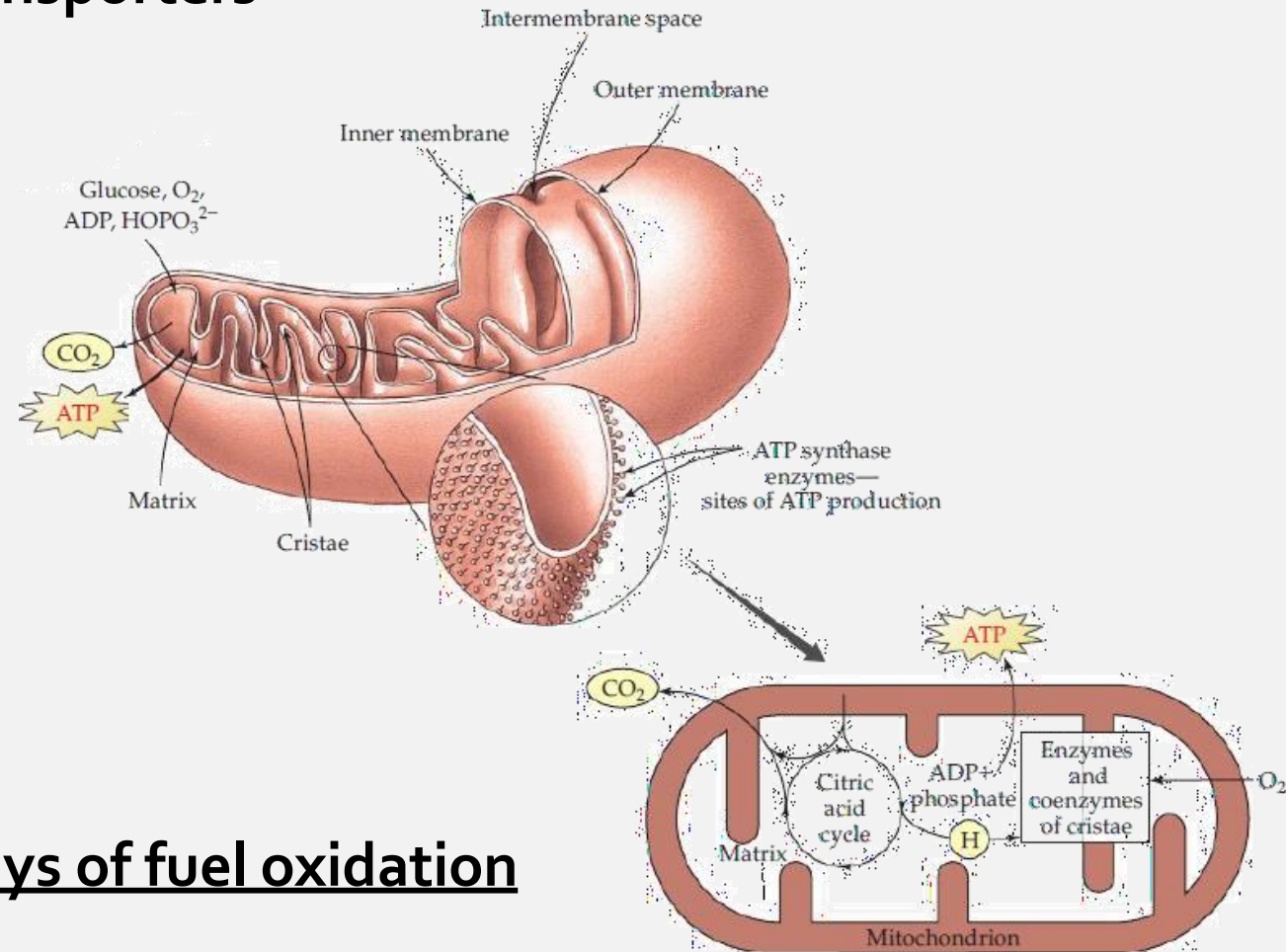


Oxidative Phosphorylation

Dr. Diala Abu-Hassan, DDS, PhD

Mitochondria

- OMM: permeable to small molecules (MW<5,000) & ions, porins (transmembrane channels)
- IMM: impermeable even to H⁺; specific transporters
- IMM bears the components of the respiratory chain and the ATP synthase
- Matrix: gel-like solution, 50% proteins, it contains pyruvate dehydrogenase complex & TCA cycle enzymes, fatty acid β -oxidation pathway, and the pathways of amino acid oxidation. mtDNA, mtRNA, mt-ribosomes
- In other words: matrix contains all pathways of fuel oxidation except glycolysis (cytosol)



Mitochondrial Membranes

- ✓ Inner membrane:
 - ✓ 22% cardiolipin
 - ✓ No cholesterol
- ✓ Outer membrane:
 - ✓ Similar to cell membrane
 - ✓ Less than 3% cardiolipin
 - ✓ 45% cholesterol

TABLE 20.3: Location of enzymes in mitochondria

Mitochondria, outer membrane:

Monoamino oxidase

Acyl CoA synthetase

Phospholipase A2

In between outer and inner membrane:

Adenylate kinase

Creatine kinase

Inner membrane, outer surface:

Glycerol-3-phosphate dehydrogenase

Inner membrane, inner surface:

Succinate dehydrogenase

Enzymes of respiratory chain

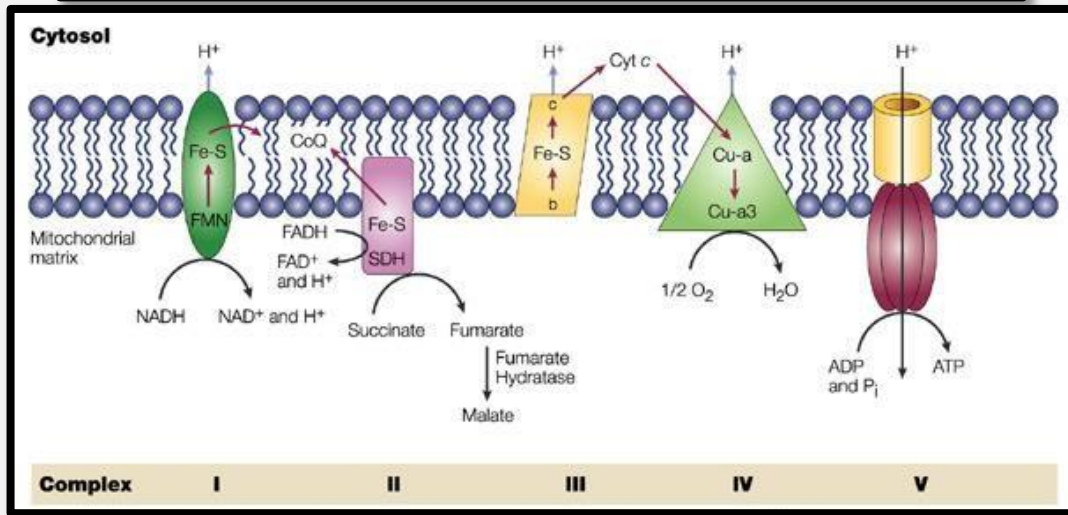
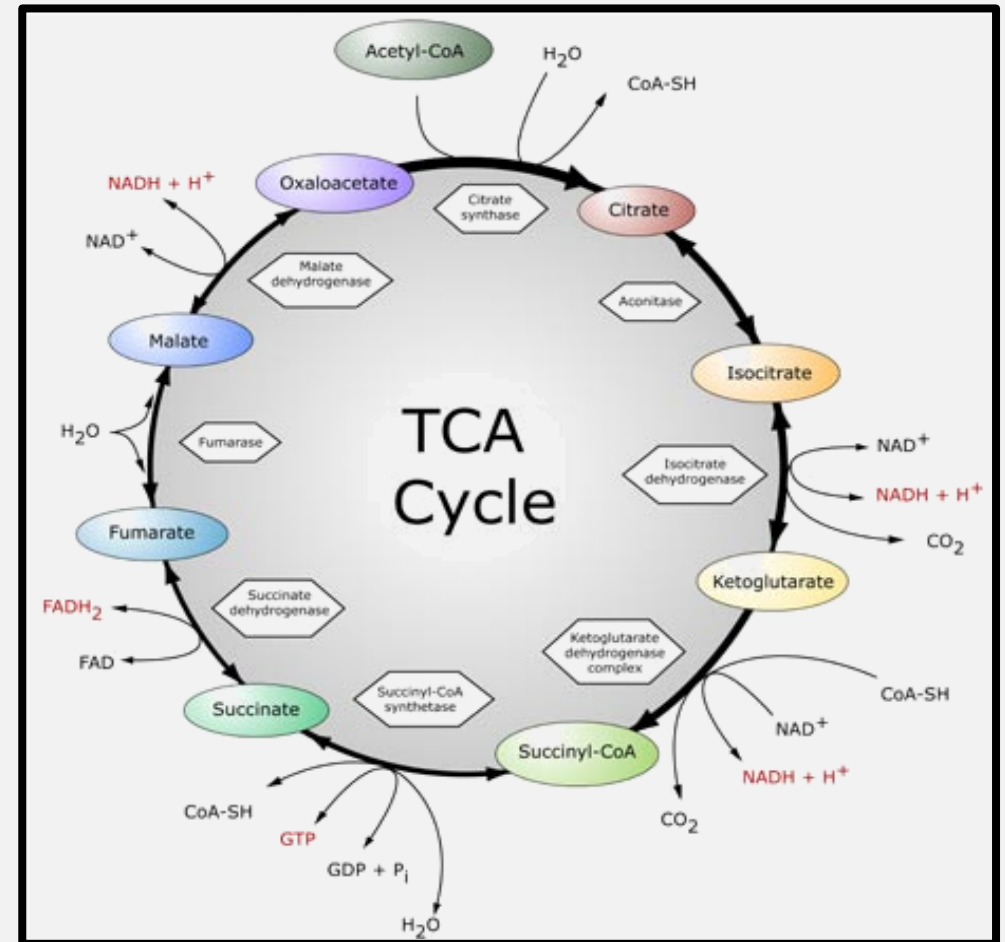
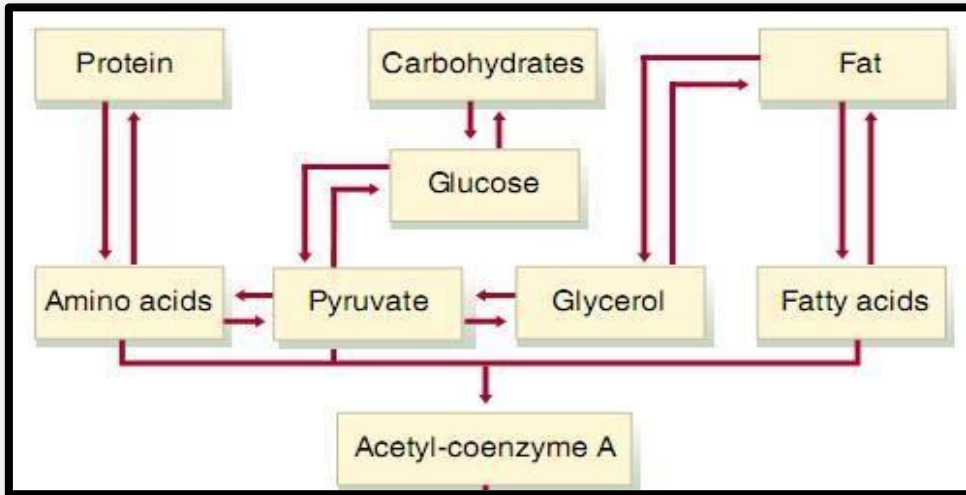
Soluble matrix:

Enzymes of citric acid cycle

Enzymes of beta oxidation of fatty acid

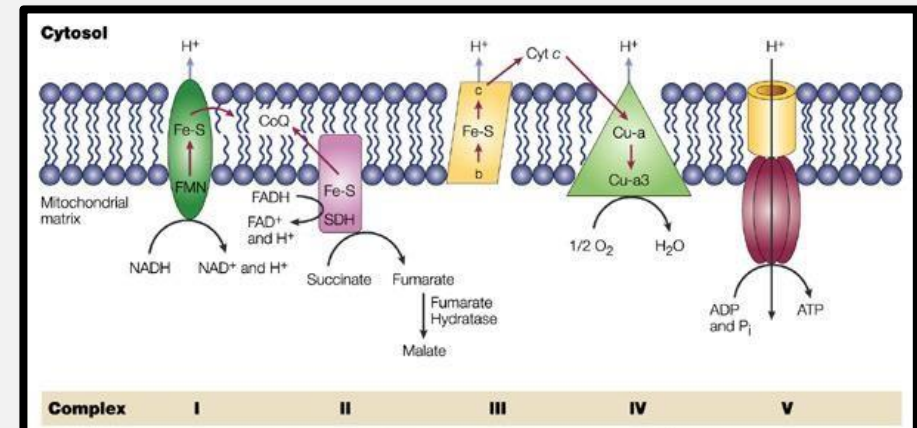
Where does Oxidative Phosphorylation occur?

