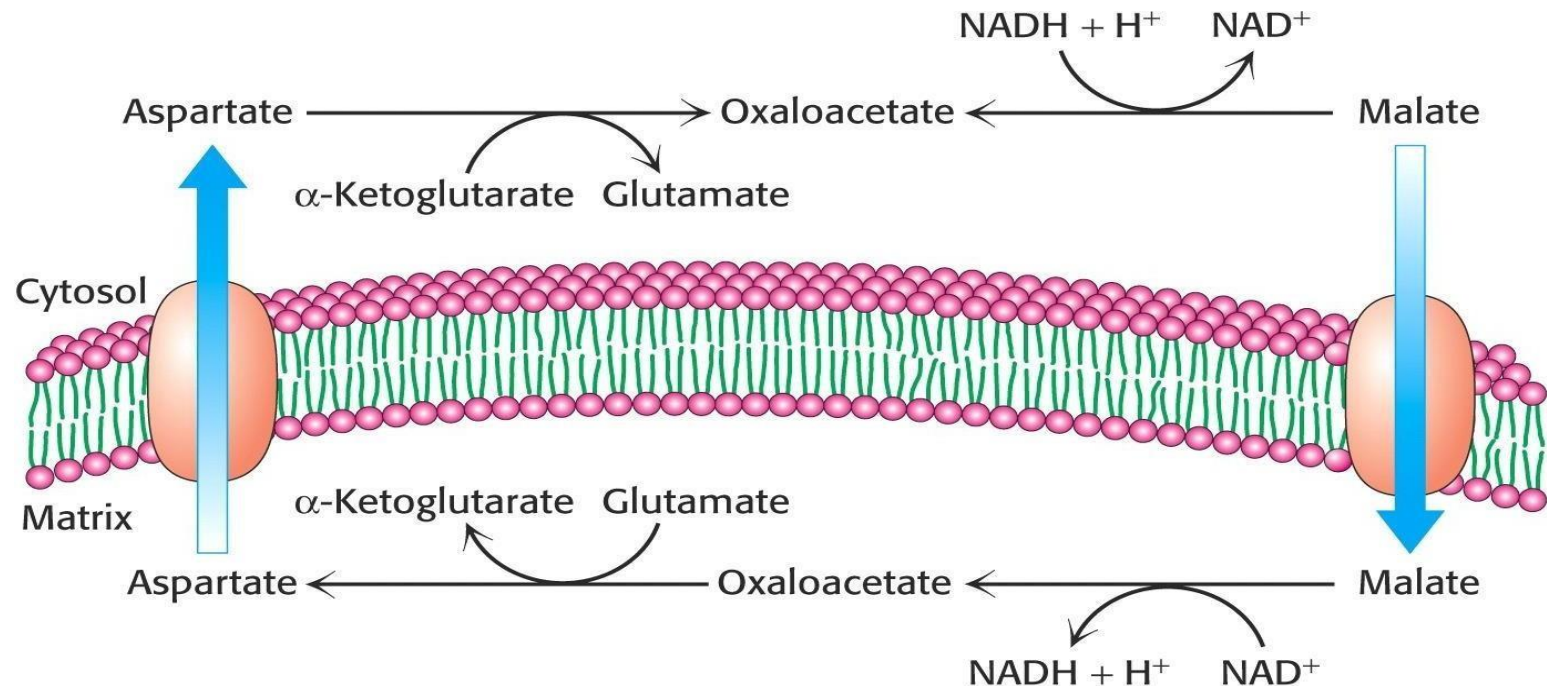
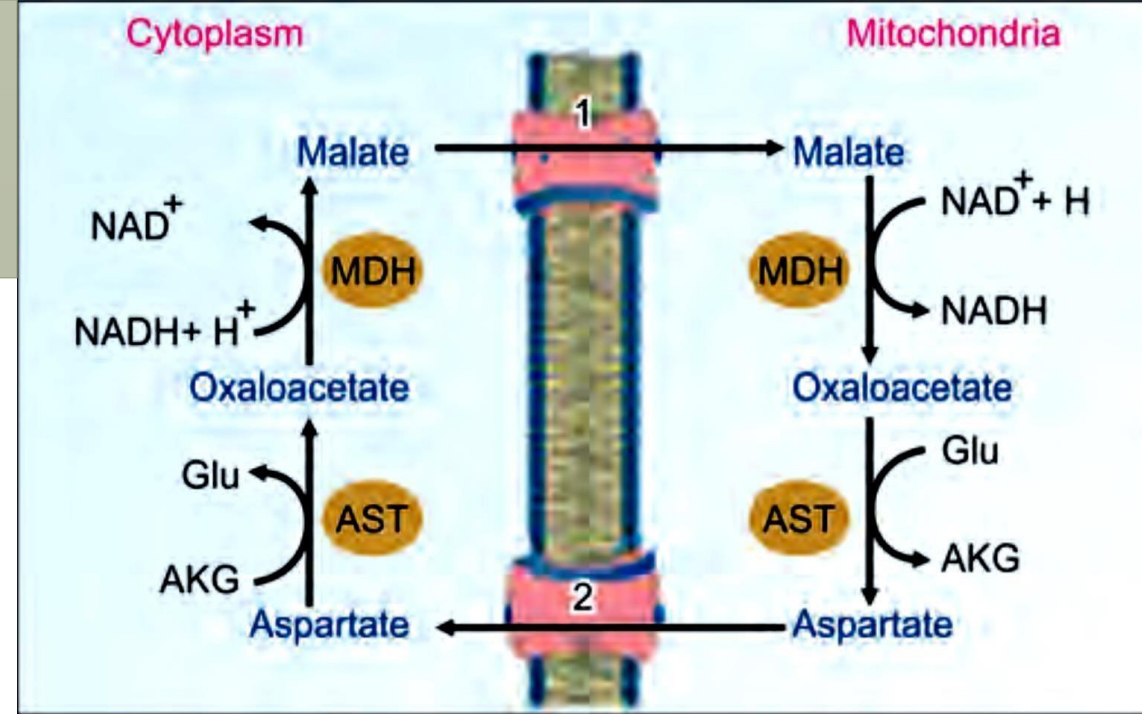
NADH itself is not transported inside, but we can extract the $2 e^-$ (energy) by oxidizing NADH and reducing dihydroxyacetone phosphate into glycerol-3-phosphate, which enters the mitochondria (see bottom left). The reverse reaction happens inside the mitochondria reducing FAD to $FADH_2$, which has a lower reducing power (lower energy) than NADH. The first reaction is catalyzed by cytosolic glycerol-3-P dehydrogenase while the second reaction by mitochondrial glycerol-3-P dehydrogenase.



