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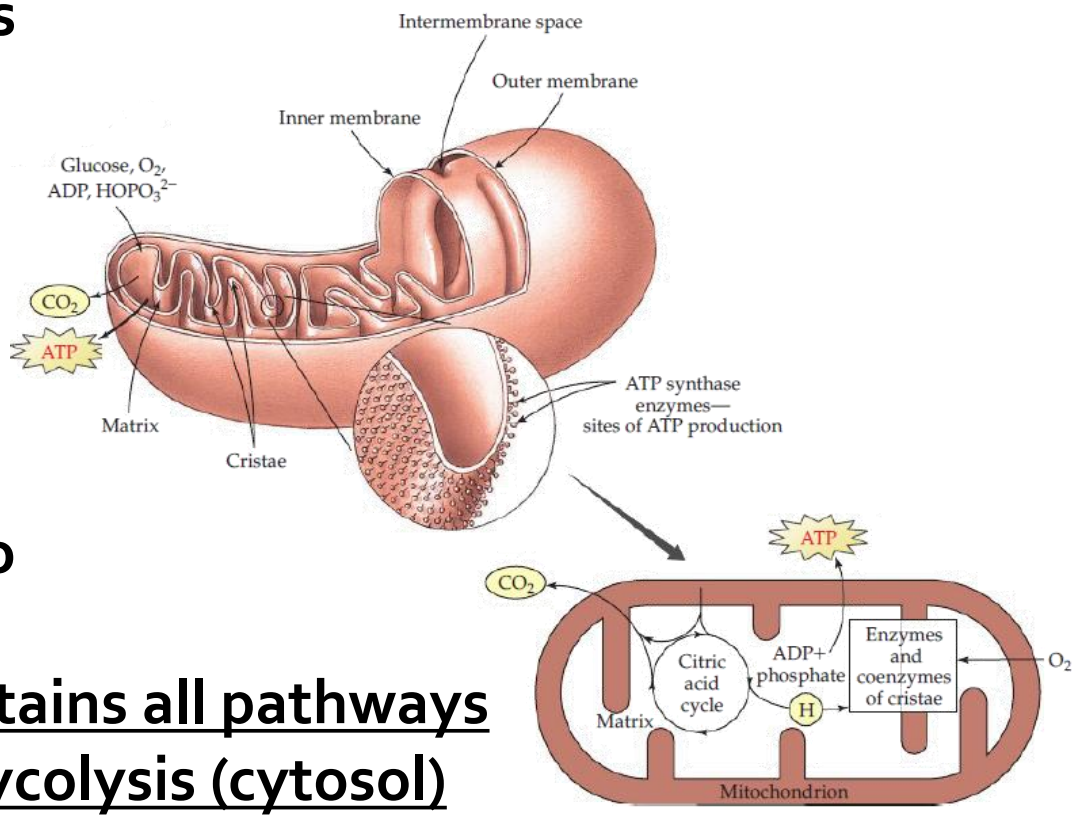
Oxidative Phosphorylation

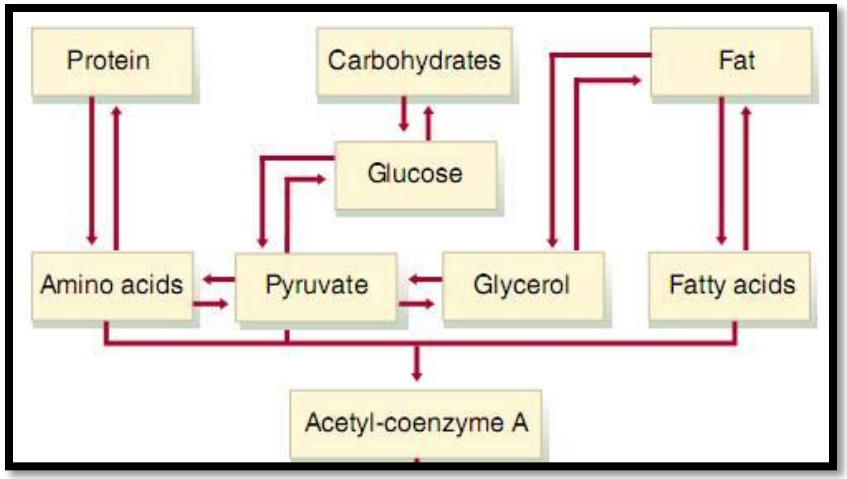
The Mitochondria

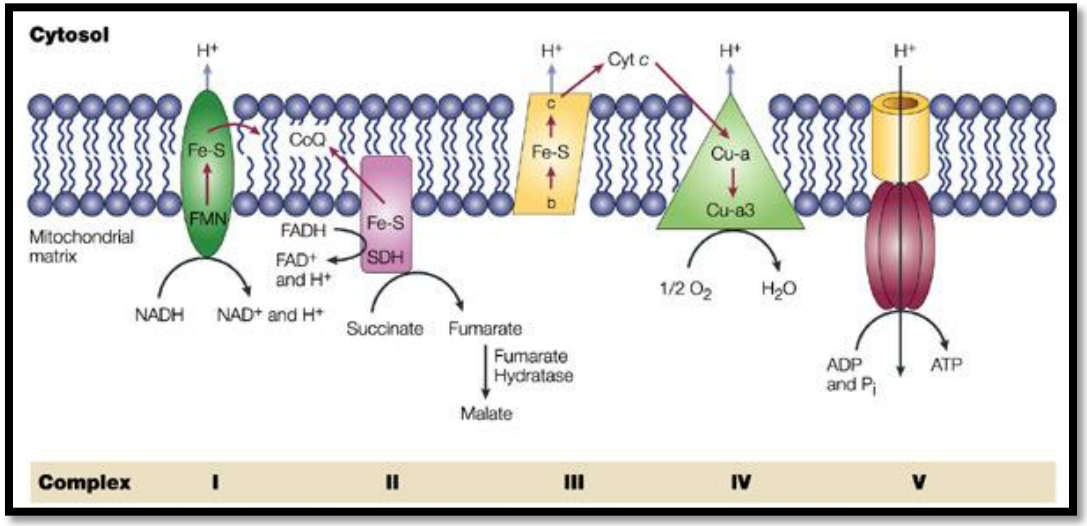
- OMM: <u>permeable</u> to small molecules (MW<5,000) & ions, <u>porins</u> (transmembrane channels)
- IMM: <u>impermeable</u> even to H+; specific transporters
- IMM bears the components of the respiratory chain and the ATP synthase
- Matrix: contains pyruvate dehydrogenase complex & TCA cycle enzymes,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

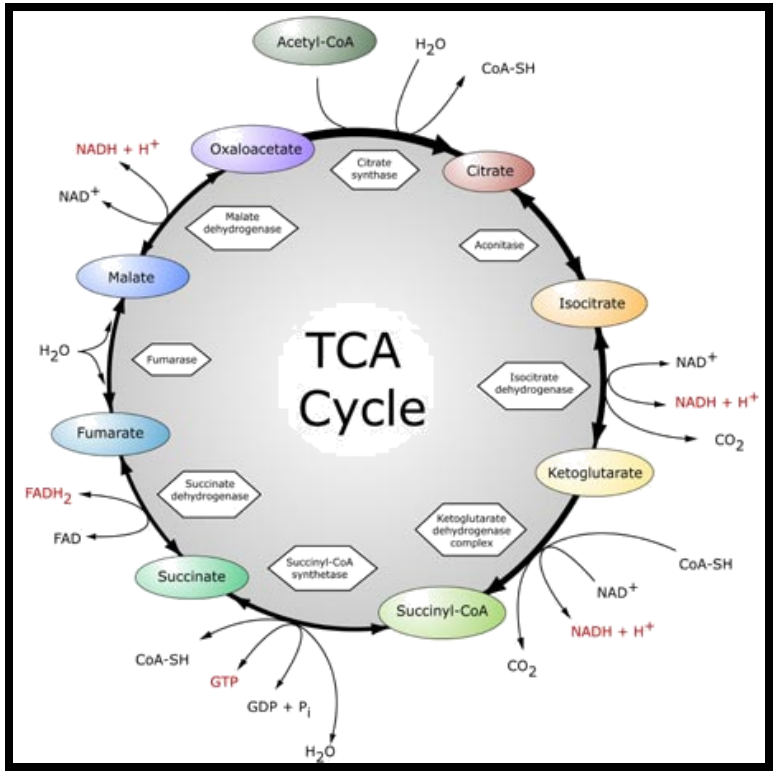


The oxidative phosphorylation, Where are we?

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

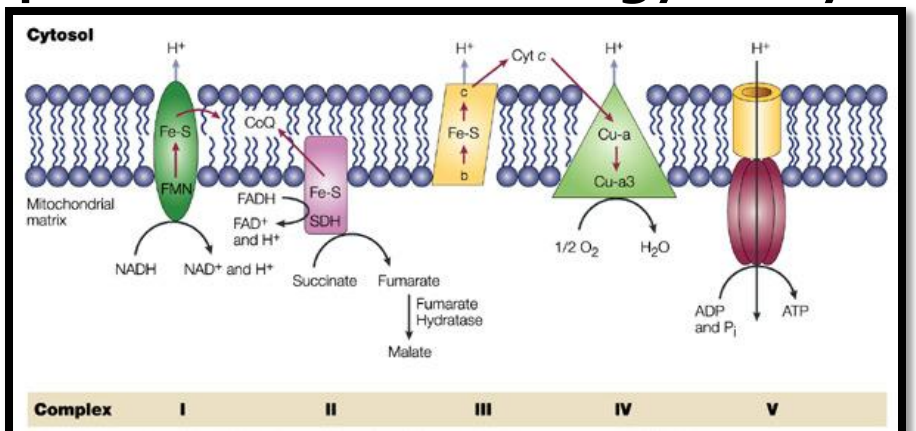




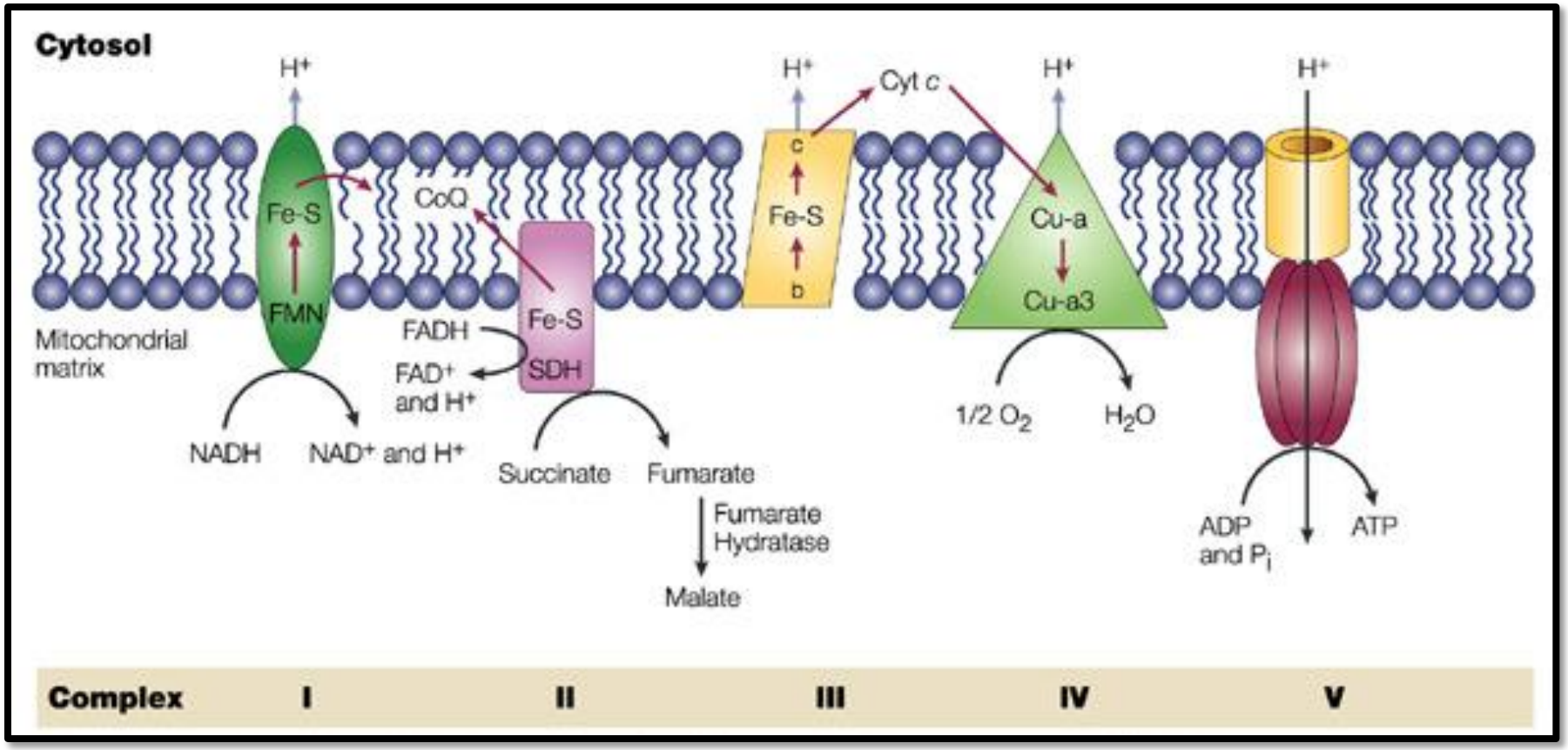


Oxidative phosphorylation (OxPhos)