- Stages: Digestion; Acetyl-CoA, TCA, OxPhos



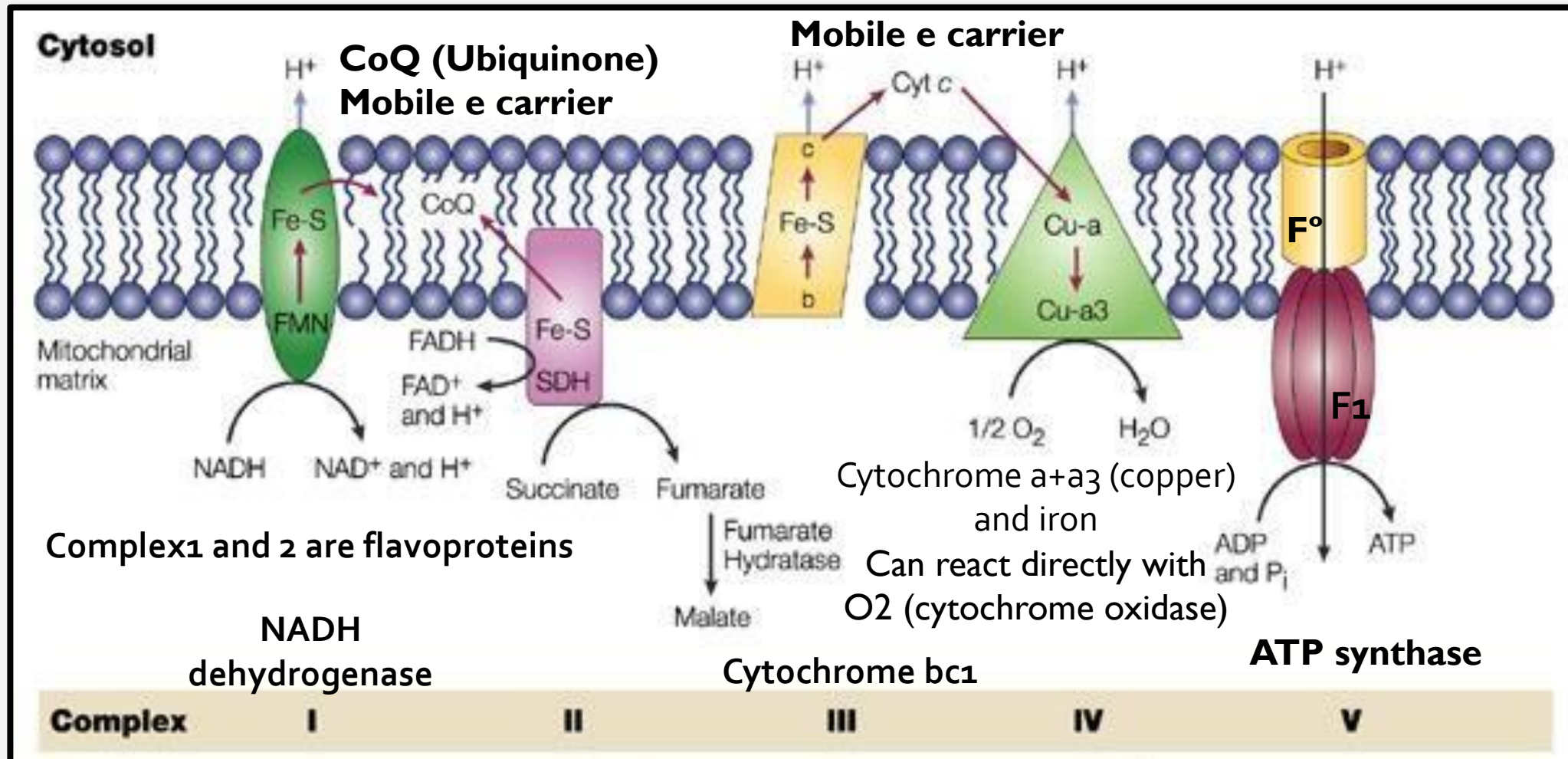
Oxidative Phosphorylation (OxPhos)

- Generation of ATP aided by the reduction of O_2
- Peter Mitchell (1961): the chemiosmotic theory
- Oxidative phosphorylation has 3 major aspects:
 - ✓ (1) It involves the flow of electrons through a chain of membrane-bound carriers (prosthetic groups)
 - ✓ (2) The free energy available (exergonic) is coupled to transport protons across a proton-impermeable membrane
 - ✓ (3) The transmembrane flow of protons down their concentration gradient provides the free energy for synthesis of ATP (ATP synthase)
- Five separate protein complexes I, II, III, IV, and V.
- Complexes I–IV each contain part of the electron transport chain.
- Each complex accepts or donates electrons to relatively mobile electron carriers, such as coenzyme Q and cytochrome c.



Oxidative Phosphorylation (OxPhos)

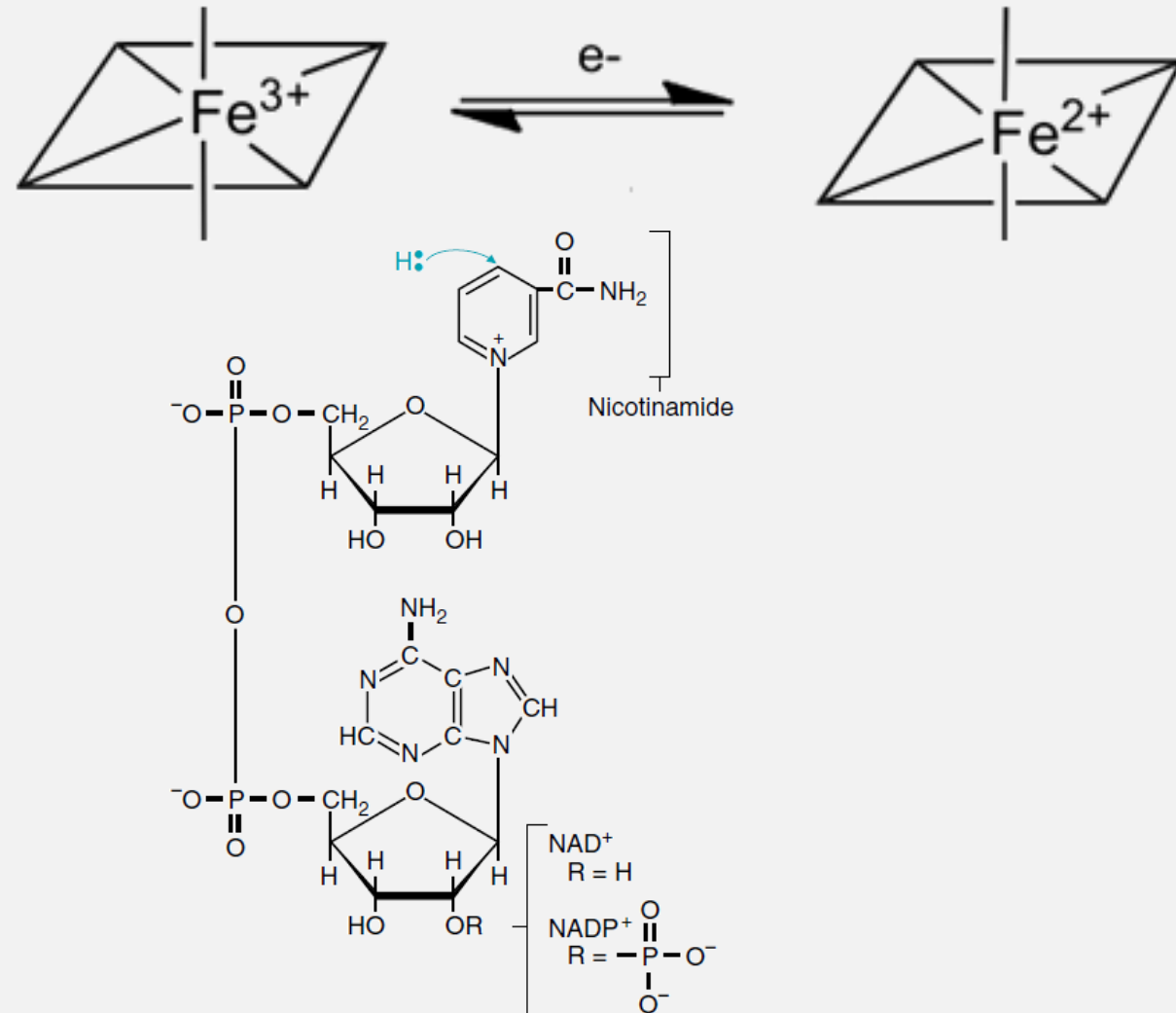
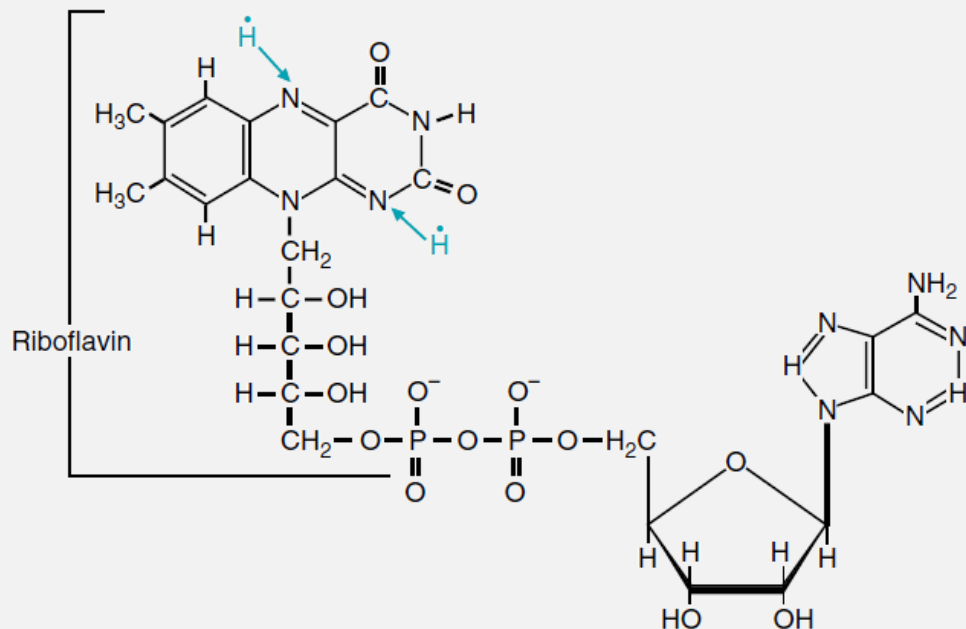
As electrons are passed down the electron transport chain, they lose much of their free energy. Part of this energy can be captured and stored by the production of ATP



Types of electron transfer (ET) through the electron transport chain (ETC)

➤ 3 types of ET occur in OxPhos:

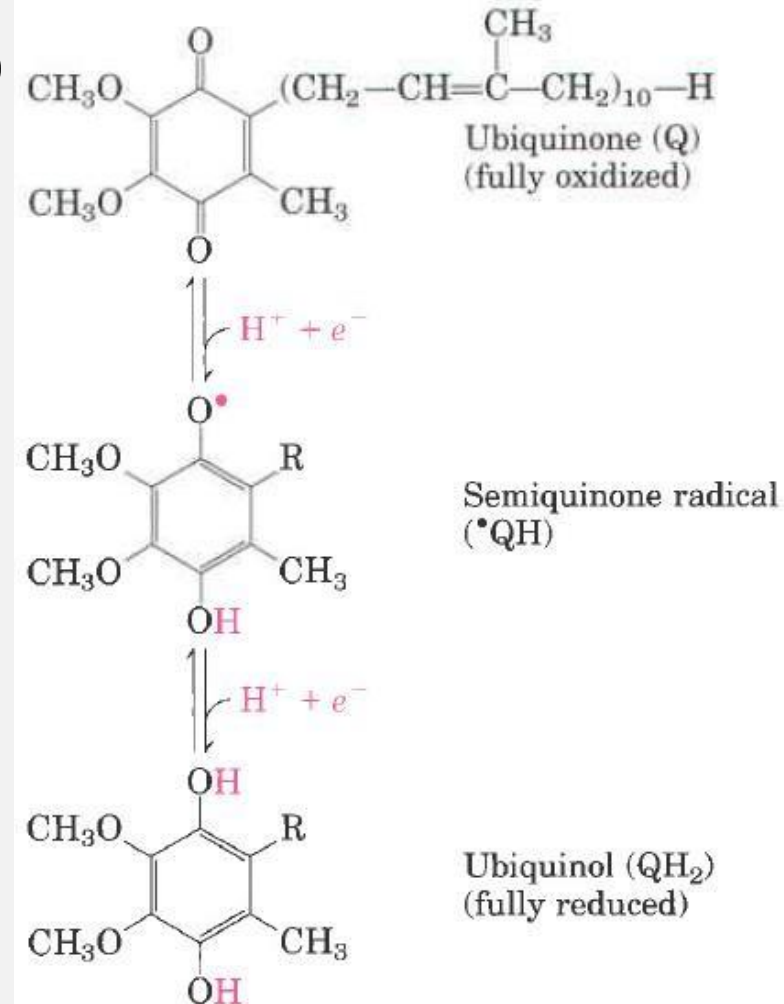
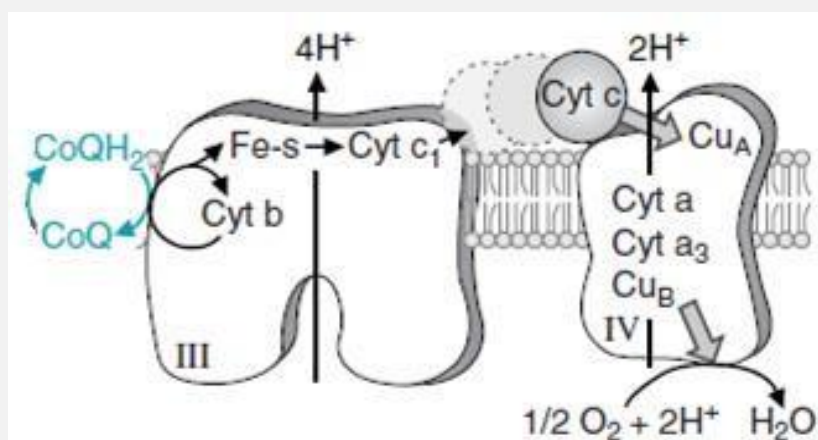
- ✓ Direct ET, as in the reduction of Fe^{3+} to Fe^{2+}
- ✓ Transfer as a hydrogen atom $\{(\text{H}^+) + (\text{e}^-)\}$
- ✓ Transfer as a hydride ion $(:\text{H}^-)$