Mitochondrial Shuttling Systems for cytosolic NADH

- 2. Malate-Aspartate shuttle by malate dehydrogenase
- operates mainly in liver, kidney and heart
- 2 membrane carriers & 4 enzymes → SEE TOP RIGHT FIGURE
- Readily reversible (vs. Glycerol 3-phosphate shuttle)
- NADH can be transferred only if the NADH/NAD⁺ ratio is higher in the cytosol than in the mitochondrial matrix
- Exchange of key intermediates between mitochondria & cytosol

SEE NEXT SLIDE FOR DETAILED EXPLANATION



Malate-Aspartate Shuttle

1. Cytosolic NADH is oxidized and OAA is reduced to Malate by MDH.
2. Malate is transported inside the mitochondria by a carrier protein.
3. Mitochondrial malate is oxidized to OAA and NAD⁺ is reduced to NADH.
 - Step 3 (the one above) is the same as step 8 of the TCA cycle.
 - This shuttling system is more complex since it is reversible and is linked to other metabolic pathways such as amino acid metabolism.
 - AST enzyme plays a role in the reversibility of this system as it is responsible for the amination of OAA in the mitochondria, producing aspartate which can be transported to the cytosol by a carrier protein.
 - AST also catalyzes the reverse reaction of deamination of aspartate to OAA in the cytosol.
 - OAA can be used to oxidize NADH to NAD⁺, repeating step 1 (the one above).
 - Notice that NADH (not FADH₂) is the mitochondrial electron carrier here.
 - This means that the full reducing power of cytosolic NADH is retained.