- Generation of ATP aided by the reduction of O_2
- Peter Mitchell (1961): the chemiosmotic theory
- Oxidative phosphorylation have 3 major aspects:
  - (1) It involves 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)

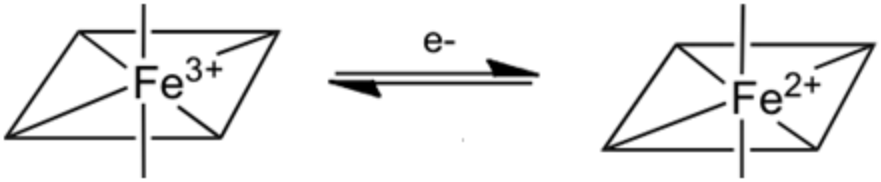


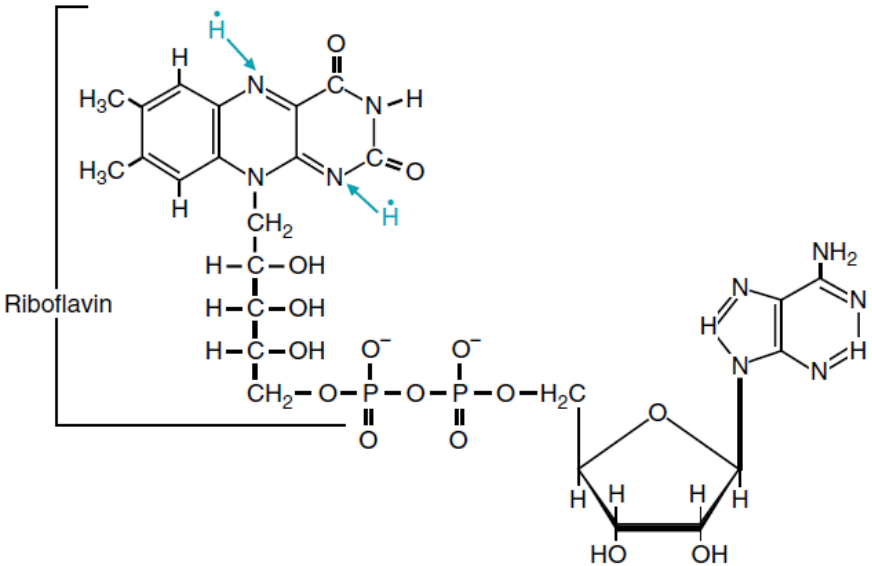
Oxidative phosphorylation (OxPhos)

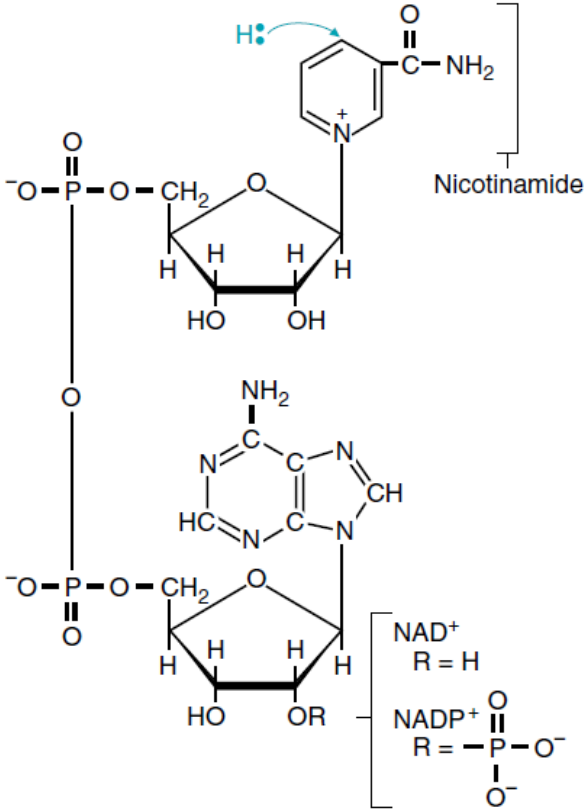


Types of electron transfer (ET) through the electron transport chain (ETC)

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







Electrons are funneled to a universal electron acceptors

- Dehydrogenases (DHs); nicotineamide adenines (NAD+ or NADP+) or flavins (FMN or FAD)

COENZYME	AS OXIDIZING AGENT	AS REDUCING AGENT
Nicotinamide adenine dinucleotide	NAD^+	$NADH/H^+$
Nicotinamide adenine dinucleotide phosphate	$NADP^+$	$NADPH/H^+$
Flavin adenine dinucleotide	FAD	$FADH_2$
Flavin mononucleotide	FMN	$FMNH_2$

- NAD-linked DHs: 2 hydrogen atoms (:H-; H+)

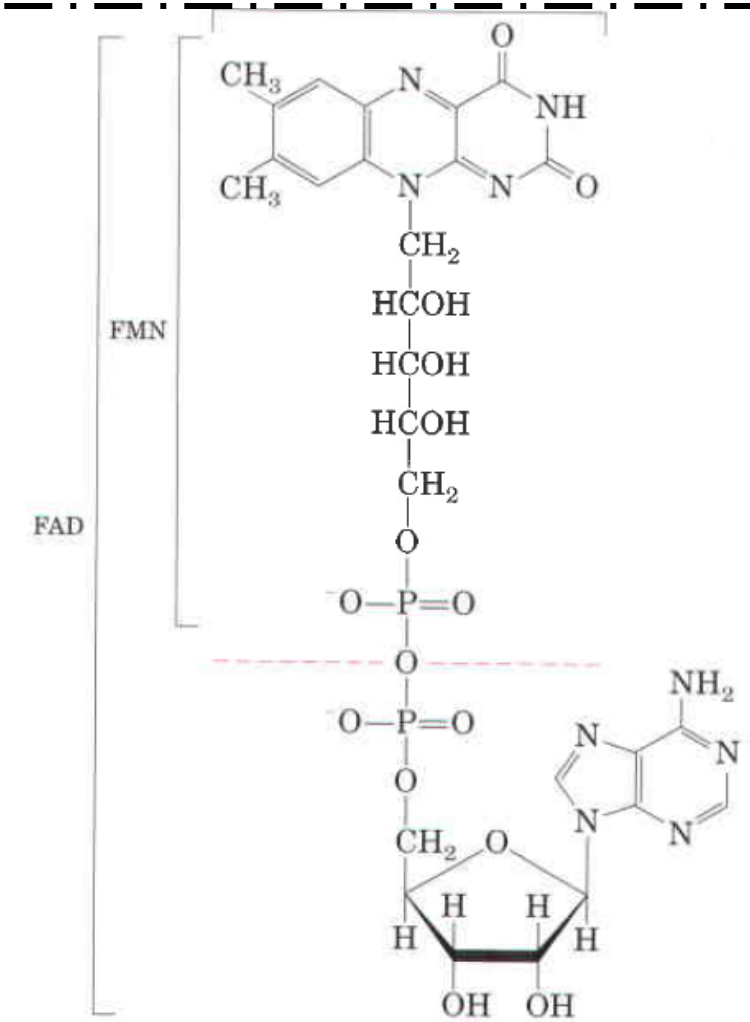
$$\text{Reduced substrate} + NAD^+ \rightleftharpoons \text{oxidized substrate} + NADH + H^+$$

$$\text{Reduced substrate} + NADP^+ \rightleftharpoons \text{oxidized substrate} + NADPH + H^+$$

- NADH and NADPH are water-soluble
- NADH: carries electrons to NADH dehydrogenase
- NADPH generally supplies electrons to anabolic reactions
- Neither can cross the IMM

Electrons are funneled to a universal electron acceptors

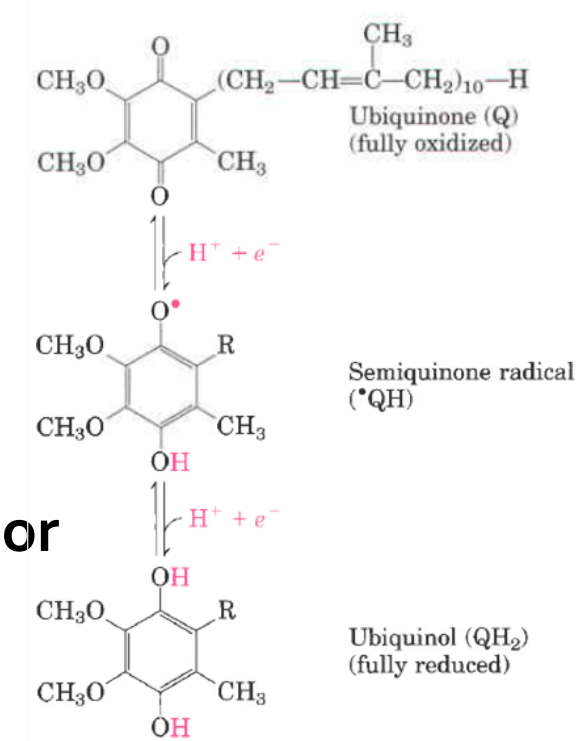
- Flavoproteins contain a very tightly, sometimes covalently, bound flavin nucleotide (FMN, FAD)
- Can accept either 1 or 2 electrons
- *ΔE°′* of a flavin nucleotide {vs. NAD(P)}, depends on the protein with which it is associated

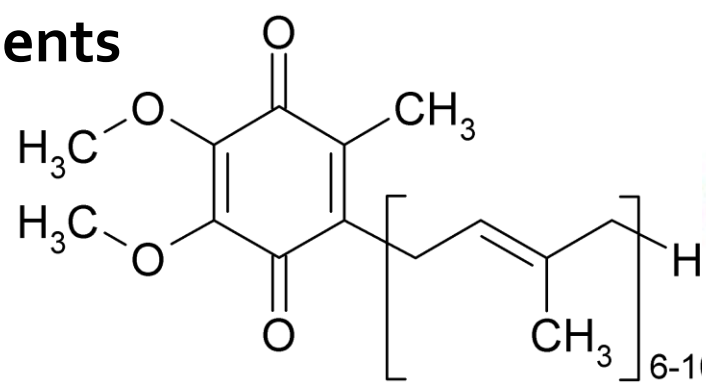


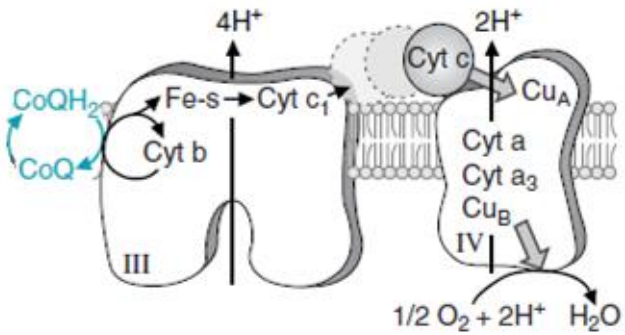
Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

Other electron-carrying molecules "Ubiquinone"

- Also called coenzyme Q, or Q
- 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