Other electron-carrying molecules

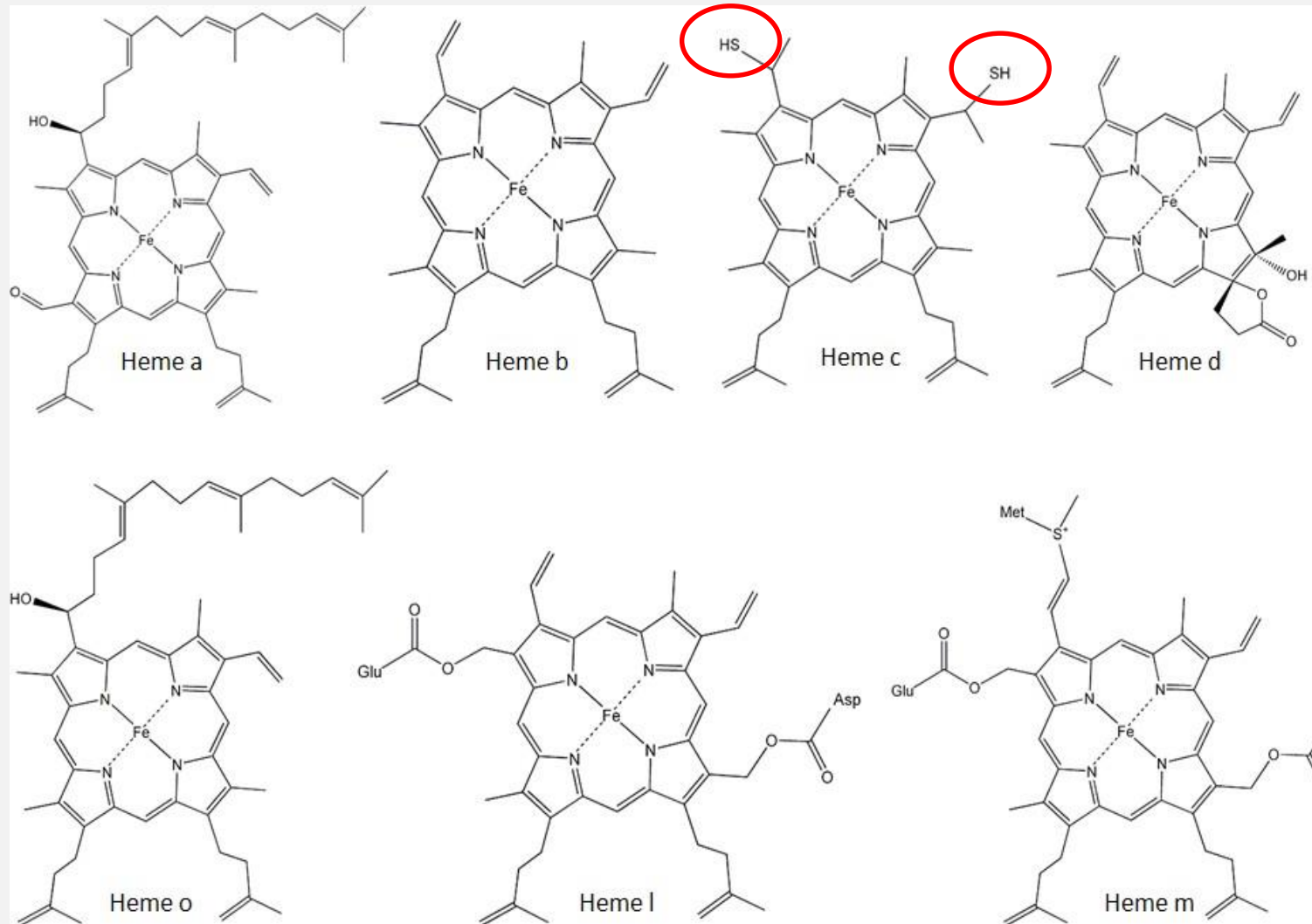
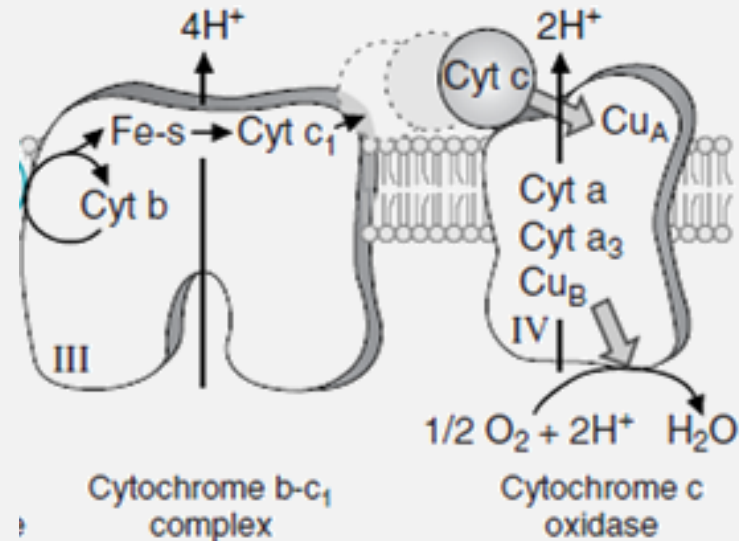
“Ubiquinone”

- Also called coenzyme Q, or Q (ubiquitous in biologic systems)
- Lipid-soluble benzoquinone with a long isoprenoid side chain
- Small & hydrophobic (freely diffusible)
- Carries electrons through the IMM
- Can accept either 1 e⁻ or 2 e⁻
- Act at the junction between a 2-electron donor and a 1-electron acceptor
- Sometimes prescribed for recovering MI patients



Other electron-carrying molecules “Cytochromes”

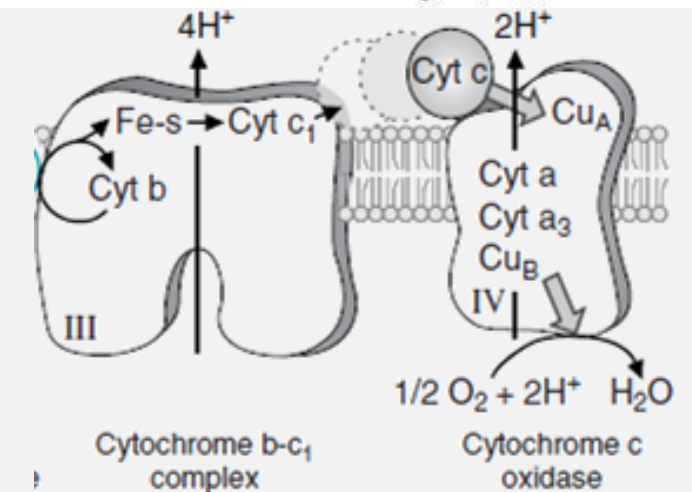
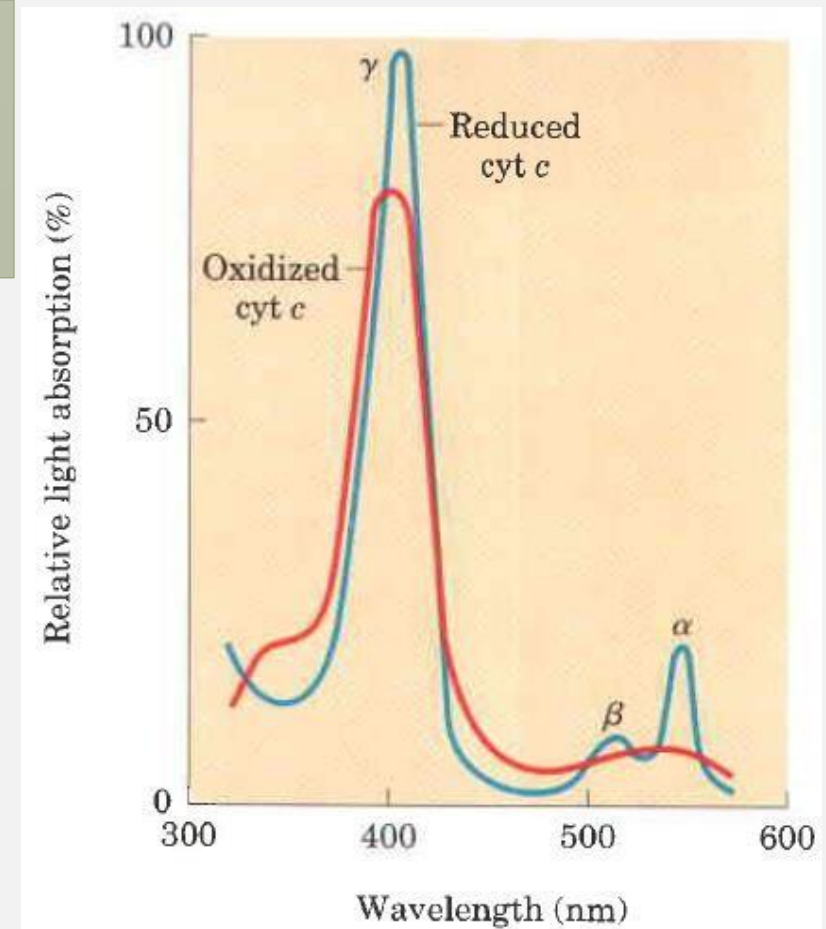
- Proteins with Fe-containing heme prosthetic groups
- Mode of binding (a, b, c)
- Mitochondria contain three classes of cytochromes (a, b, & c)



Other electron-carrying molecules “Cytochromes”

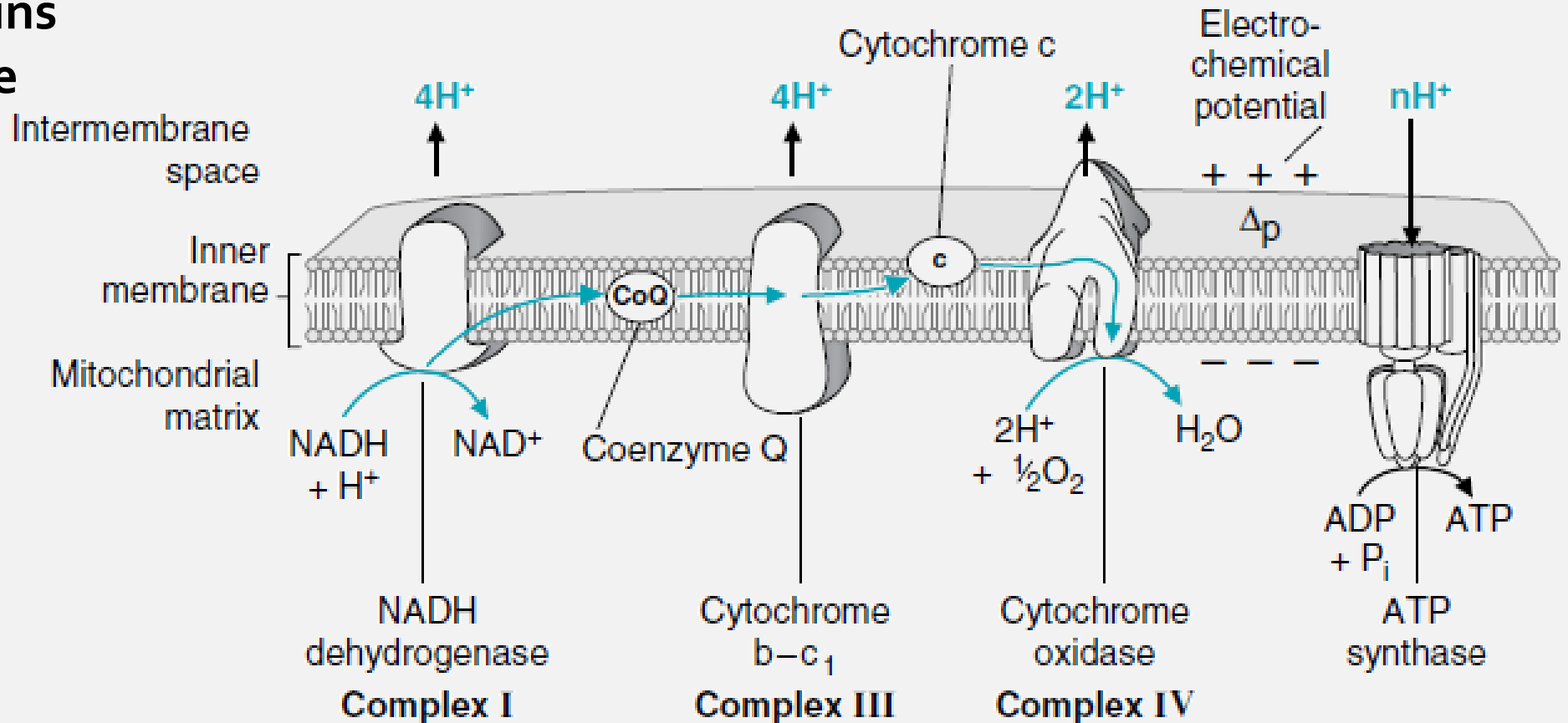
- Light absorption: Each cytochrome in its reduced (F^{+2}) state has 3 absorption bands in the visible range
- α band : near 600 nm in type a; near 560 nm in type b, & near 550 nm in type c
- Some cytochromes are named by the exact α band wavelength:
 - ✓ Cytochrome b_{562} ; Cytochrome c_{550} ; Cytochrome c_{551}
- Heme can carry one electron
- $\Delta E^{o'}$ depends on the protein
- Cytochromes a, b & c are transmembrane (c is the exception)

Cyto. c is associated with the outer face of the IMM, and is a mobile carrier of electrons