MDH → Malate Dehydrogenase

AST → Aspartate Aminotransferase

Examples on NADH producing enzymes

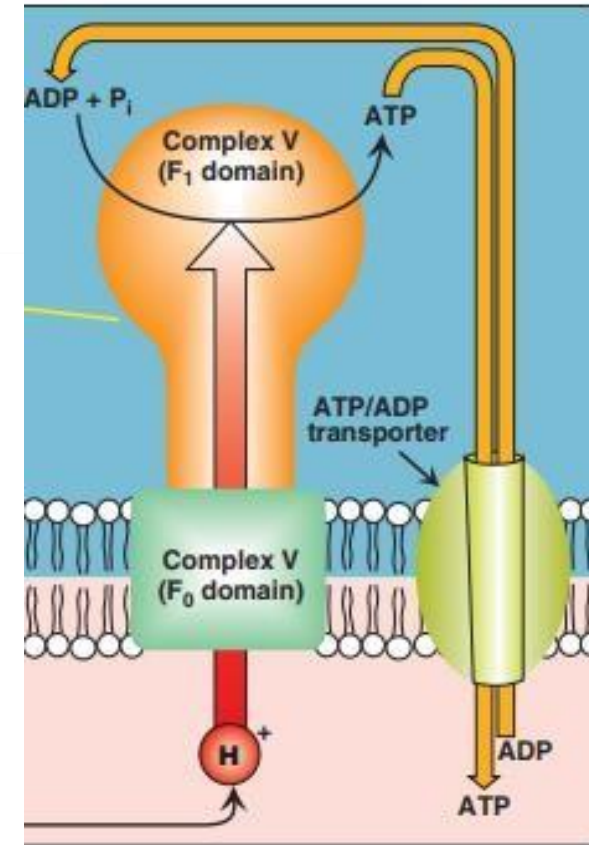
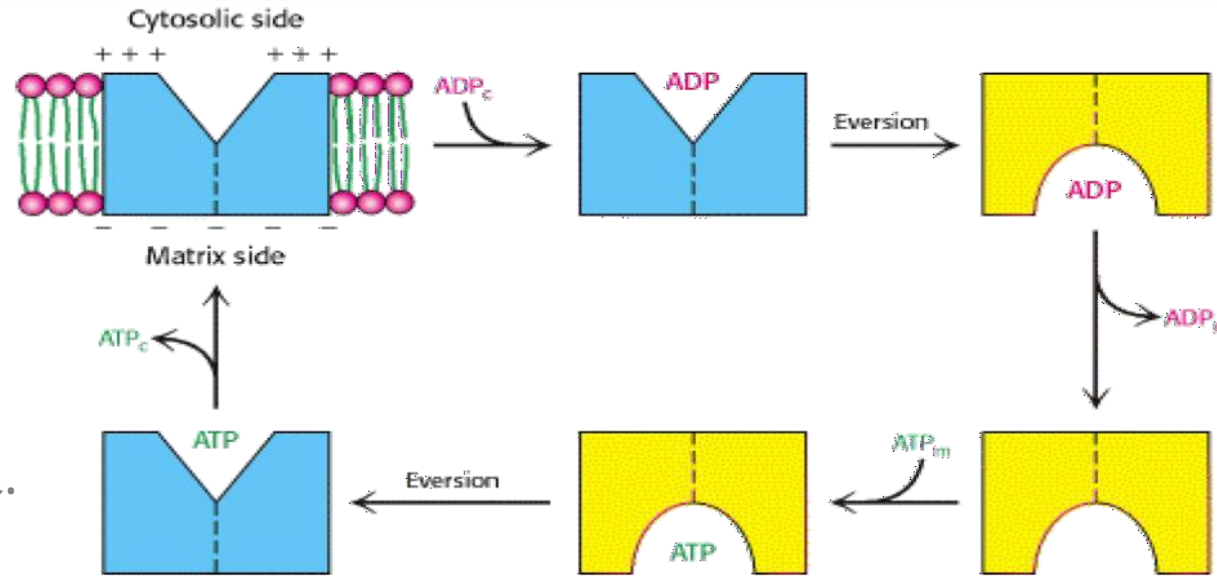
→ DON'T WORRY ABOUT ALL THESE FOR NOW ←