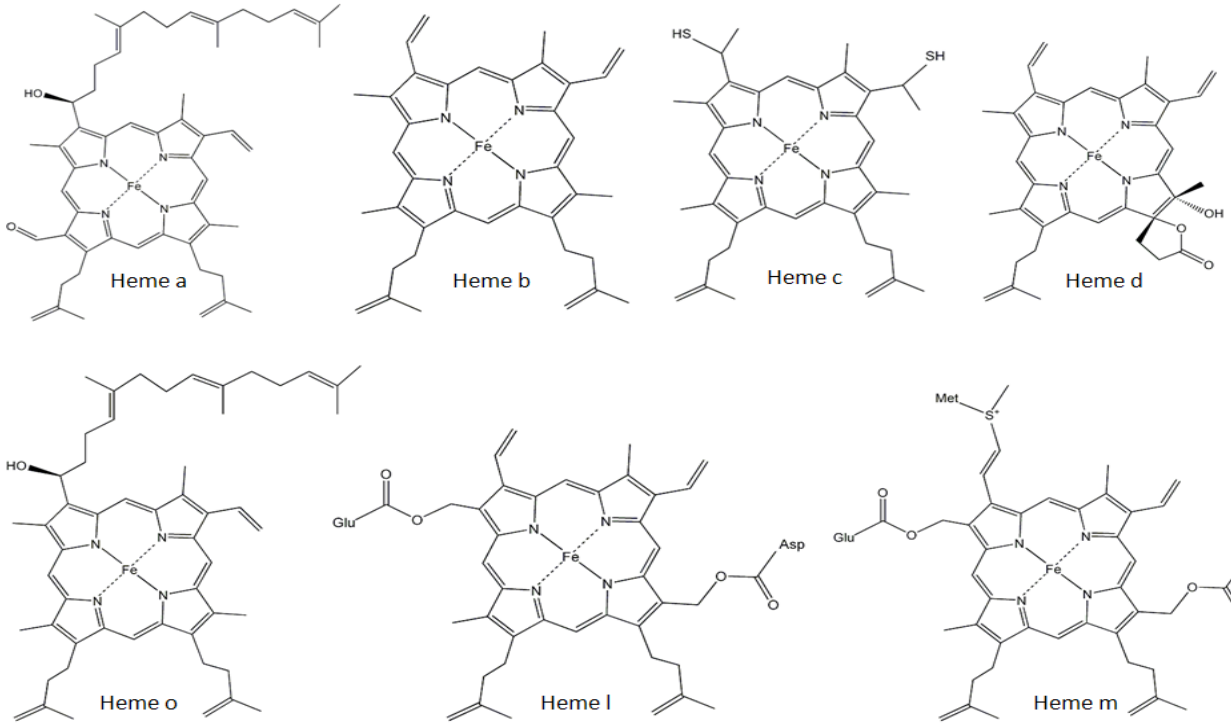


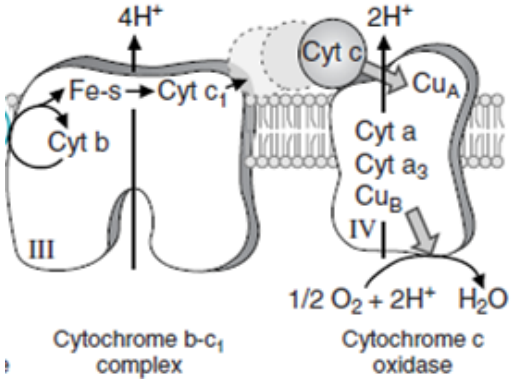




Other electron-carrying molecules "Cytochromes"

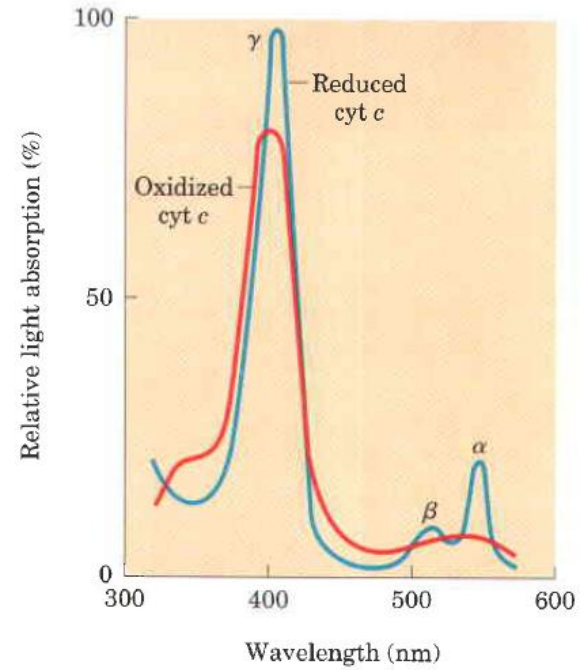
- Proteins with characteristic strong absorption of visible light (Fe-containing heme prosthetic groups)
- Classification based on light absorption
- Mode of binding (a, b, c)
- Mitochondria contain three classes o f cytochromes (a , b, & c)

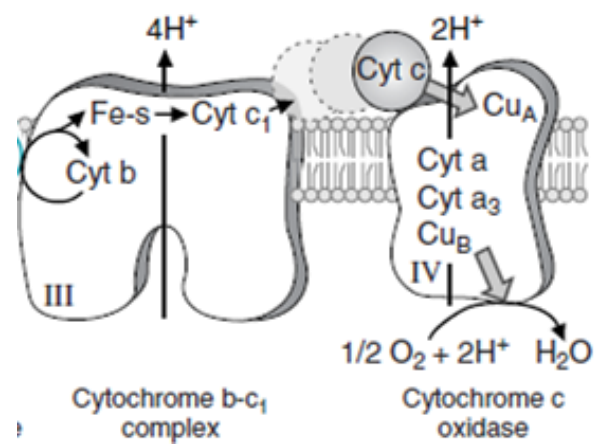




Other electron-carrying molecules "Cytochromes"

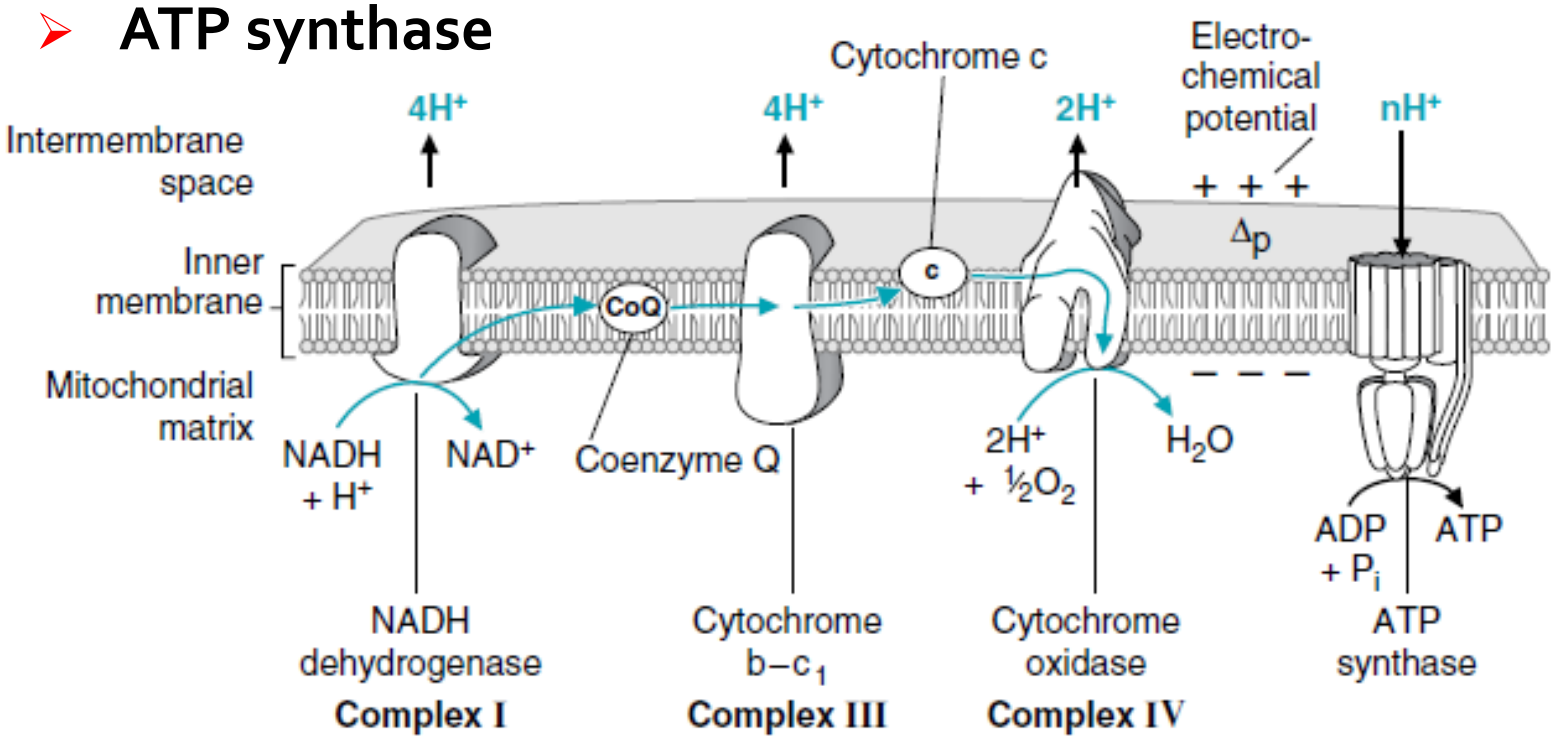
- Light absorption: Each cytochrome in its reduced (F^{+2}) state has 3 absorption bands in the visible range
- α band : near 600 nm in type a; near 560 nm in type b, & near 550 nm in type c
- Some cytochromes are named by the exact α band wavelength:
  - ✓ Cytochrome b_{562}; Cytochrome c_{550}; Cytochrome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



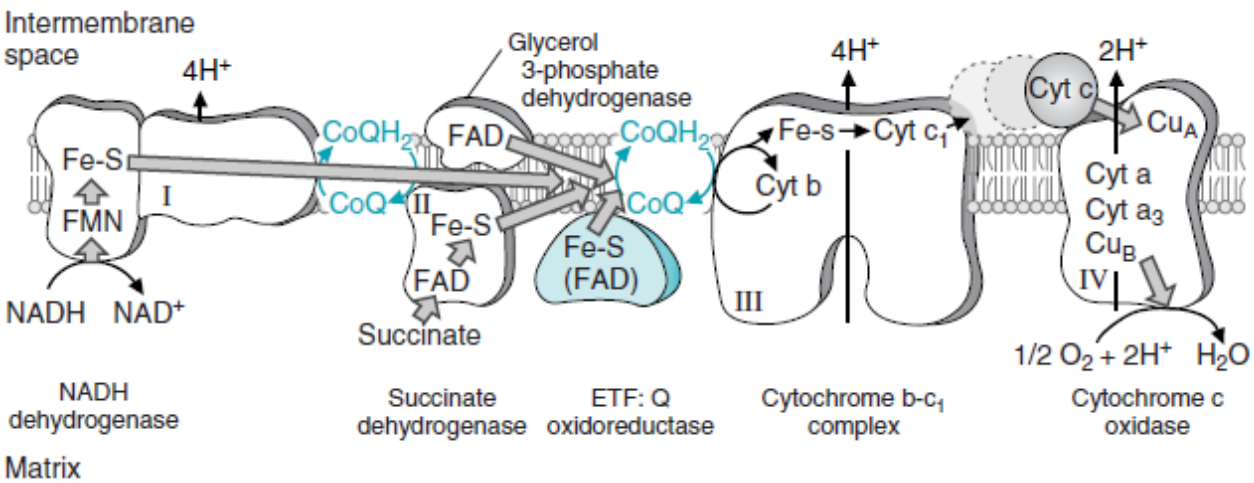


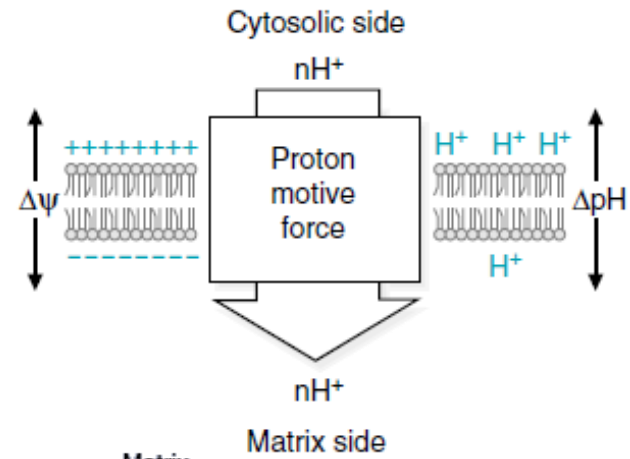
Requirements of OxPhos

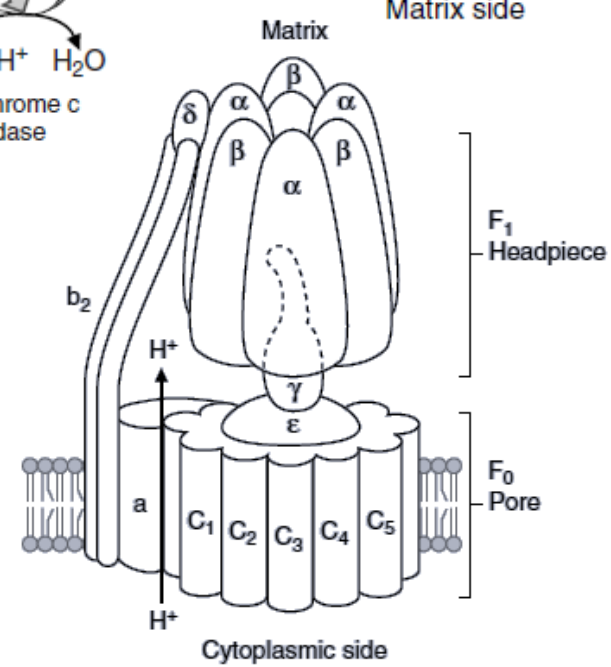
- Redox reaction: electron donor (NADH or FADH2) & electron acceptor (O2)
- An intact IMM
- ETC of proteins
- ATP synthase