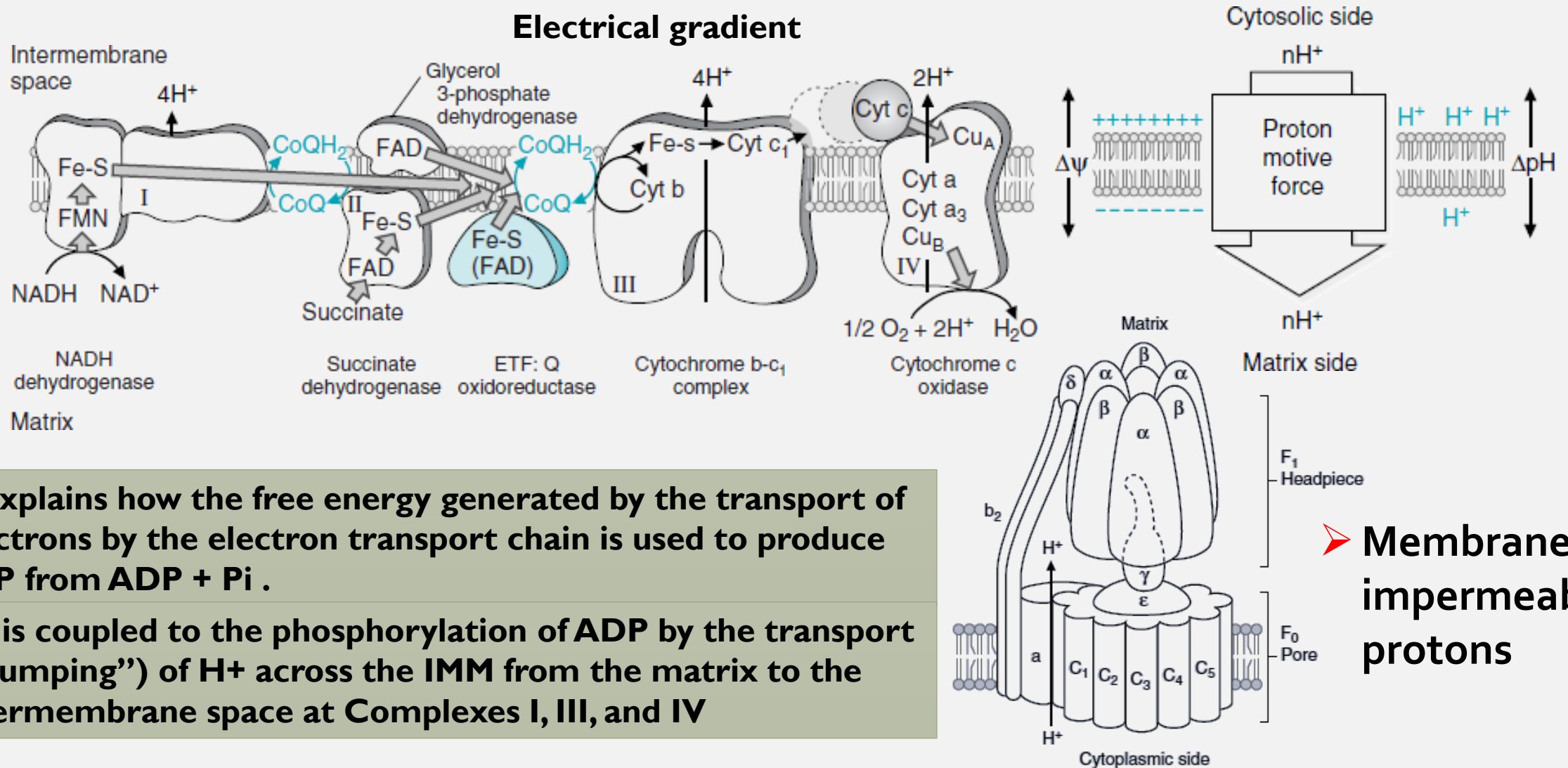


Requirements of OxPhos

- Redox reaction: electron donor (NADH or FADH₂) & electron acceptor (O₂)
- An intact IMM
- ETC of proteins
- ATP synthase

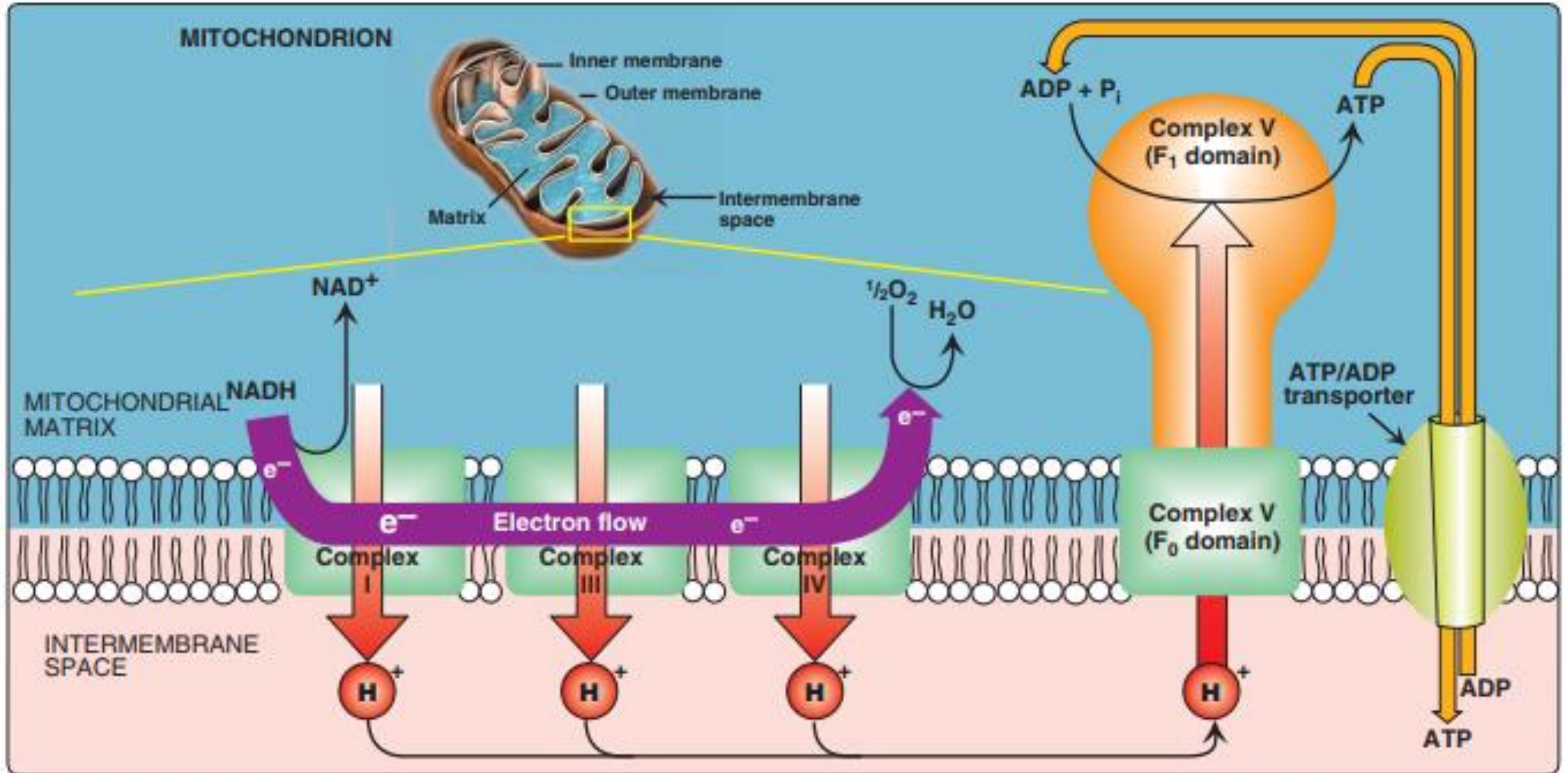


ET to O₂, How does this occur? “the Chemiosmotic (Mitchell) hypothesis”



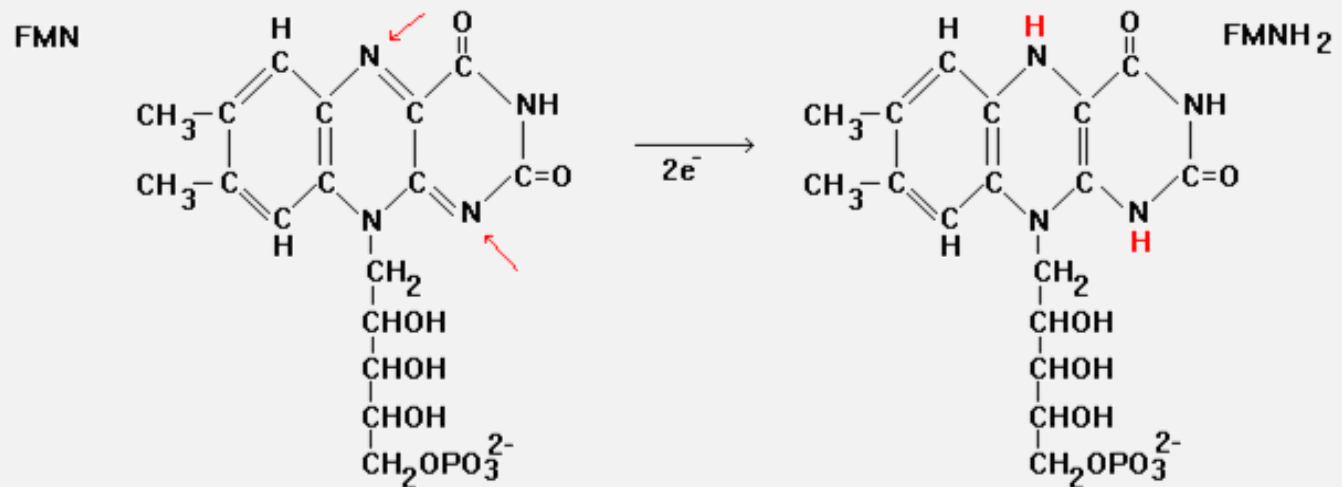
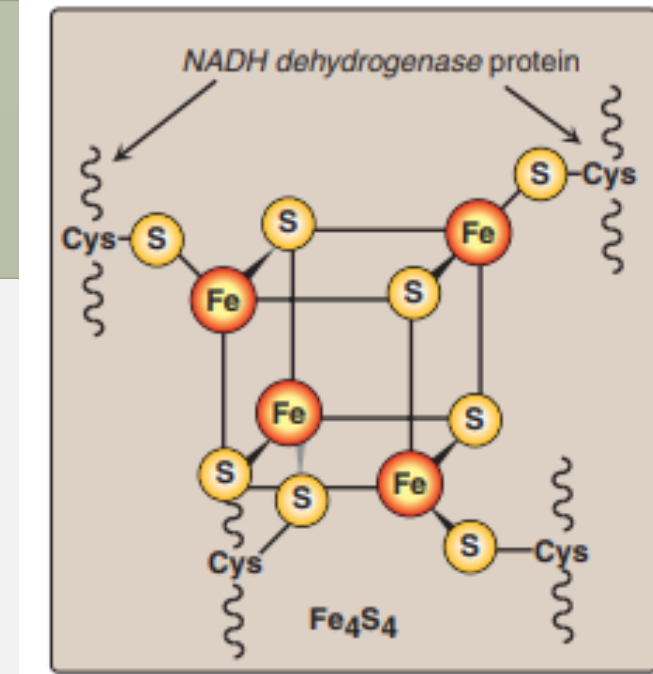
- ✓ It explains how the free energy generated by the transport of electrons by the electron transport chain is used to produce ATP from ADP + Pi .
- ✓ ET is coupled to the phosphorylation of ADP by the transport (“pumping”) of H⁺ across the IMM from the matrix to the intermembrane space at Complexes I, III, and IV

Electron transport chain coupled to the transport of H⁺



Redox Components of the ETC Complex I (NADH dehydrogenase)

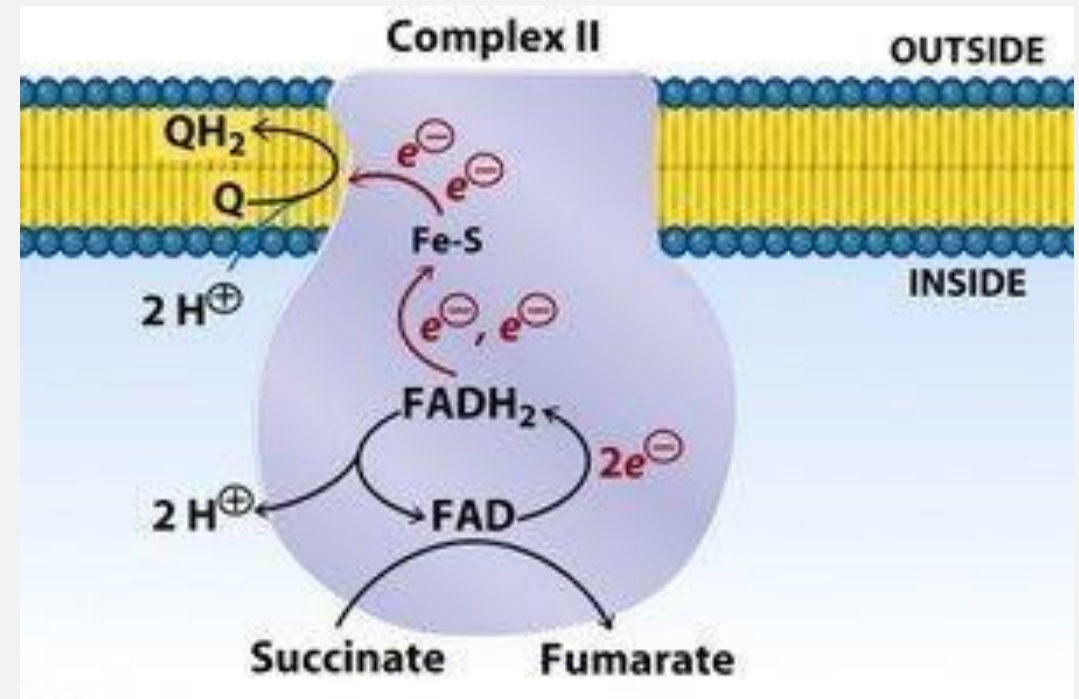
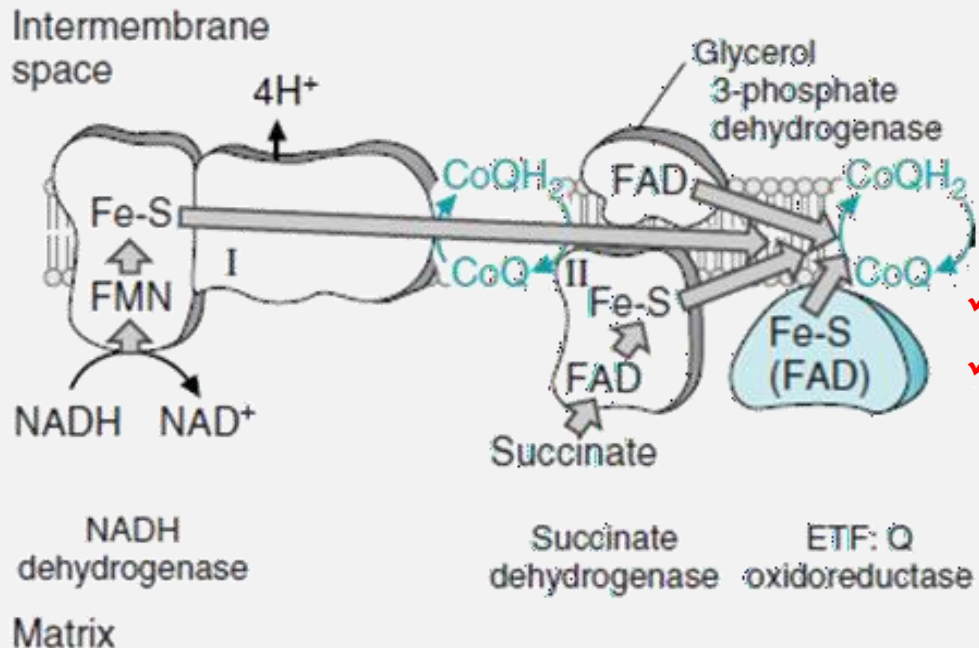
- NADH-Q oxidoreductase
- More than 25 polypeptide chain
- A huge flavoprotein membrane-spanning complex
- The FMN is tightly bound
- Seven Fe-S centers of at least two different types
- Fe-S centers, transfer of the hydrogen atoms to coenzyme Q
- Binds NADH & CoQ
- 4 H⁺