Box 37.3: NAD⁺ dependent enzymes

1. Lactate dehydrogenase (lactate → pyruvate) (see Fig. 9.14)
2. Glyceraldehyde-3-phosphate dehydrogenase (glyceraldehyde-3-phosphate → 1,3-bisphosphoglycerate) (see Fig.9.10)
3. Pyruvate dehydrogenase (pyruvate → acetyl CoA) (see Fig.9.22)
4. Alpha ketoglutarate dehydrogenase (alpha ketoglutarate → succinyl CoA) (see Fig.19.2)
5. Beta hydroxyacyl CoA dehydrogenase (beta hydroxyacyl CoA → beta ketoacyl CoA) (see Step 3, Fig.12.9)
6. Glutamate dehydrogenase (Glutamate → alpha ketoglutarate) (see Fig.15.9)

Mitochondrial Shuttling Systems for ATP/ADP

Focus on the figure:
ANT moves ADP into the mitochondria and moves ATP out of the mitochondria to carry cellular functions.
Examine the conformational changes responsible for this coupled transport mechanism.



- ATP-ADP Translocase (adenine nucleotide translocase or ANT)
- The flows of ATP and ADP are coupled (ADP enters only if ATP exits, and vice versa)
- Highly abundant (14% of IMM proteins)
- Contains a single nucleotide-binding site (alternates)
- Similar affinity to ATP and ADP
- A phosphate carrier is responsible for transporting Pi from the cytosil into mitochondria.
- Inhibition leads to subsequent inhibition of cellular respiration

For any feedback, scan the code or click on it.



Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:
(numbered in order as cited in the text)

Lippincott Illustrated Reviews, 8th Ed.
Pages 81 → 87.

﴿يَأْتِيهَا الَّذِينَ ءَامَنُوا لَا تَتَّخِذُوا الْيَهُودَ وَالنَّصْرَىٰ أَوْلِيَاءَ بَعْضُهُمْ
أَوْلِيَاءُ بَعْضٍ ؕ وَمَن يَتَوَلَّهُمْ مِنكُمْ فَإِنَّهُ مِنهٖم ۗ إِنَّ اللَّهَ لَا يَهْدِي الْقَوْمَ
الظَّالِمِينَ ﴿٥١﴾ فَتَرَى الَّذِينَ فِي قُلُوبِهِم مَّرَضٌ يُسْرِعُونَ فِيهِمْ
يَقُولُونَ نَخْشَىٰ أَن تُصِيبَنَا دَآئِرَةٌ ۚ فَعَسَىٰ اللَّهُ أَن يَأْتِيَ بِالْفَتْحِ أَوْ أَمْرٍ
مِّنْ عِنْدِهِ ۖ فَيُصِيبِحُوا عَلَىٰ مَا أَسْرُوا فِي ۖ أَنفُسِهِمْ تَدْمِينٌ ﴿٥٢﴾
وَيَقُولُ الَّذِينَ ءَامَنُوا أَهٗتُولَآءِ الَّذِينَ أَقْسَمُوا بِاللَّهِ جَهْدَ أَيْمَانِهِمْ
إِنَّهُمْ لَمَعَكُمْ حَبِطَتِ أَعْمَالُهُمْ فَأَصْبَحُوا خَاسِرِينَ ﴿٥٣﴾

اللهم انصر إخواننا وأعنا على نصرتهم
واخذل اللهم من خذلهم