ET to O2, how does the process occurs? "The chemi-osmotic theory"



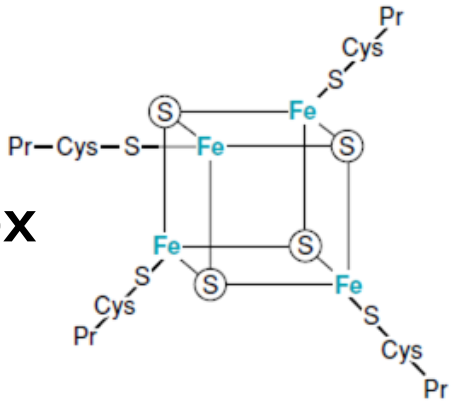


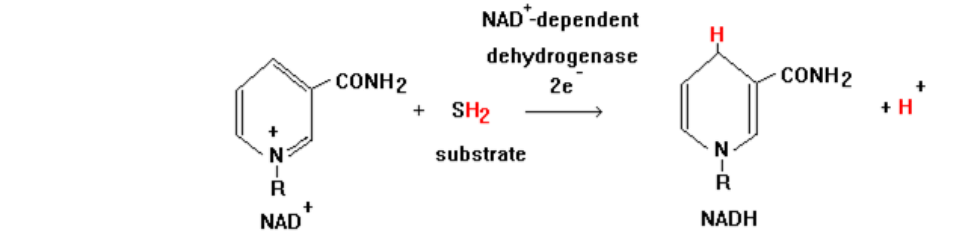


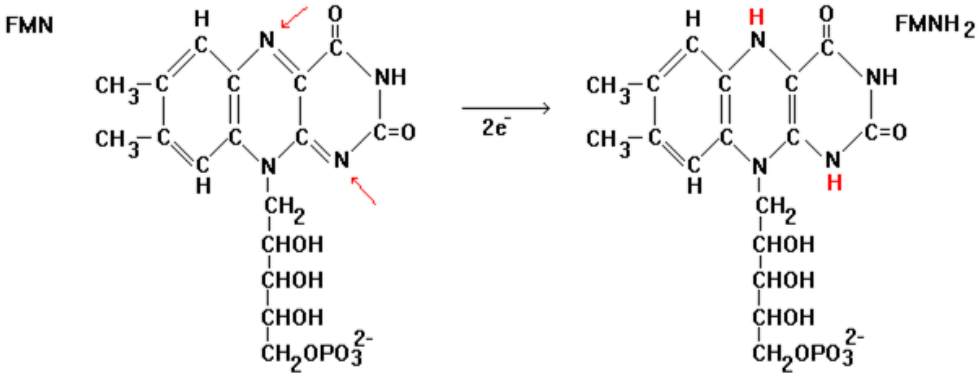
- Membrane is impermeable to protons
- ≈ - 16 kcal with each step

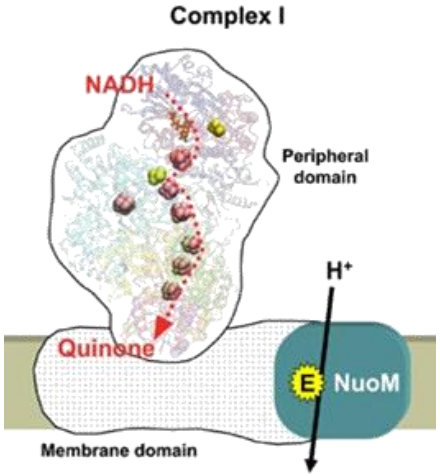
Oxi–Red Components of the ETC
"NADH Dehydrogenase" – Complex I

- 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
- Drop in energy ≈ 13 to 14 kcal
- Binds NADH & CoQ
- 4 H^+

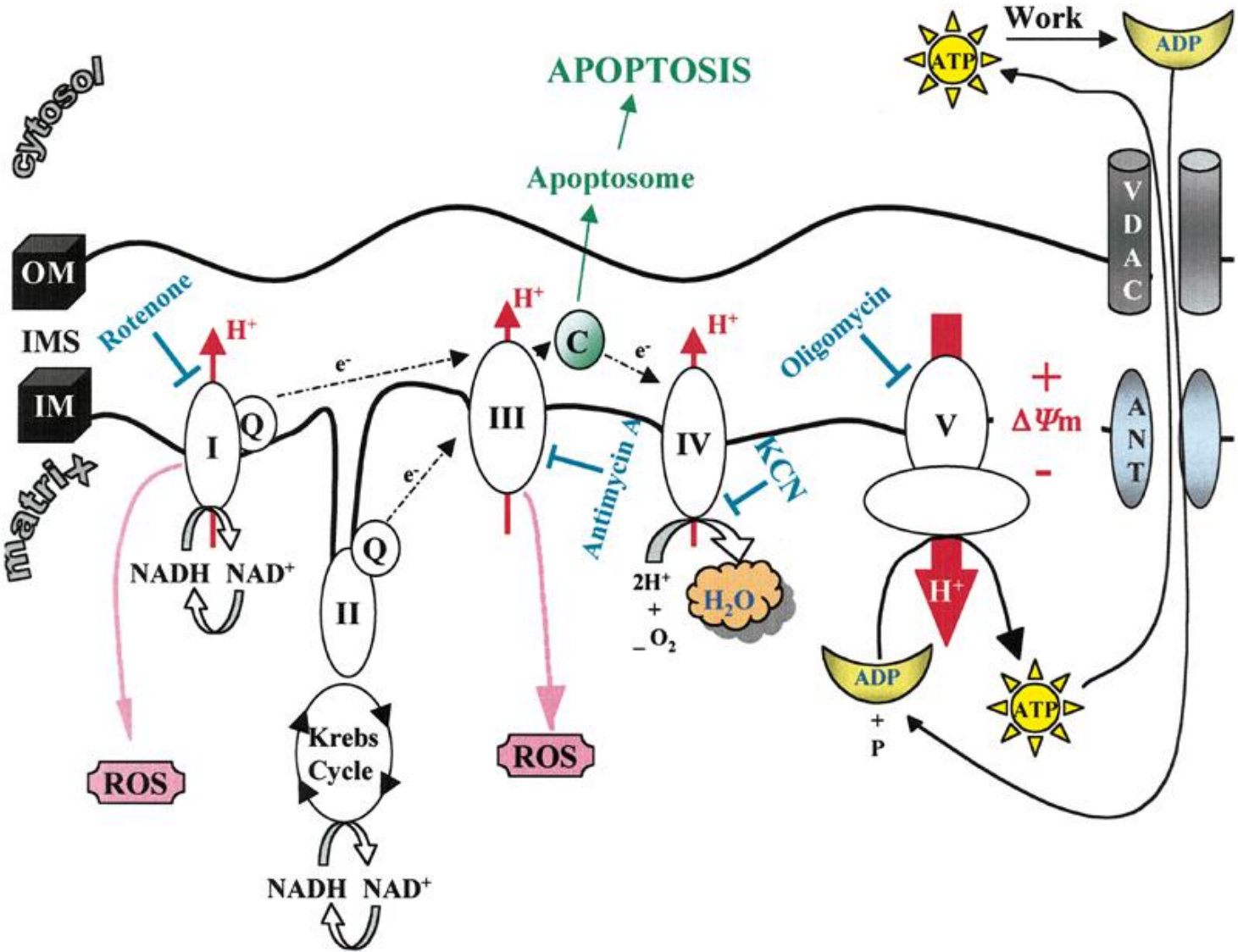






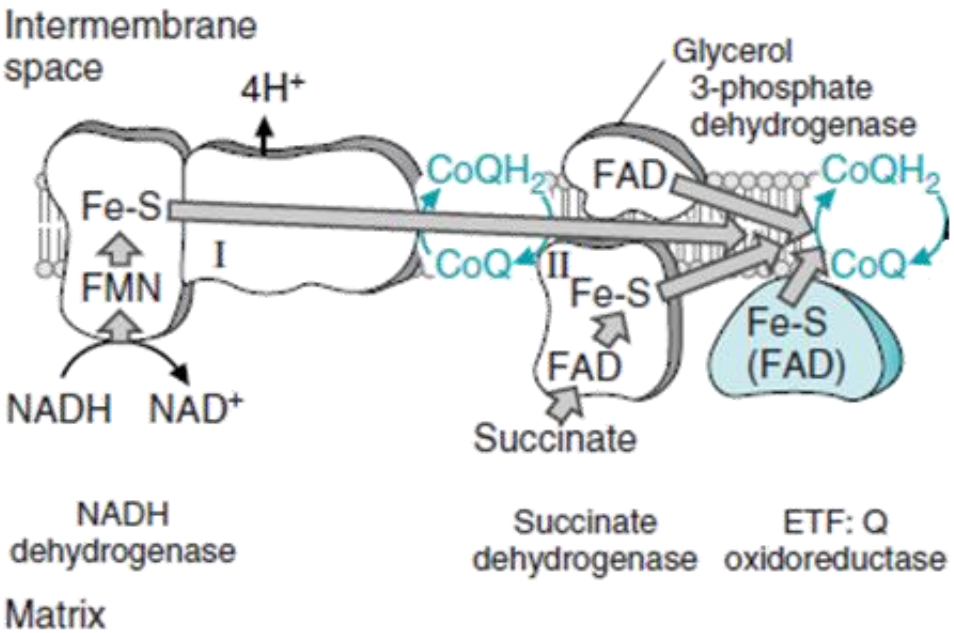


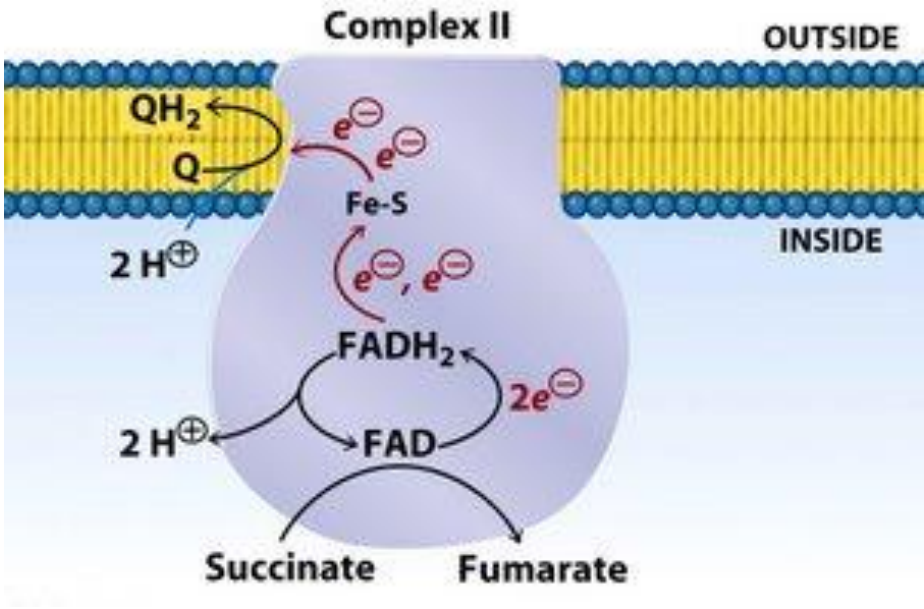
Oxi–Red Components of the ETC
"Succinate Dehydrogenase" – Complex II