Redox Components of the ETC

Complex II (Succinate dehydrogenase)

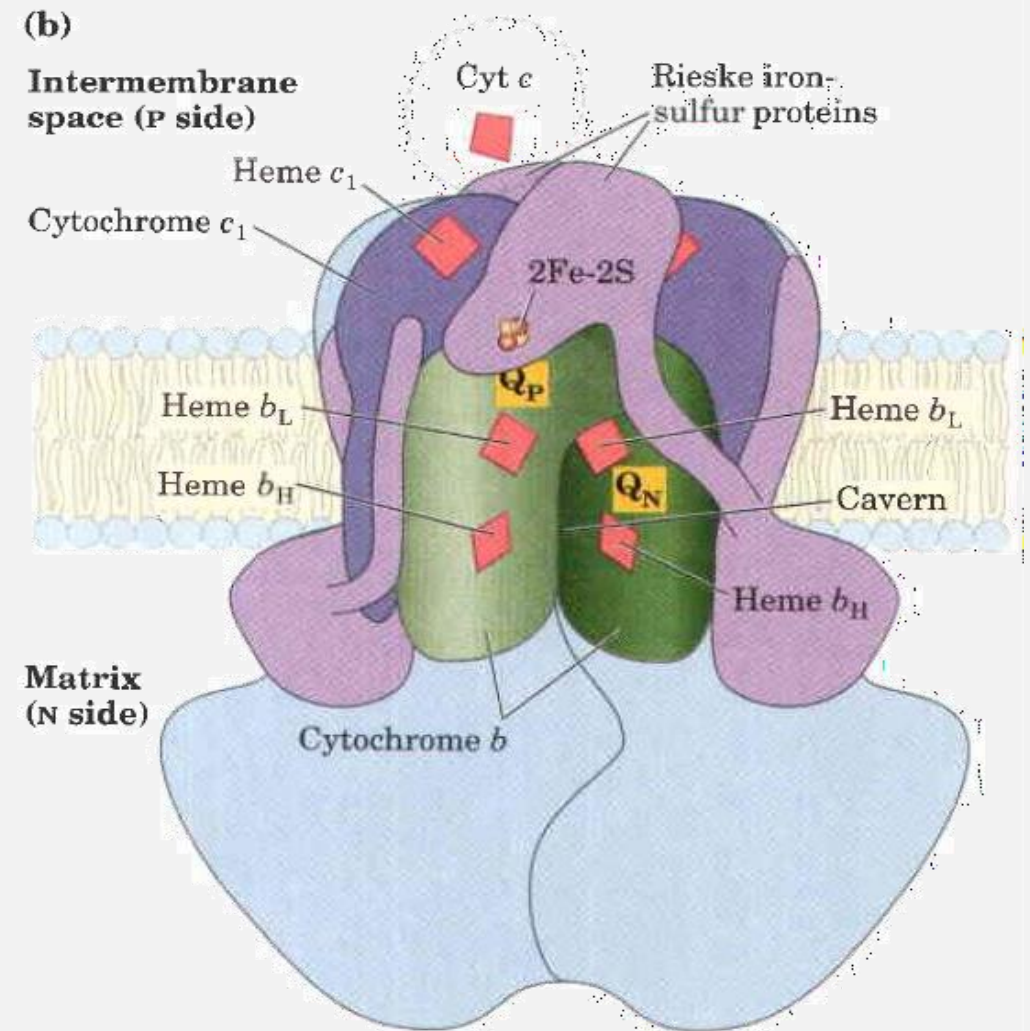
- Flavoprotein, iron sulfur centers
- TCA cycle
- ✓ Electron Transfer Flavoproteins, ETF-CoQ oxidoreductase (ex. fatty acid oxidation)
- ✓ ≈ 0 kcal, no proton transport



- ✓ Substrates oxidized by FAD-linked enzymes bypass complex-I
- ✓ Three major enzyme systems:
 - ✓ Succinate dehydrogenase
 - ✓ Fatty acylCoA dehydrogenase
 - ✓ Mitochondrial glycerol phosphate dehydrogenase

Redox Components of the ETC Complex III (Cytochrome bc₁)

- 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⁺



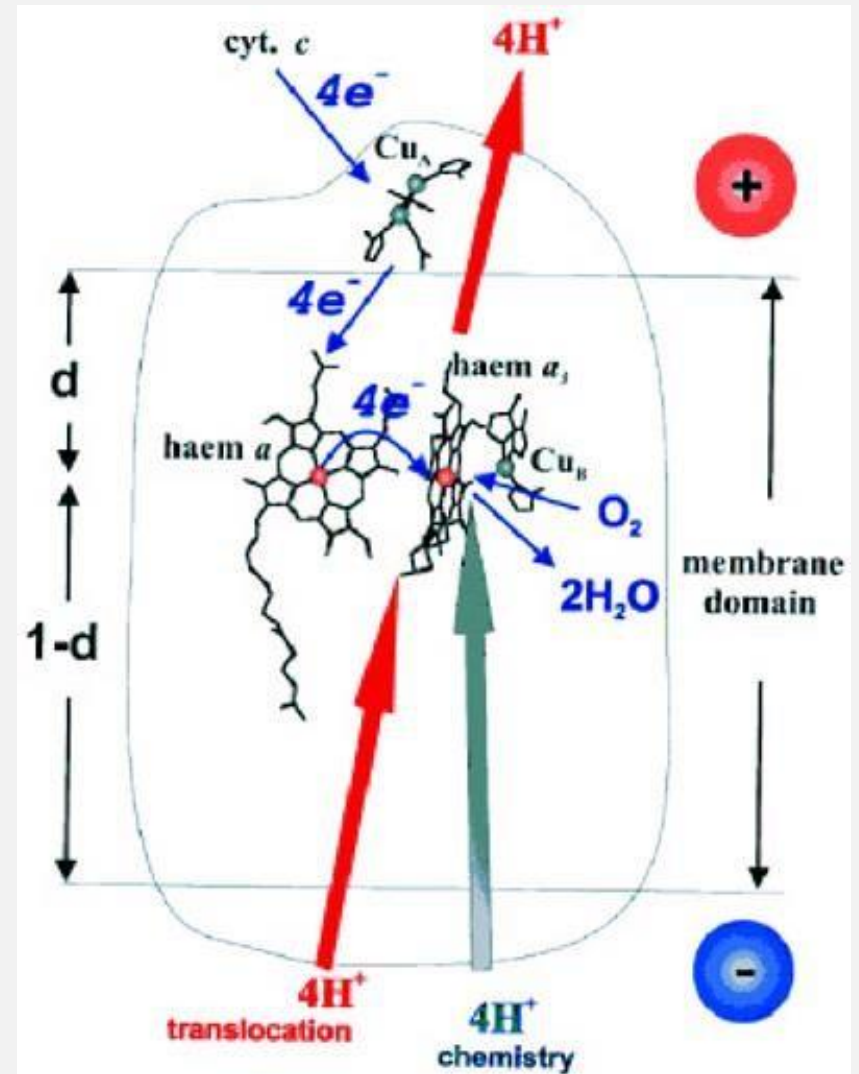
Redox Components of the ETC

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 reduced to 2 H₂O (2H⁺/2e⁻)

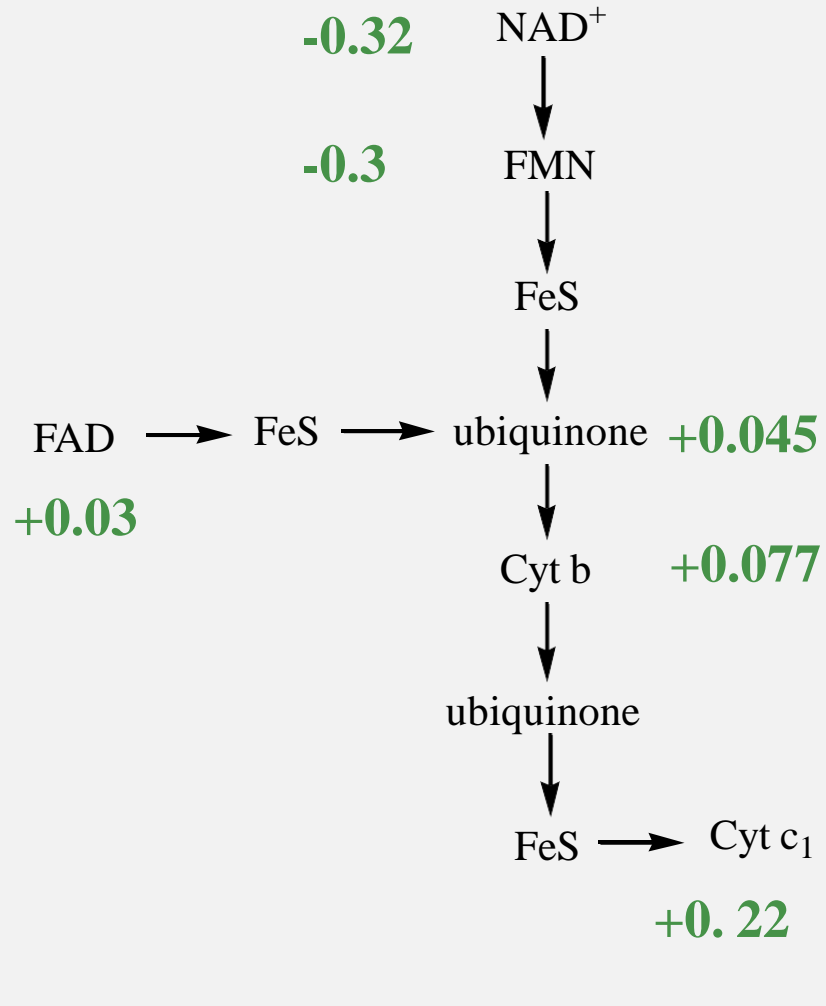


- Cytochrome oxidase has a much lower K_m for O₂ than myoglobin and hemoglobin
- ✓ Partial reduction of O₂ is hazardous



How can we prove the right arrangement of ET?

➤ 1. Measuring the standard reduction potentials



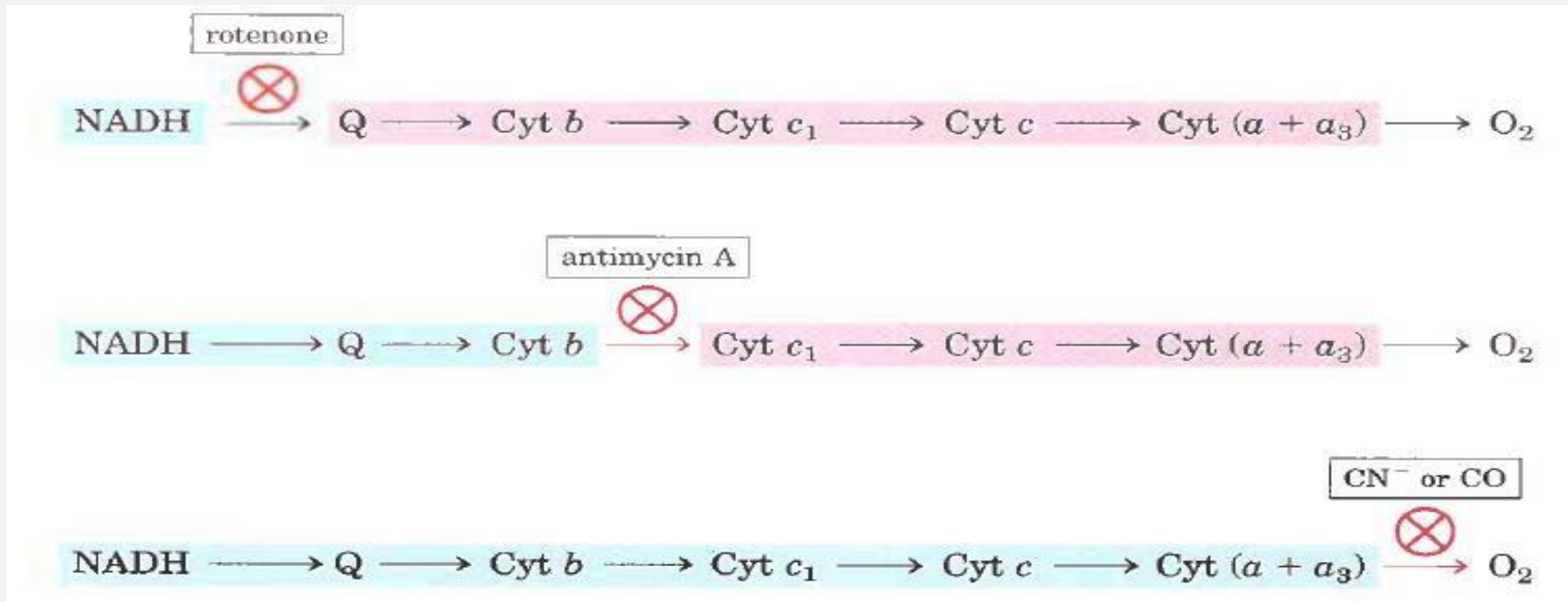
Redox reaction (half-reaction)	E'° (V)
$2H^+ + 2e^- \longrightarrow H_2$	-0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	-0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	-0.324
$NADH \text{ dehydrogenase (FMN)} + 2H^+ + 2e^- \longrightarrow NADH \text{ dehydrogenase (FMNH}_2\text{)}$	-0.30
$Ubiquinone + 2H^+ + 2e^- \longrightarrow ubiquinol$	0.045
$Cytochrome b (Fe^{3+}) + e^- \longrightarrow cytochrome b (Fe^{2+})$	0.077
$Cytochrome c_t (Fe^{3+}) + e^- \longrightarrow cytochrome c_t (Fe^{2+})$	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

NADH → Q → cytochrome b → cytochrome c_1 → cytochrome c → cytochrome a → cytochrome a_3 → O_2

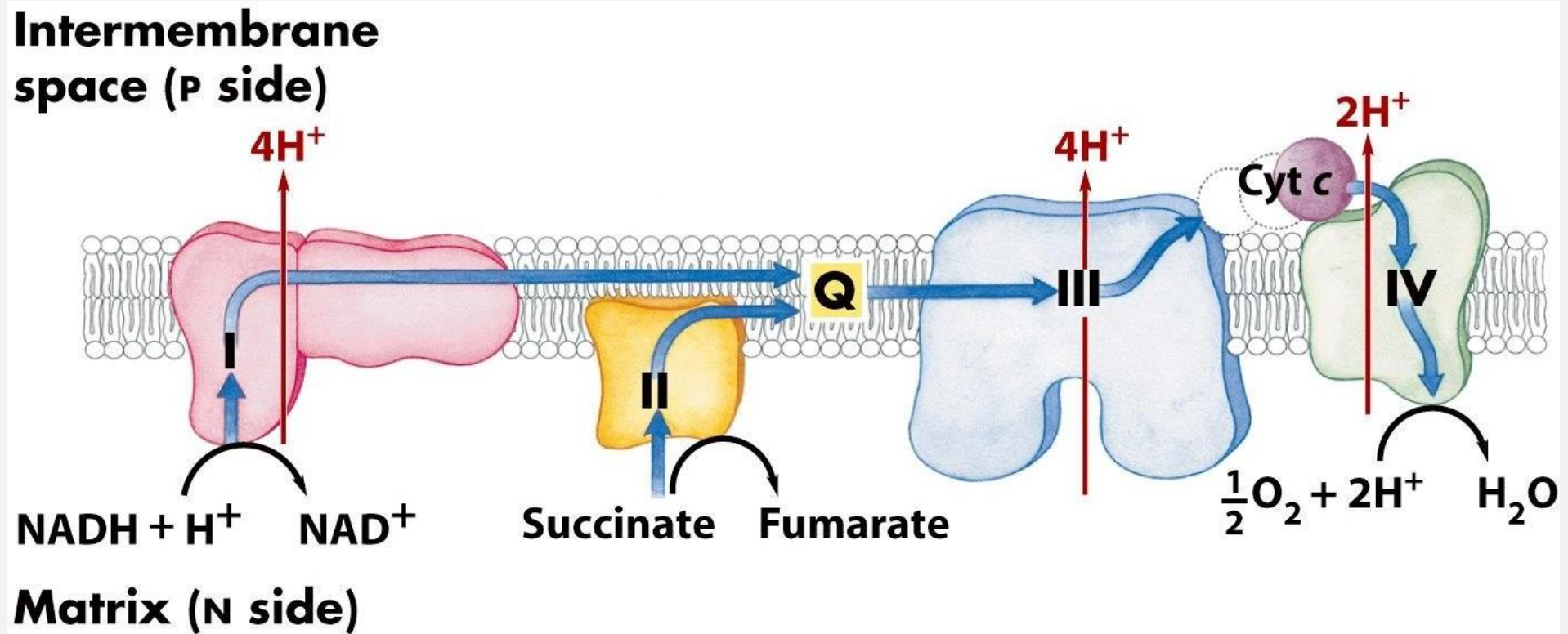
How can we prove the right arrangement of ET?

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

- 2. Reduction of the entire ETC with no O₂
- 3. Addition of inhibitors



Pumping of Protons

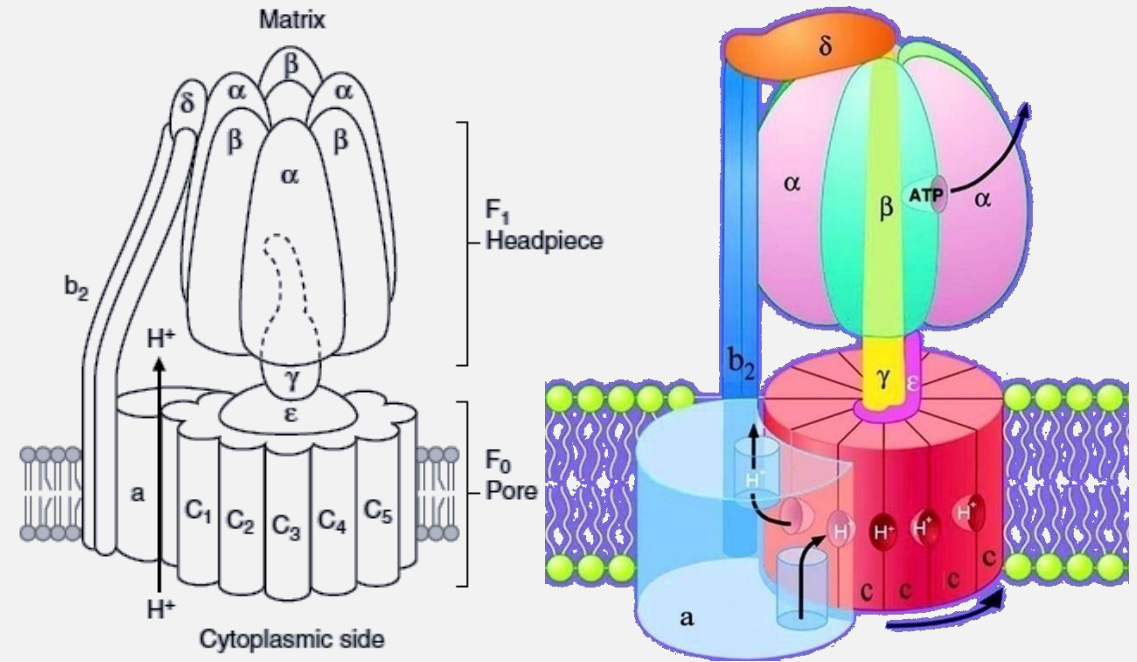


➤ For every 2 electrons passing:

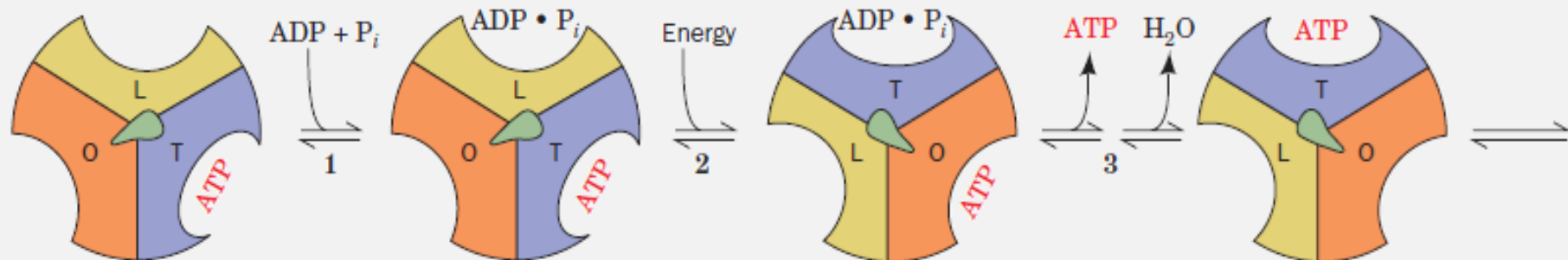
- 4H^+ (complex I); 0H^+ (complex II); 4H^+ (complex III), 2H^+ (complex IV)

ATP Synthase

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



Proton passage drives the rotation of F_o dissipating the pH and electrical gradients. F_o 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.



Energy yield of the ETC

- NADH, -53 kcal, ATP?
- FADH₂, -41 kcal, ATP?
- ΔG° for the phosphorylation of ADP to ATP is +7.3 kcal/mol
- ΔG° is so negative, never reversible
- Electron transport chain is our major source of heat



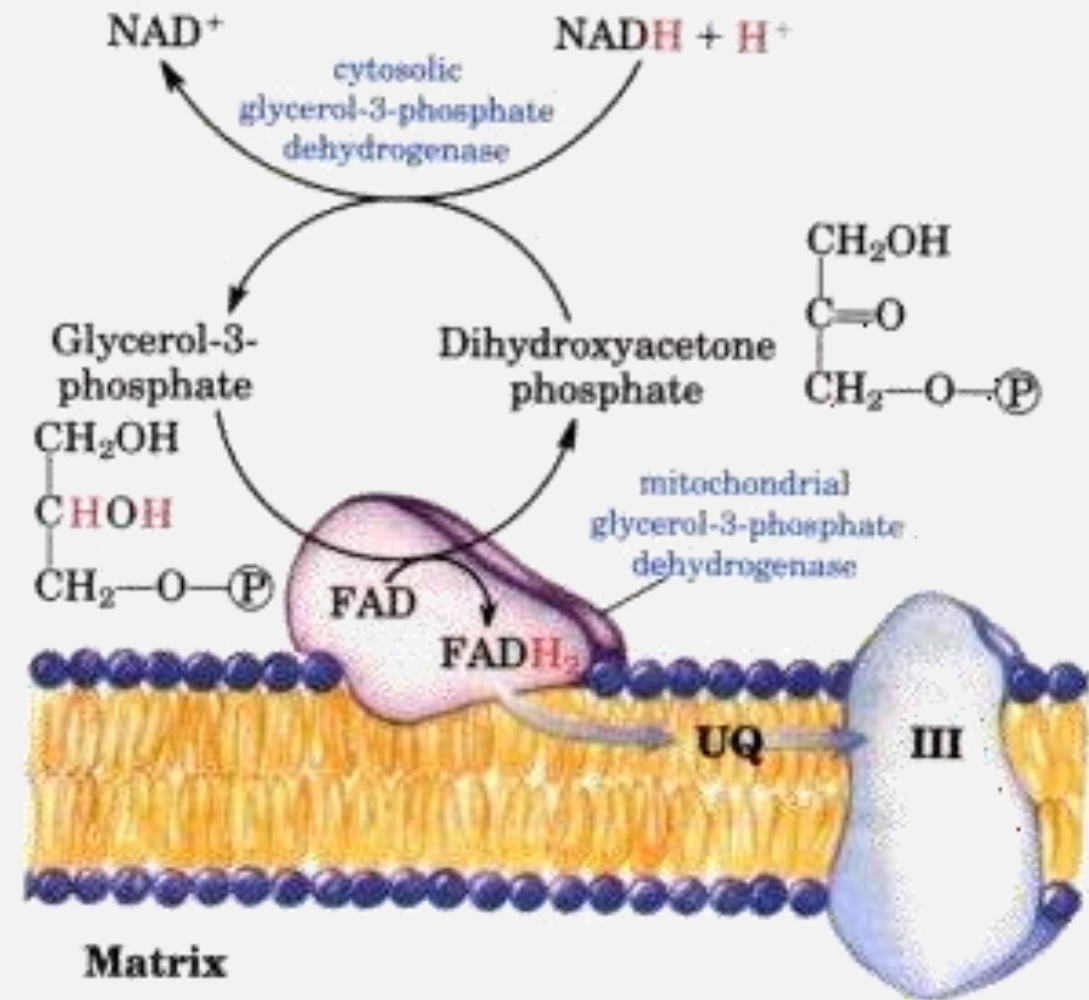
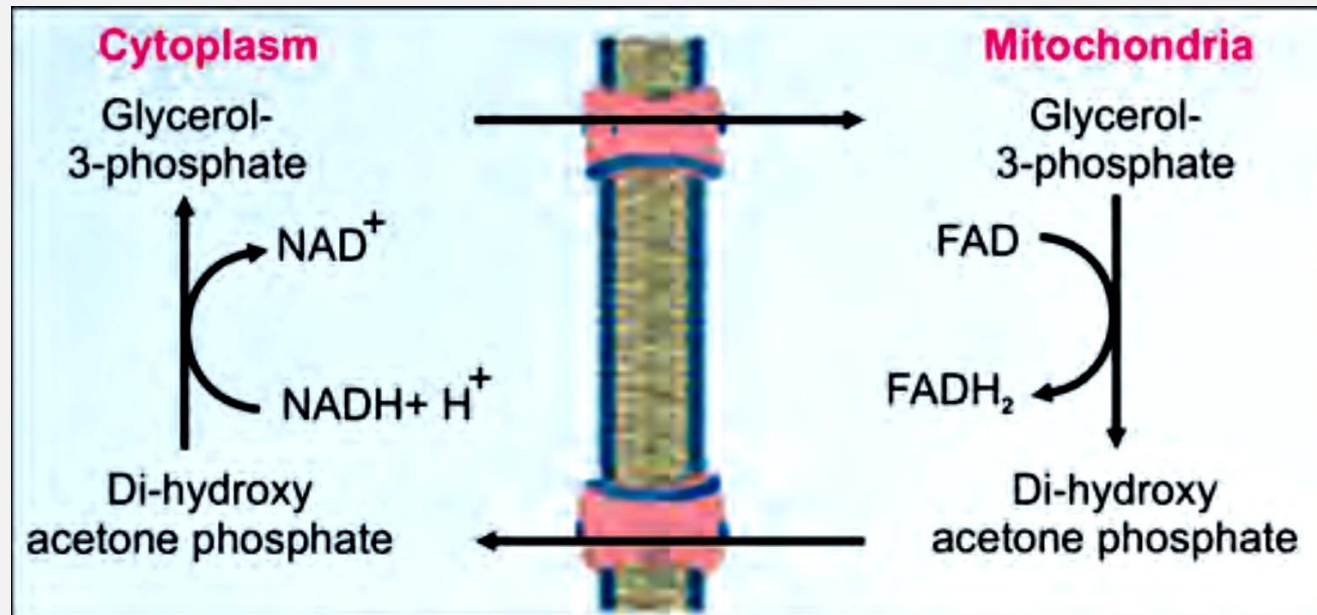
shutterstock.com · 481856635