Oxi–Red Components of the ETC
"Succinate Dehydrogenase" – Complex II

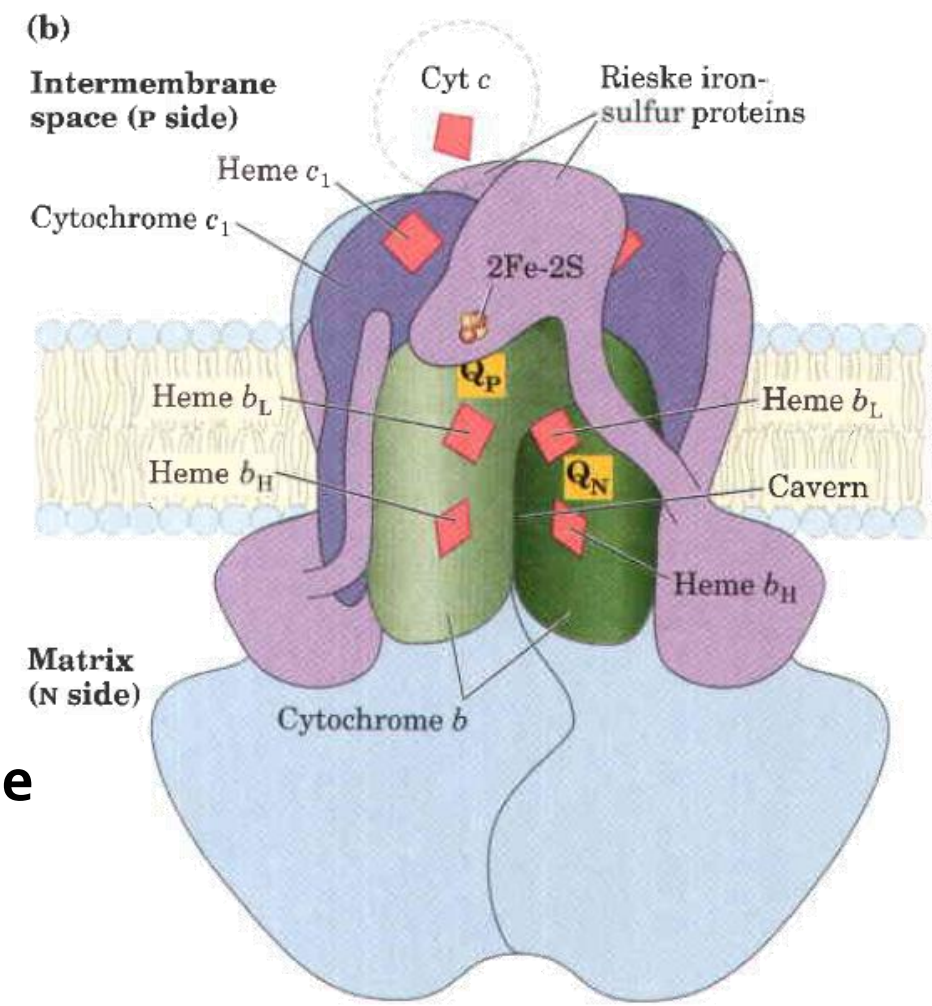
- Succinate Dehydrogenase & other flavoproteins
- TCA cycle
  - ✓ ETF-CoQ oxidoreductase (ex. fatty acid oxidation)
  - ✓ ≈ 0 kcal, H+?





Oxi–Red Components of the ETC "Cytochrome bc1" – Complex III

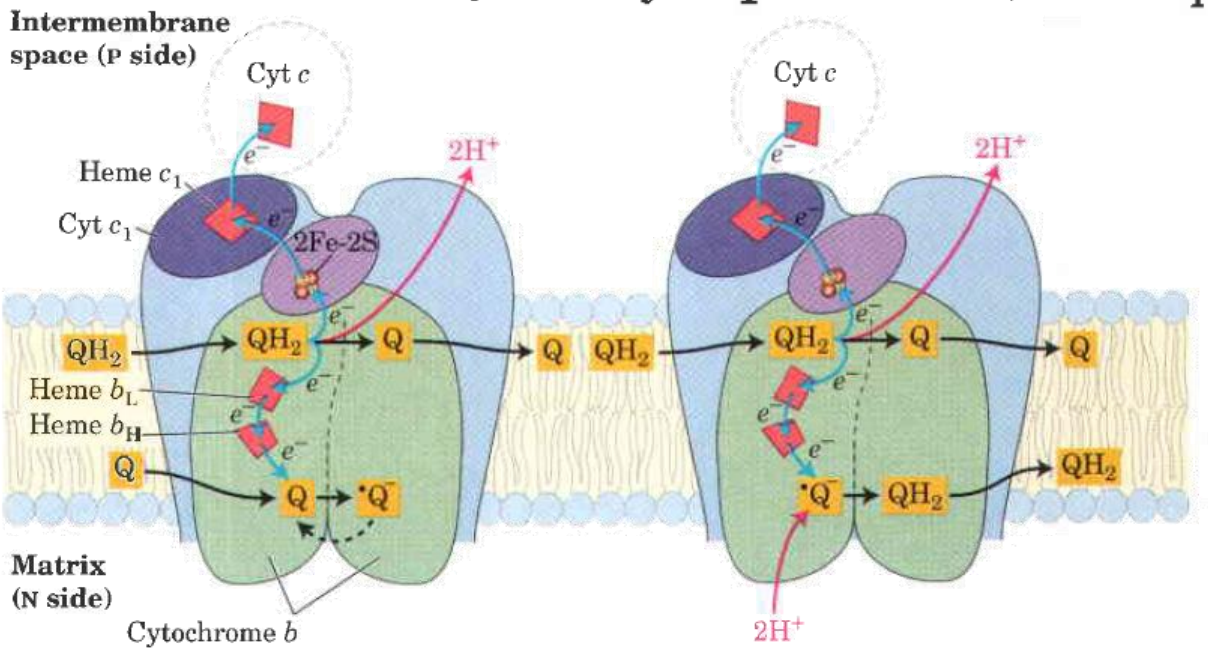
- Also called: Q-cytochrome c Oxidoreductase
- Catalyzes the transfer of electrons from QH2 to cytochrome c
- 11 subunits including two cytochrome subunits
- Contains iron sulfur center
- Contain three heme groups in two cytochrome subunits
- b_L and b_H in cytochrome b; c type in cytochrome c1
- Contain two CoQ binding sites
- 4H+



The Q-cycle

$$QH_2 + 2\ \text{cyt}\ c_1\ (\text{oxidized}) + 2H_N^+ \longrightarrow Q + 2\ \text{cyt}\ c_1\ (\text{reduced}) + 4H_P^+$$

- Partial reduction is hazardous
- Accommodates the switch between 2e-/1e-
- Explains the measured stoichiometry of 4 H+/2e-



$$QH_2 + Q + \text{cyt}\ c_1\ (\text{oxidized}) \longrightarrow {}^{\bullet}Q^- + Q + 2H_P^+ + \text{cyt}\ c_1\ (\text{reduced})$$

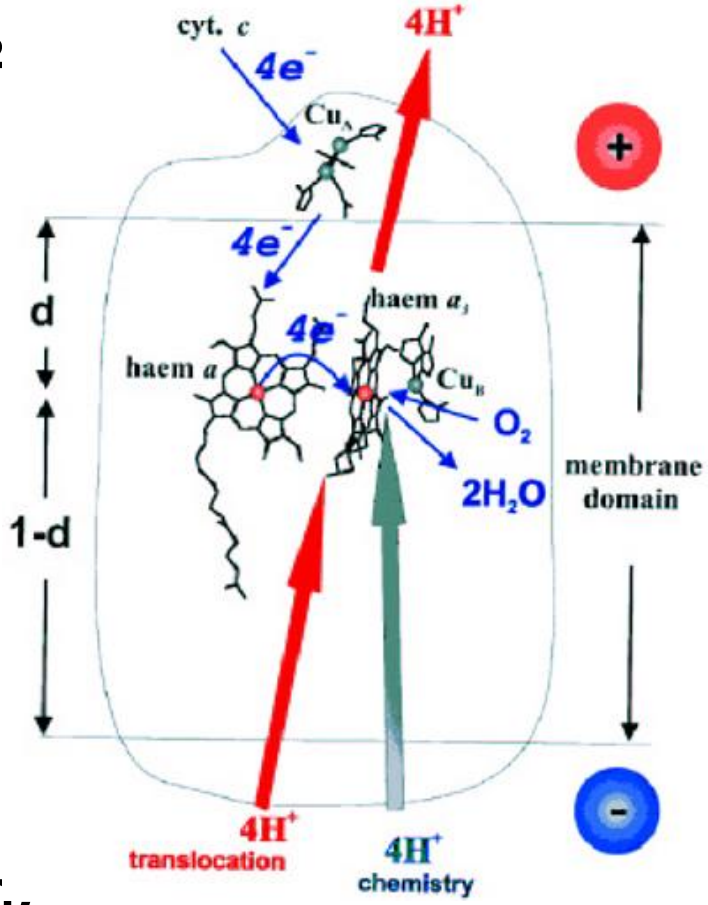
$$QH_2 + {}^{\bullet}Q^- + 2H_N^+ + \text{cyt}\ c_1\ (\text{oxidized}) \longrightarrow QH_2 + 2H_P^+ + Q + \text{cyt}\ c_1\ (\text{reduced})$$

Net equation: $QH_2 + 2\ \text{cyt}\ c_1\ (\text{oxidized}) + 2H_N^+ \longrightarrow Q + 2\ \text{cyt}\ c_1\ (\text{reduced}) + 4H_P^+$

Oxi–Red Components of the ETC "Cytochrome c oxidase" – Complex IV

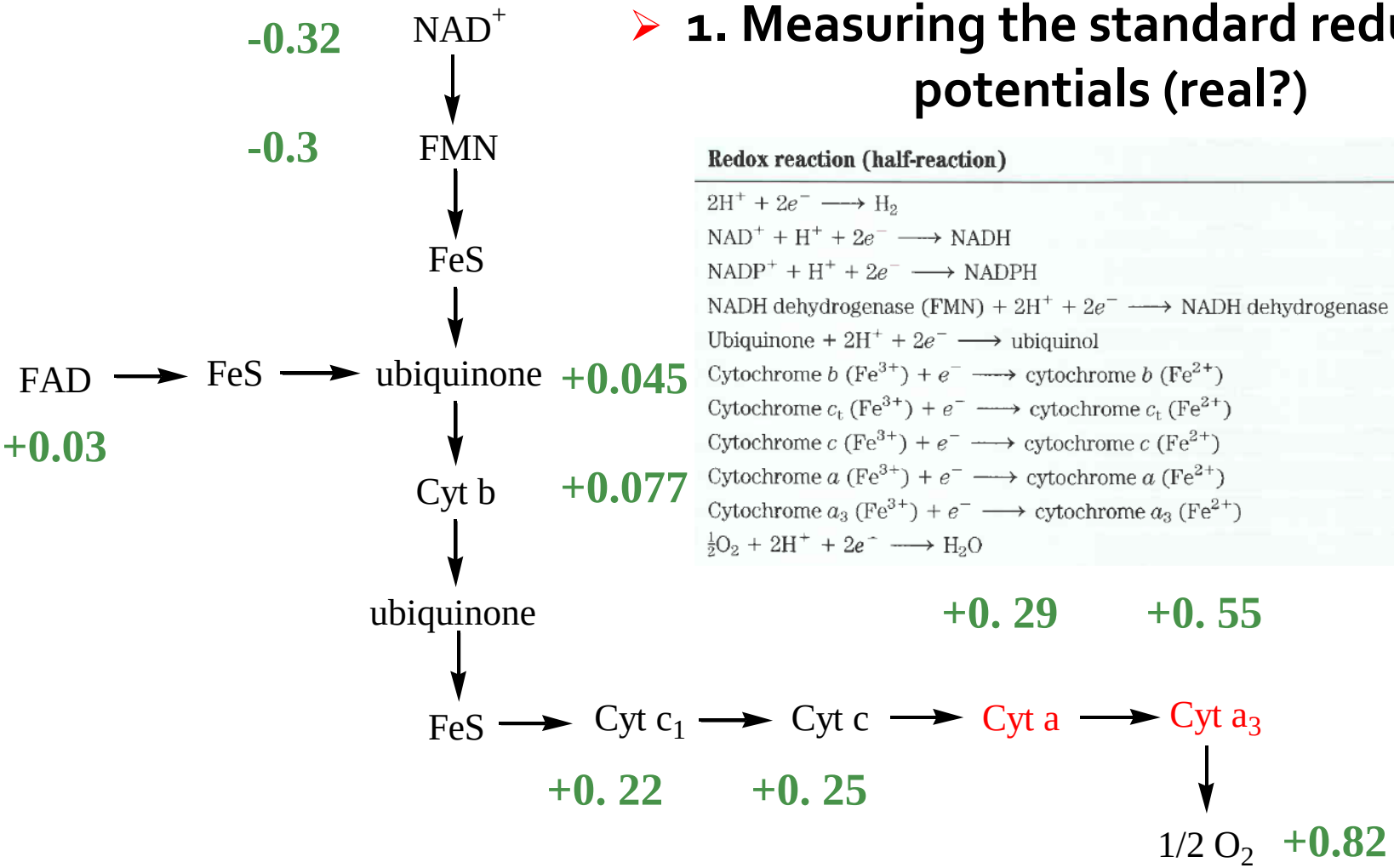
- Passes electrons from Cytocrome c to O2
- Contains cytochrome *a* and *a*3
- Contains two copper sites
- Contains oxygen binding sites
- O2 must accept 4 electrons to be reduced to 2 H2O (2H+/2e-)
- Cytochrome c is one electron carrier

  $$\text{Cyt } c_{red} + 4H^+ + O_2 \rightarrow \text{Cyt } c_{ox} + 2H_2O$$
- Cytochrome oxidase has a much lower Km for O2 than myoglobin (hemoglobir., myoglobin, complex IV)
- Partial reduction of O2 is hazardous



The right arrangement
How can we prove it?

- **1. Measuring the standard reduction potentials (real?)**



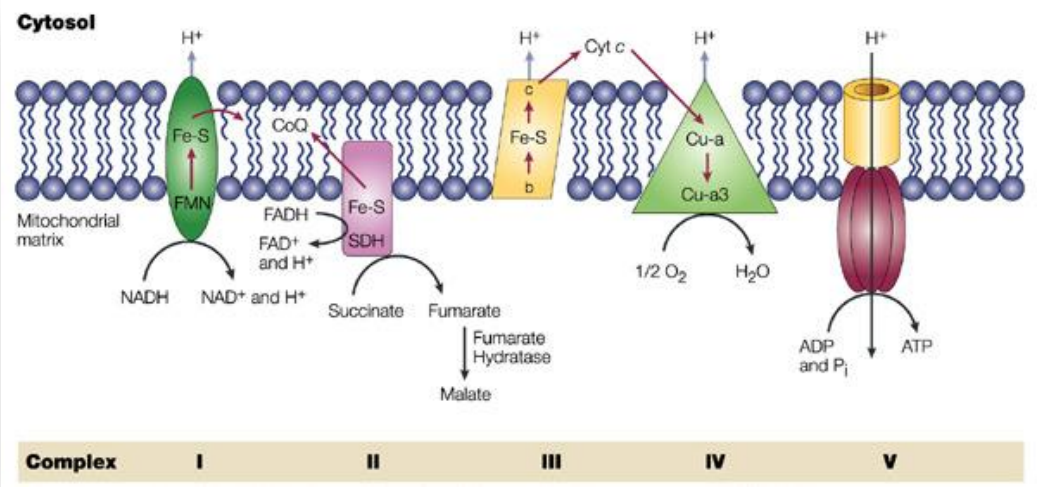
Redox reaction (half-reaction)	E'° (V)
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol	0.045
Cytochrome b (Fe^{3+}) + $e^- \longrightarrow$ cytochrome b (Fe^{2+})	0.077
Cytochrome c_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

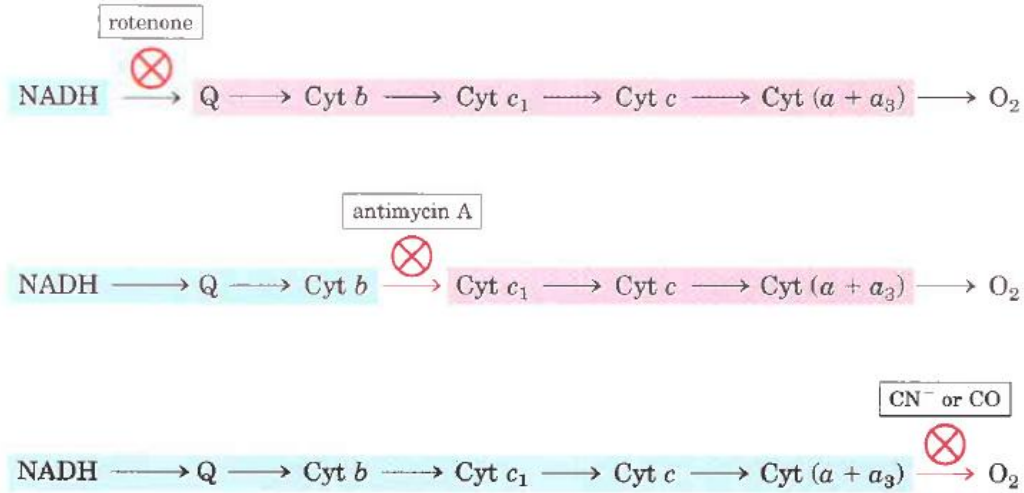
NADH → Q → cytochrome b → cytochrome c1 → cytochrome c → cytochrome a → cytochrome a3 → O2

The right arrangement
How can we prove it?

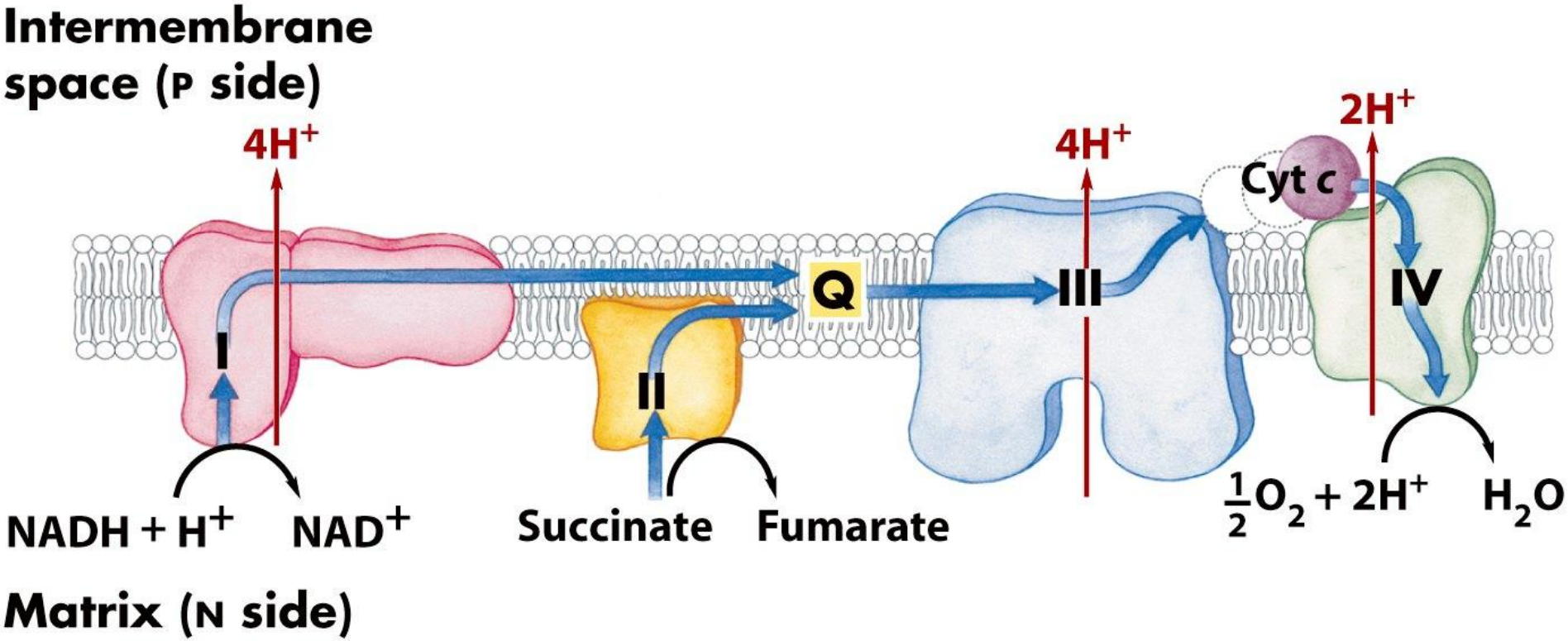
NADH → Q → cytochrome b → cytochrome c1 → cytochrome c → cytochrome a → cytochrome a3 → O2

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





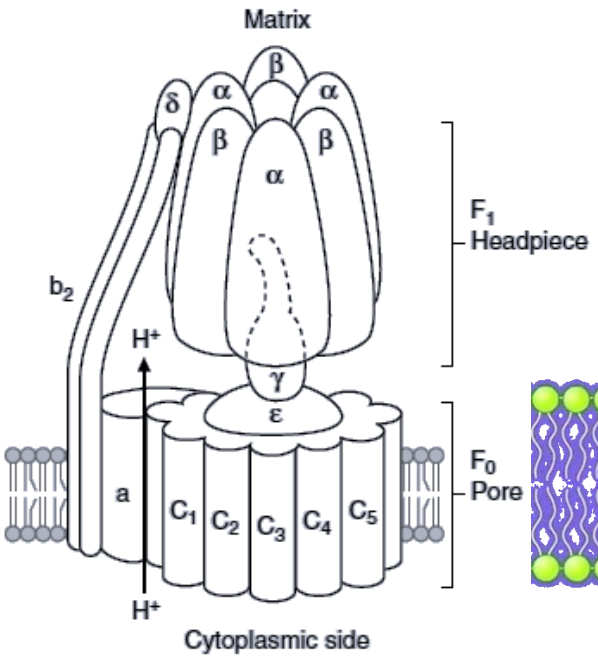
Pumping of Protons

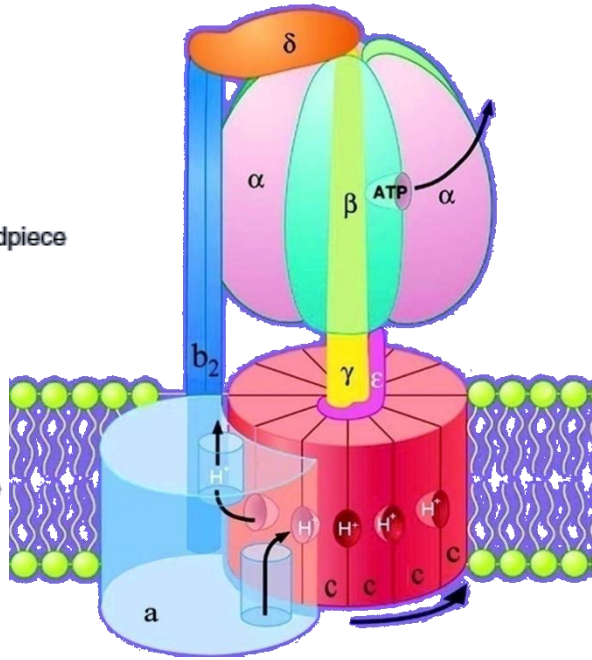


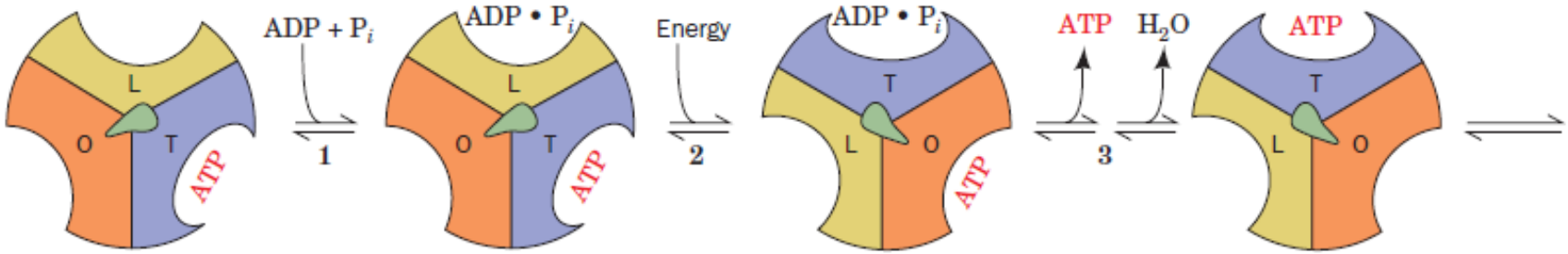
- For every 2 electrons passing:
- $4H^+$ (complex I); $0H^+$ (complex II); $4H^+$ (complex III), $2H^+$ (complex IV)