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

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

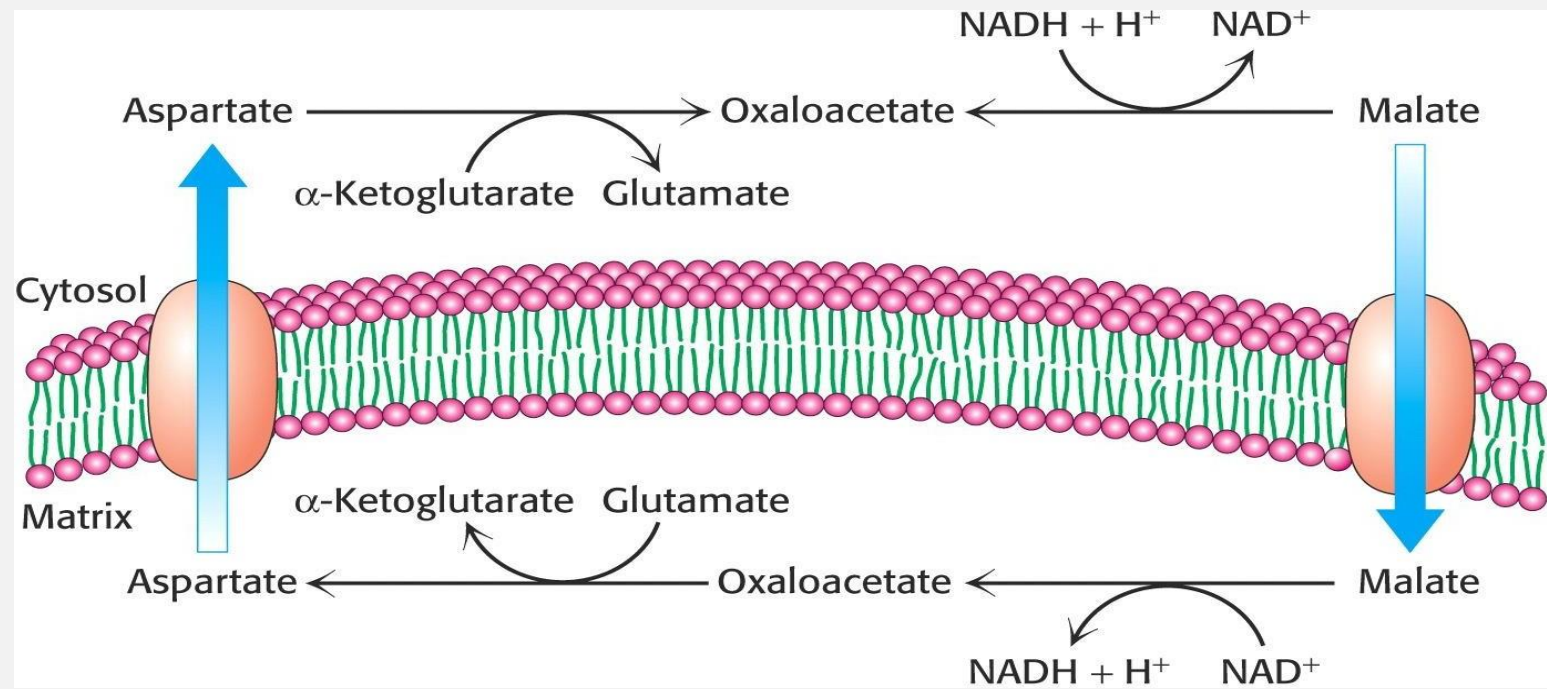
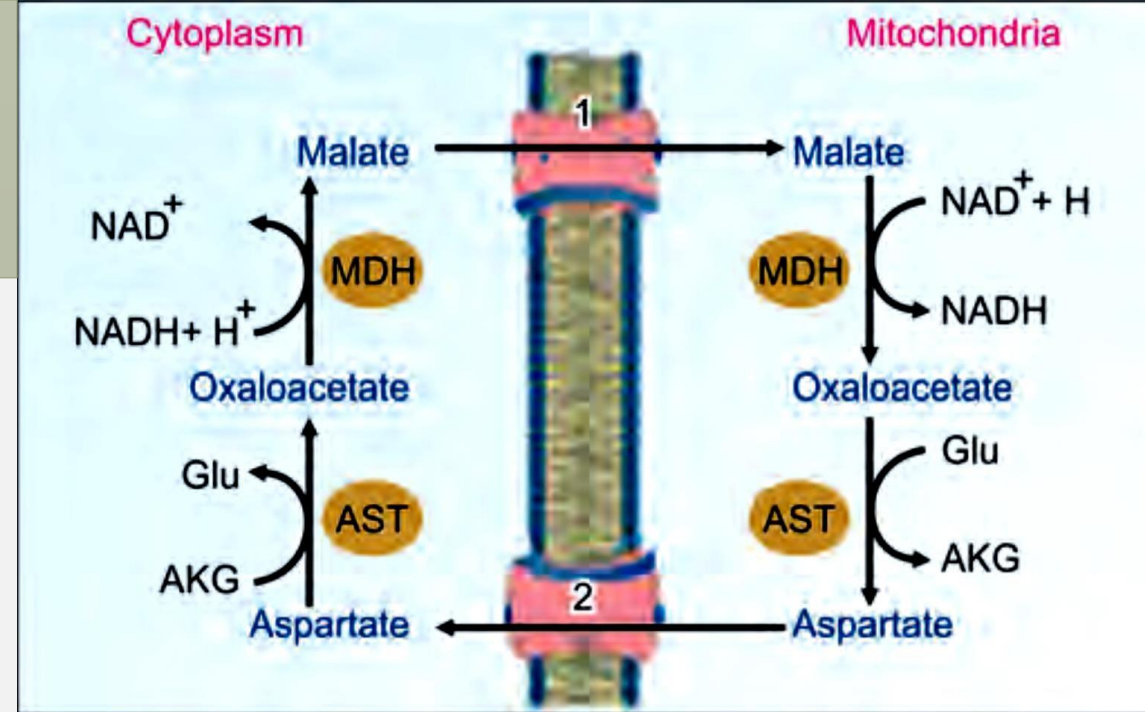
Mitochondrial Shuttling Systems for cytosolic NADH

- 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



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
- 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

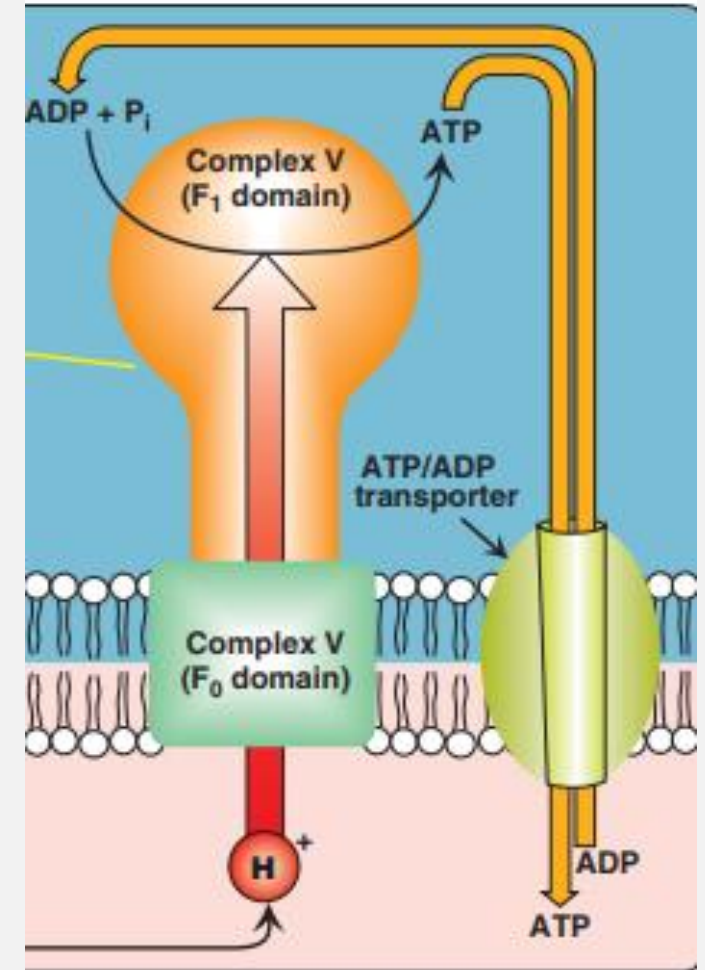
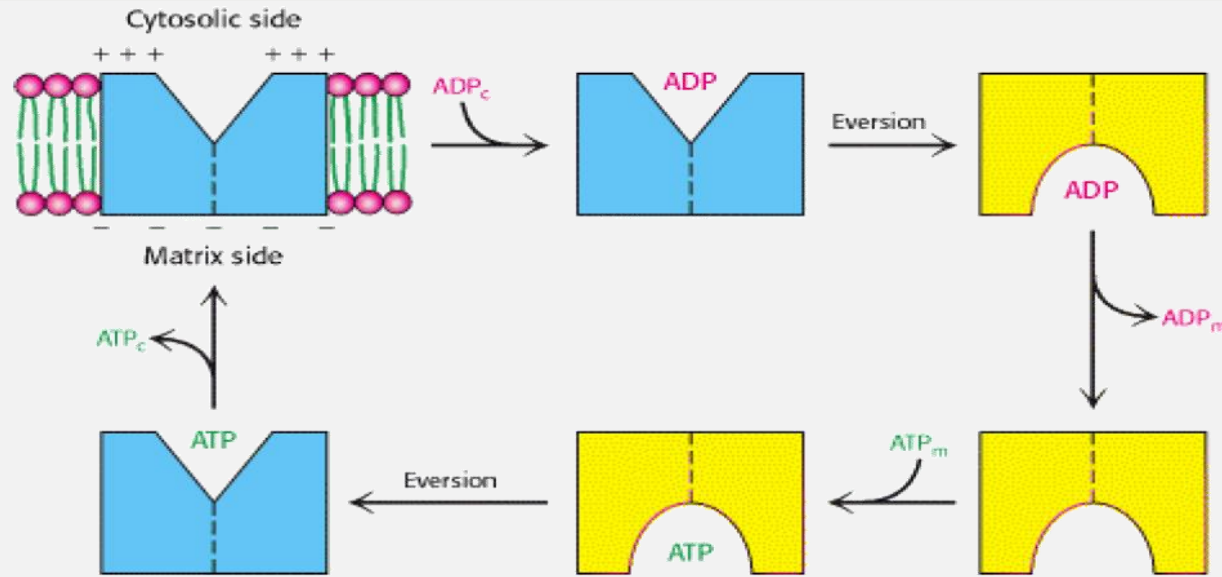


Examples on NADH producing enzymes

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



- 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 cytosol into mitochondria.
- Inhibition leads to subsequent inhibition of cellular respiration

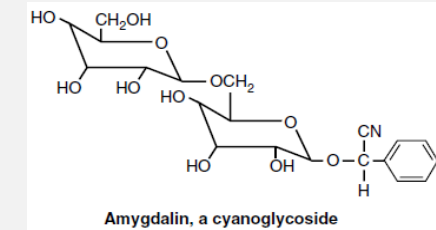
Regulation-The need for ATP

- What OxPhos needs? (NADH, O₂, ADP, and Pi)
- ET is tightly coupled to phosphorylation (simultaneously)
- ADP is the most important factor in determining the rate
- The regulation of the rate of oxidative phosphorylation by the ADP level is called respiratory control or acceptor control



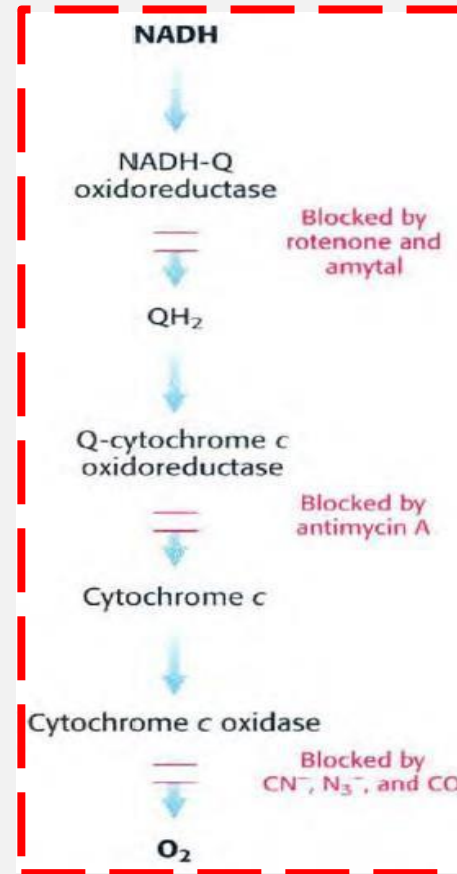
The rate of oxygen consumption by mitochondria increases markedly when ADP is added and then returns to its initial value when the added ADP has been converted into ATP

Regulation-inhibition (coupling)



Anit-cancerous drug

- Can occur at any stage
- Specific inhibitors:
 - ✓ Cyanoglycosides such as amygdalin (misnomer B17) are present in edible plant pits
 - ✓ Oligomycin prevents the influx of H⁺ through ATP synthase (tight coupling)



الصفحة الرئيسية < مصلحات

أشهر جرائم القتل العائلية في المملكة

جراسا نيوز -

جراسا-نعرض فيما يلي قائمة بأشهر جرائم القتل العائلية التي حدثت في الاردن خلال السنوات الماضية ، والتي كان لكل منها وقع الصدمة حين وقوعها لما تمثله من فعل غريب على المجتمع وأعرافه ، فضلا عن مخالفتها الشرائع السماوية والقوانين النافذة والطبيعة الإنسانية بعامة.