ATP Synthase

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

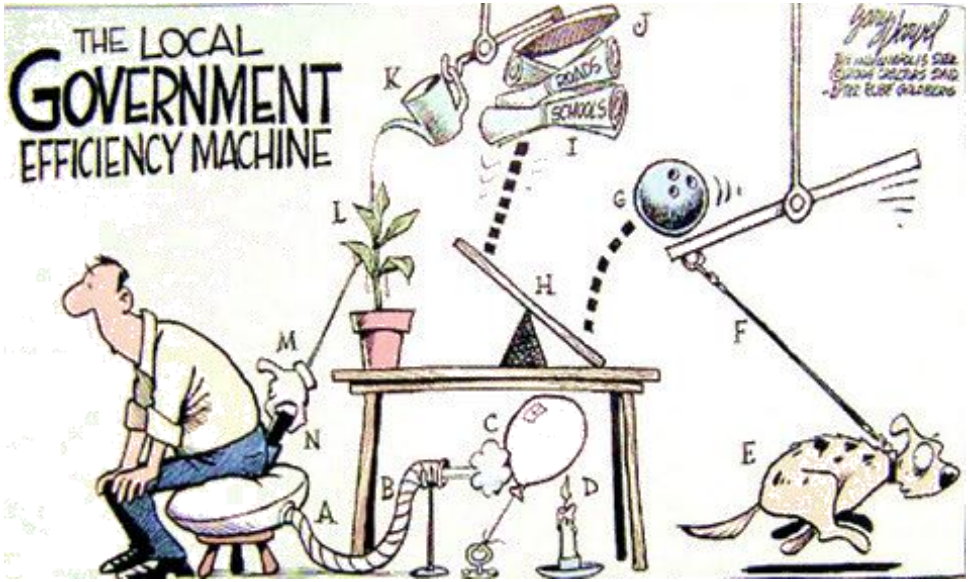






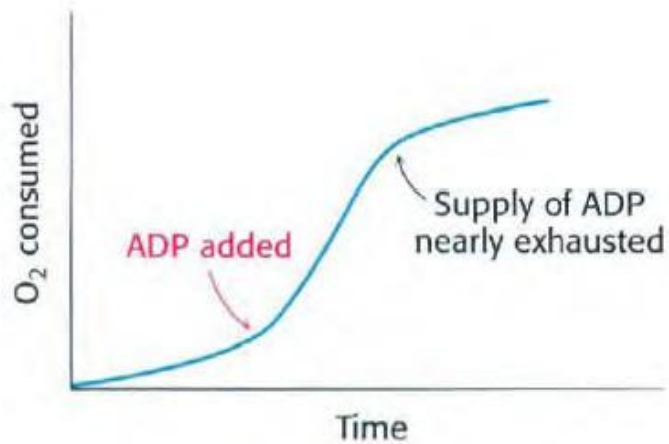
Energy yield from the ETC

- NADH, -53 kcal, ATP?
- FADH2, -41 kcal, ATP?
- $\Delta G^{o\prime}$ is so negative, never reversible
- ATP machine efficiency, (anions, Ca^{+2}, heat, phosphate, substrates)
- Electron transport chain is our major source of heat

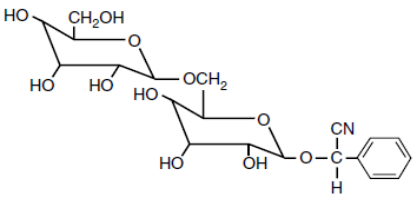


Regulation – the need for ATP

- What OxPhos needs? (NADH, O2, ADP, and Pi)
- In skeletal muscles, 20% drop in ATP concentration
- In the heart, Ca^{+2} activates TCA enzymes for extra push (NADH, ATP), no drop
- ET is tightly coupled to phosphorylation (simultaneously)
- <u>ADP is the most important factor</u> in determining the rate
- The regulation of the rate of oxidative phosphorylation by the ADP level is called <u>respiratory control</u>



Regulation – inhibition (coupling)

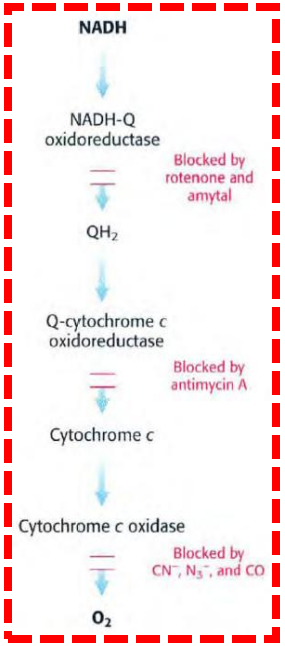


Amygdalin, a cyanoglycoside

Anit-cancerous drug

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

Specific inhibitor	Target
Rotenone (insecticide) & Amytal (sedative)	NADH-Q oxidoreductase
Antimycin A (antibiotic)	Q-cytochrome c oxidoreductase
Cyanide (CN-), Azide (N3-), & (CO)	Cytochrome c oxidase
Oligomycin (antibiotic)	ATP synthase



الصفحة الرئيسية > محليات

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.

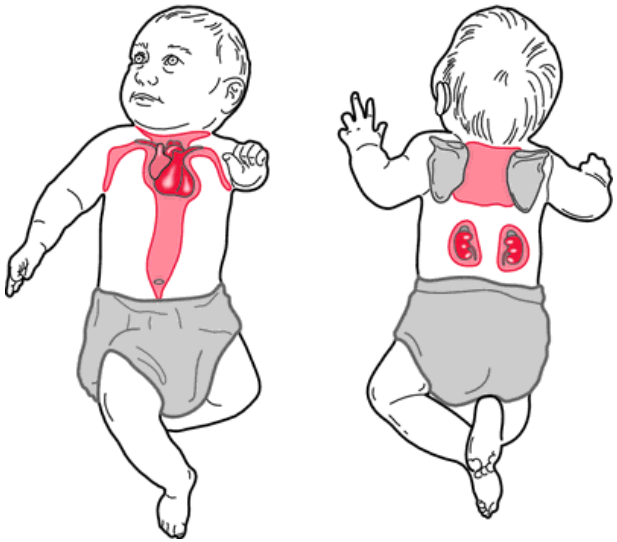
قضية السيانيد

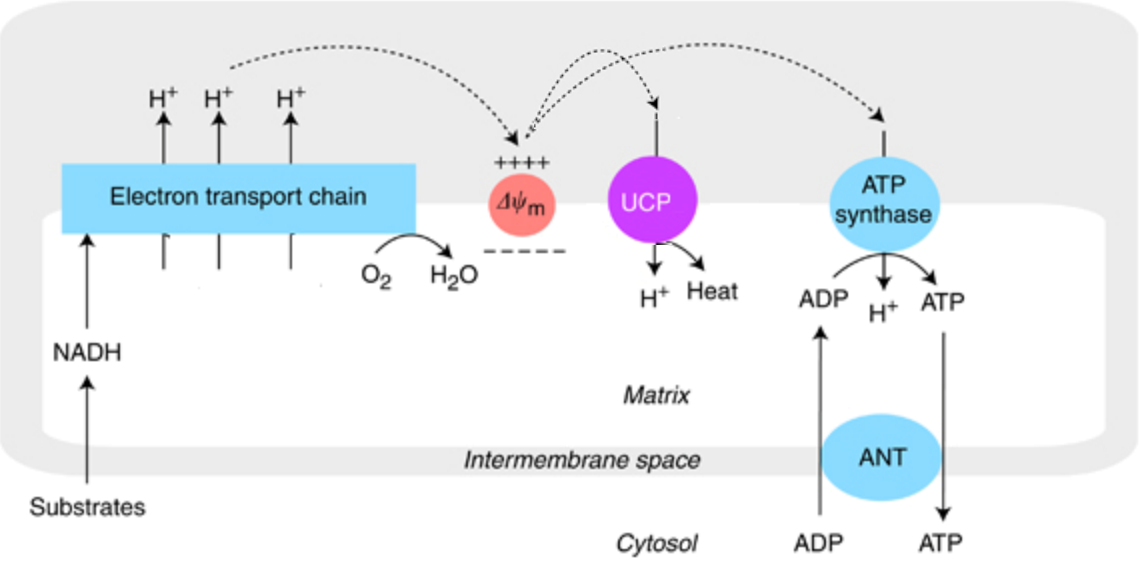
أول جريمة من نوعها يرتكبها أب ضد ولديه ، اذ قام الاب بوضع مادة السيانيد في كأس الحليب وطلب من طفليه ان يشربا منه ، حيث فارقا الحياة بعد 10 دقائق من مغادرة الام المنزل لتعود وتجدهما جثتين هامدتين.

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

Regulation – Uncoupling
Regulated - Uncoupling proteins (UCPs)

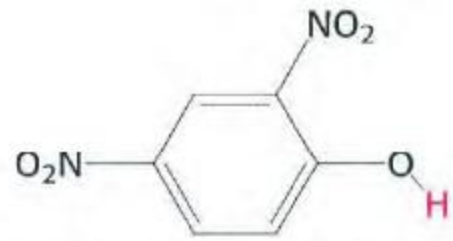
- Short-circuiting ATP synthase
- UCP1 (thermogenin):
  - ✓ Brown adipose tissue, non-shivering thermogenesis
  - ✓ Infants: neck, breast, around kidneys
  - ✓ Fatty acids directly activates UCP1
- UCP2 (most cells); UCP3 (skeletal muscle); {UCP4, UCP5} (brain)
- Obesity tendency in some populations



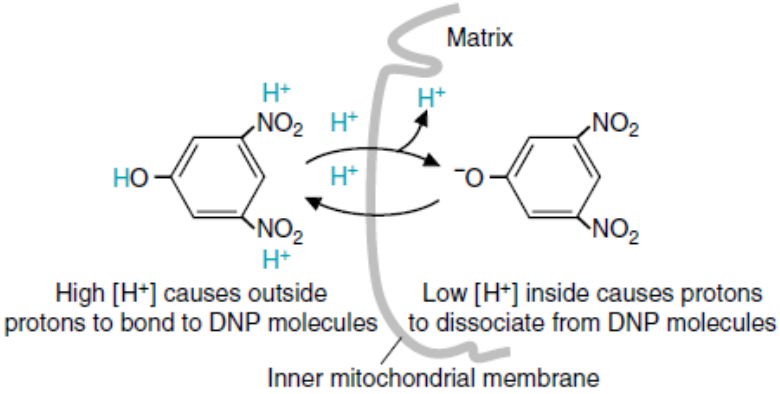


Regulation – Uncoupling
Unregulated – chemical uncouplers

- What is uncoupling?
- How does it occur? Dissipation of PMF
- What is the result?
- Is it physiological or not?
- 2,4-dinitrophenol (DNP) & other acidic aromatic compounds
- What changes happen? ↑ O2 consumption, ↑NADH oxidation
- Soviet soldiers were given DNP, FDA banned DNP (1938)



2,4-Dinitrophenol (DNP)



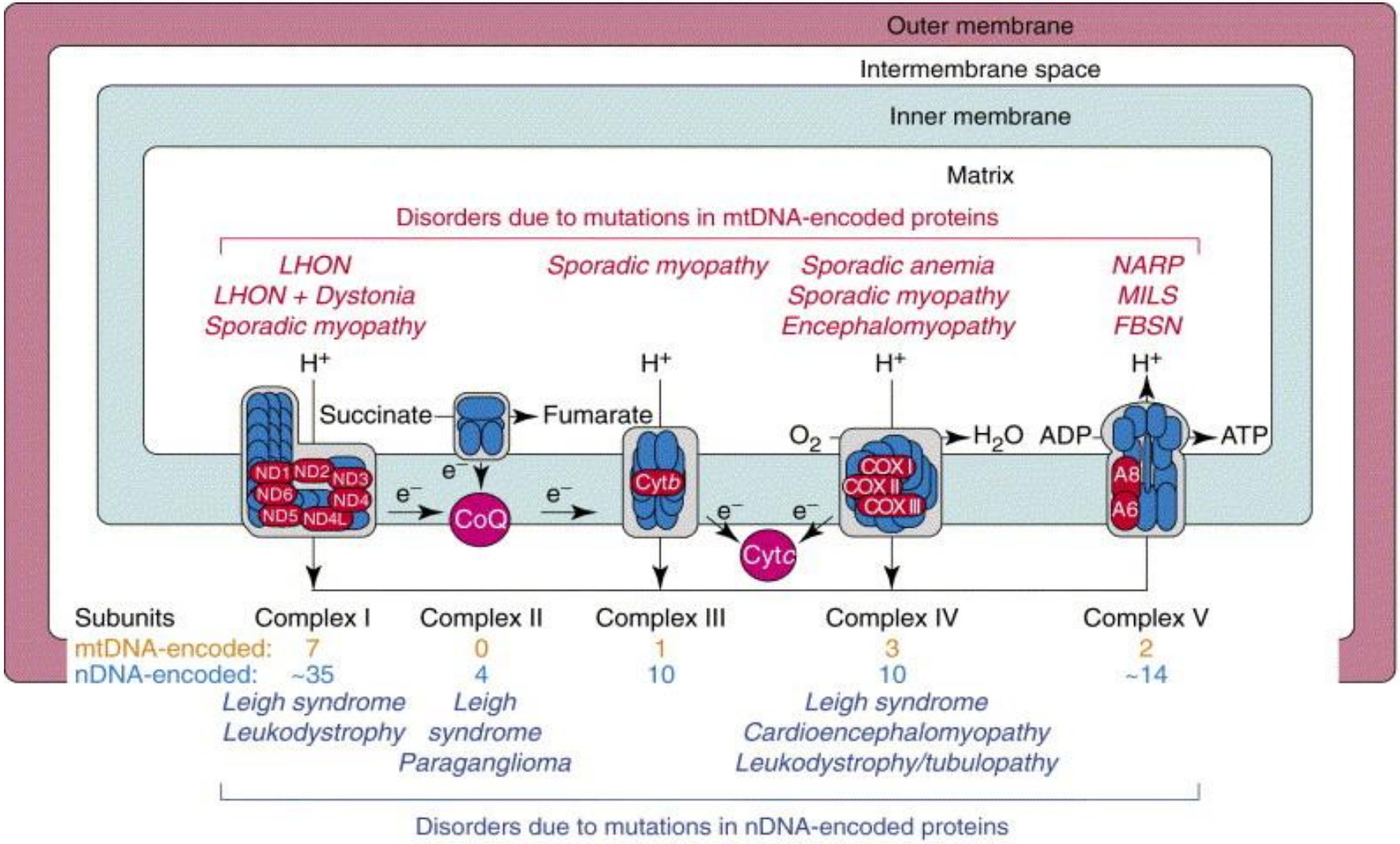
OxPhos Diseases (Genetic)

- 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 (F0)
  - ✓ Also encodes necessary components for translation of its own mRNA: a large and small rRNA and tRNAs
  - ✓ Maternal inheritance, replicative segregation & heteroplasmy
  - ✓ Accumulation of somatic mutations with age
  - ✓ Highest ATP demands: CNS, heart, skeletal muscle, and kidney, liver

OxPhos Diseases (Genetic)

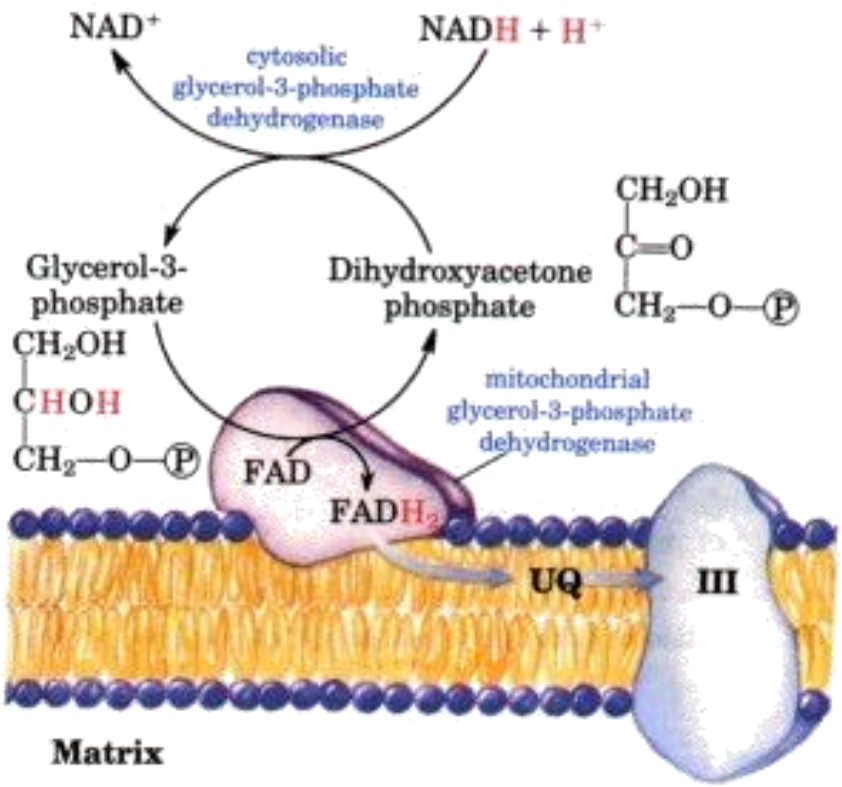
- B. Nuclear Genetic Disorders of Oxidative Phosphorylation
  - ✓ 1,000 proteins (50% of the mitochondria is protein)
  - ✓ Usually autosomal recessive
  - ✓ Expressed in all tissues
  - ✓ Phenotypic expression with high ATP demand

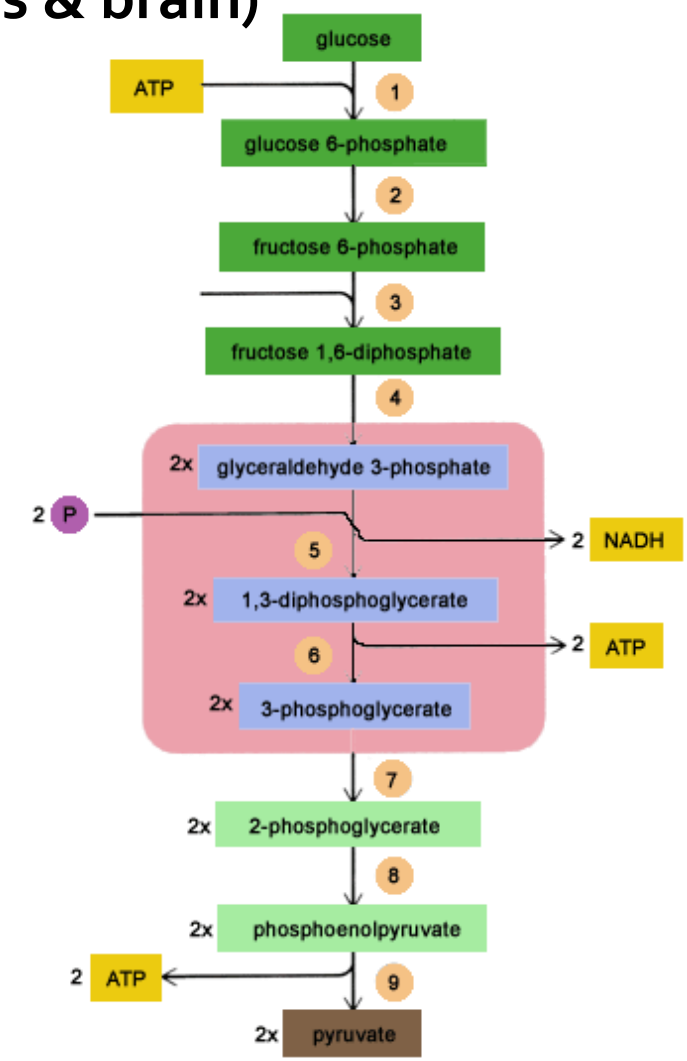
OxPhos Diseases (Genetic)



Mitochondrial shuttling systems "Cytosolic NADH"

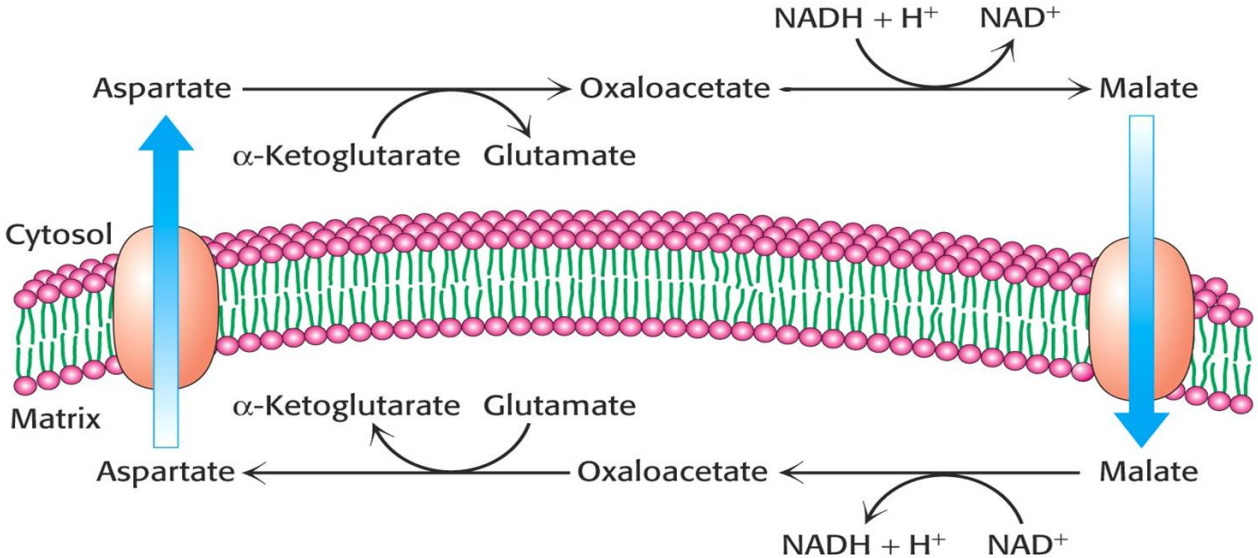
- Glycerol 3-phosphate shuttle (Sk. muscles & brain)
- Glycolytic pathway as an example
- How NADH passes?
- ATP yield?





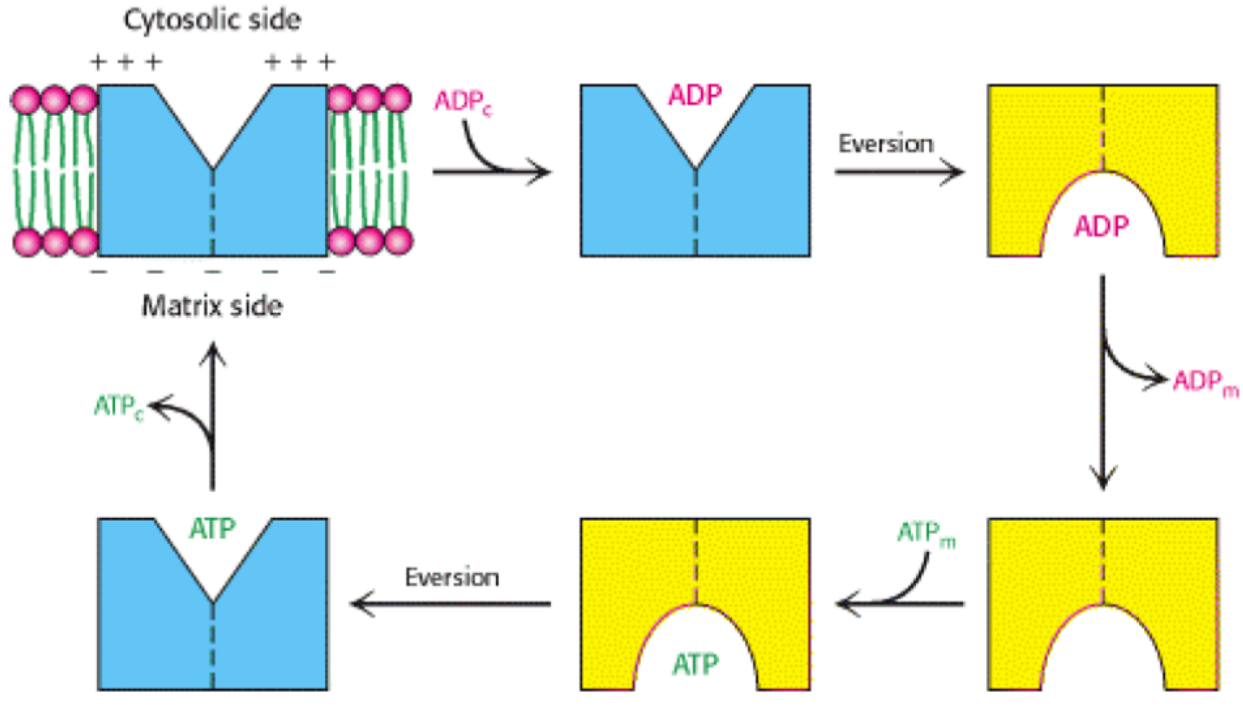
Mitochondrial shuttling systems "Cytosolic NADH"

- Malate-Aspartate shuttle
- Heart & liver
- 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



Mitochondrial shuttling systems "ATP/ADP"

- ATP-ADP Translocase (also called 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
- Endergonic (25% of ETC)
- Inhibition leads to subsequent inhibition of cellular respiration