قضية السيائيد

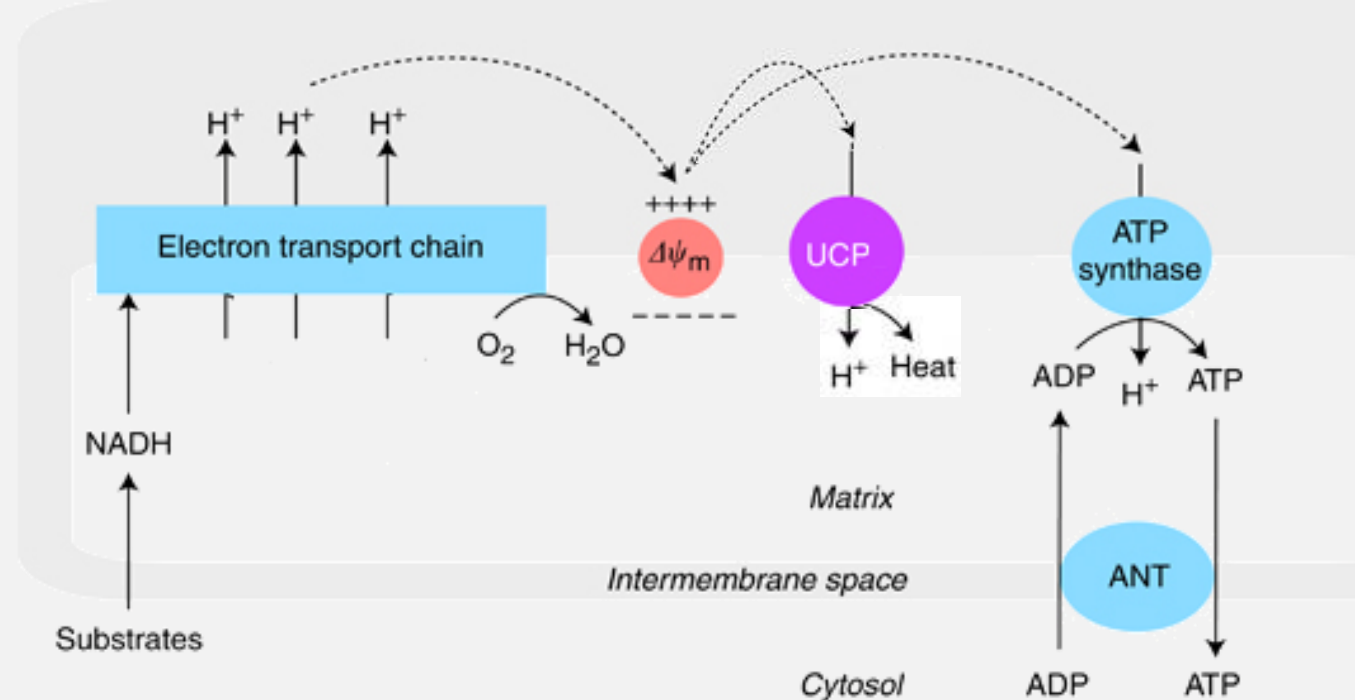
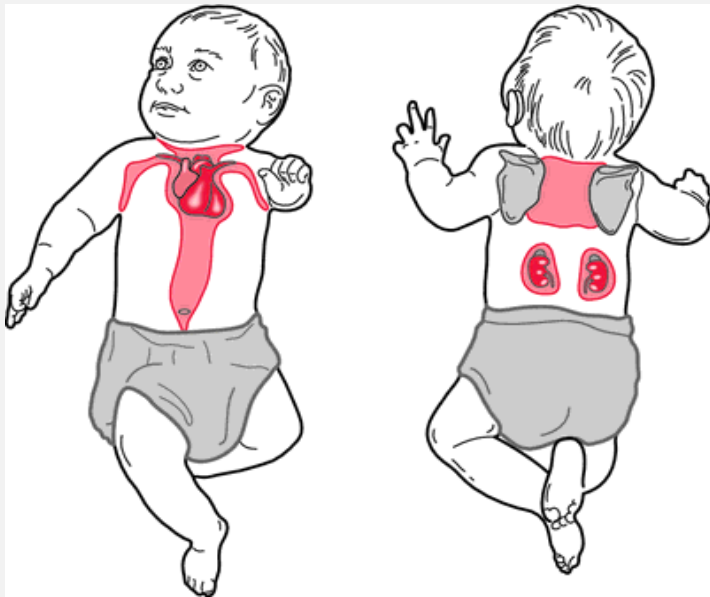
أول جريمة من نوعها يرتكبها أب ضد ولديه ، إذ قام الاب بوضع مادة السيائيد في كأس الحليب وطلب من طفليه ان يشربا منه ، حيث فارقا الحياة بعد 10 دقائق من مغادرة الام المنزل لتعود وتجدهما جثتين هامدتين.

وقد ادین الاب بعقوبة الاعدام شنقا الا ان والده اسقط الحق الشخصي كونه وليا عن الطفلين وحكم عليه بالاشغال المؤبدة.

Specific inhibitor	Target
Rotenone (insecticide) & Amytal (sedative)	NADH-Q oxidoreductase
Antimycin A (antibiotic)	Q-cytochrome c oxidoreductase
Cyanide (CN ⁻), Azide (N ₃ ⁻), & (CO)	Cytochrome c oxidase
Oligomycin (antibiotic)	ATP synthase

Regulation-Regulated Uncoupling Proteins (UCPs)

- Short-circuiting ATP synthase
- UCP₁ (thermogenin):
 - ✓ Brown adipose tissue, non-shivering thermogenesis
 - ✓ Infants: neck, breast, around kidneys
 - ✓ Fatty acids directly activates UCP₁
- UCP₂ (most cells); UCP₃ (skeletal muscle); {UCP₄, UCP₅} (brain)
- Obesity tendency in some populations



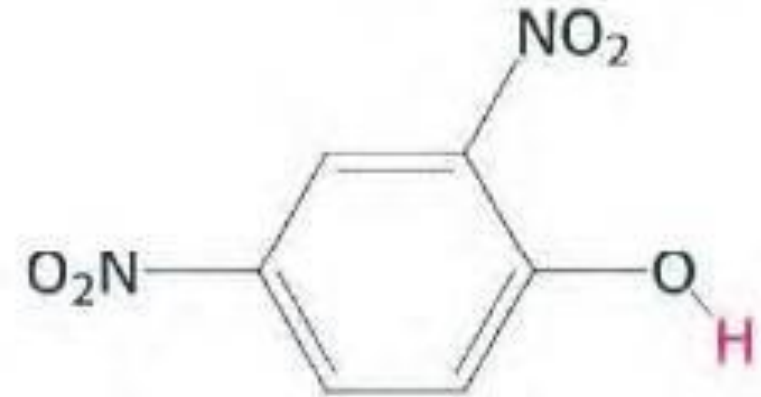
UCP mutations and cardiometabolic diseases risk



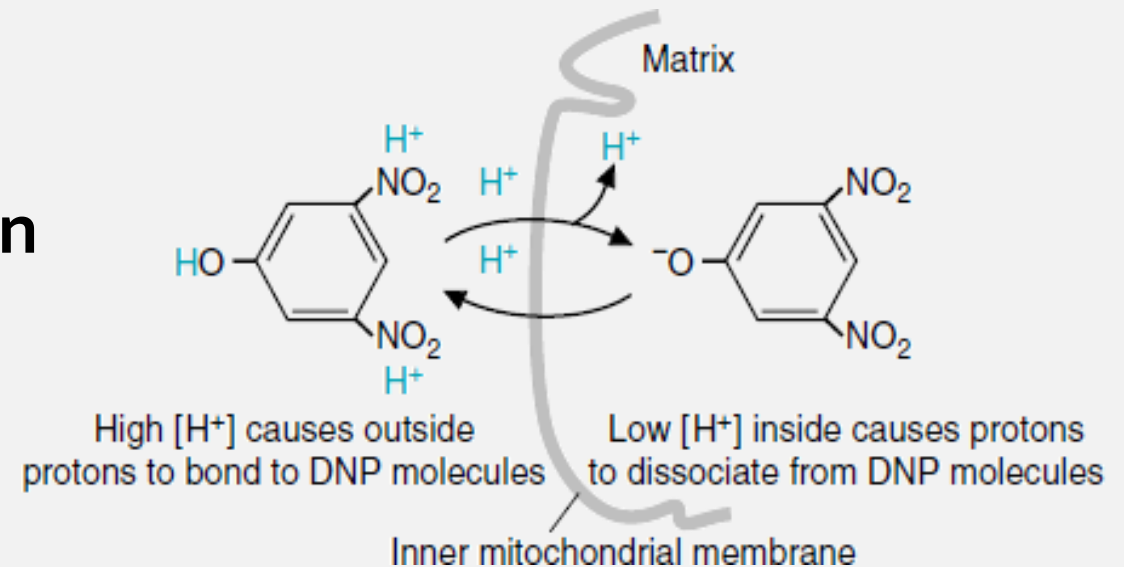
World map showing the investigation of the association of *Ucp1* polymorphisms with CMDs or CMD risk factors

Regulation-Unregulated Chemical Uncouplers (nonphysiological)

- 2,4-dinitrophenol (DNP) & other acidic aromatic compounds
- DNP disrupts the tight coupling of electron transport and phosphorylation in mitochondria
- It carries protons across the inner mitochondrial membrane.
- How does it occur? Dissipation of PMF
- Results in increased oxygen consumption and oxidation of NADH but no ATP production
- FDA banned DNP in 1938



2,4-Dinitrophenol (DNP)



OxPhos Diseases (Genetic)

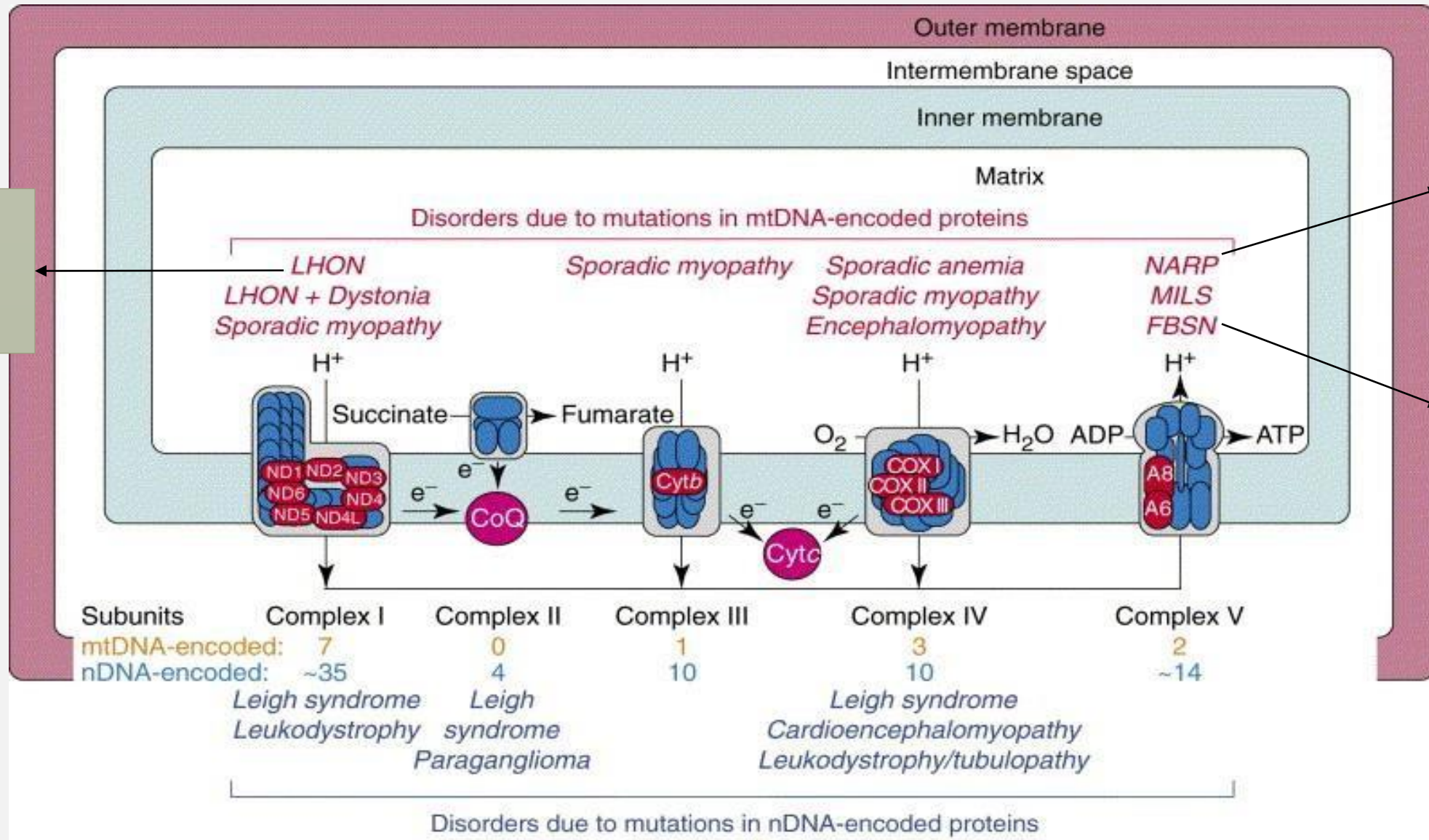
➤ A. Mitochondrial DNA and OXPHOS Diseases

- ✓ Small (16,569) base pair, double-stranded, circular DNA
- ✓ Encodes 13 subunits: 7 (I) , 1 (III), 3 (IV), 2 (Fo)
- ✓ Also encodes necessary components for translation of its own mRNA: a large and small rRNA and tRNAs
- ✓ mtDNA has a mutation rate ~10 times more than nuclear DNA
- ✓ Maternal inheritance, replicative segregation & heteroplasmy
- ✓ Accumulation of somatic mutations with age
- ✓ Highest ATP demands: CNS, heart, skeletal muscle, and kidney, liver are affected more

➤ B. Nuclear Genetic Disorders of Oxidative Phosphorylation

- ✓ 1,000 proteins
- ✓ Usually autosomal recessive
- ✓ Expressed in all tissues
- ✓ Phenotypic expression with high ATP demand

OxPhos Diseases (Genetic)



Leber's hereditary optic neuropathy

Neuropathy, ataxia, and retinitis pigmentosa

Familial infantile bilateral striatal necrosis