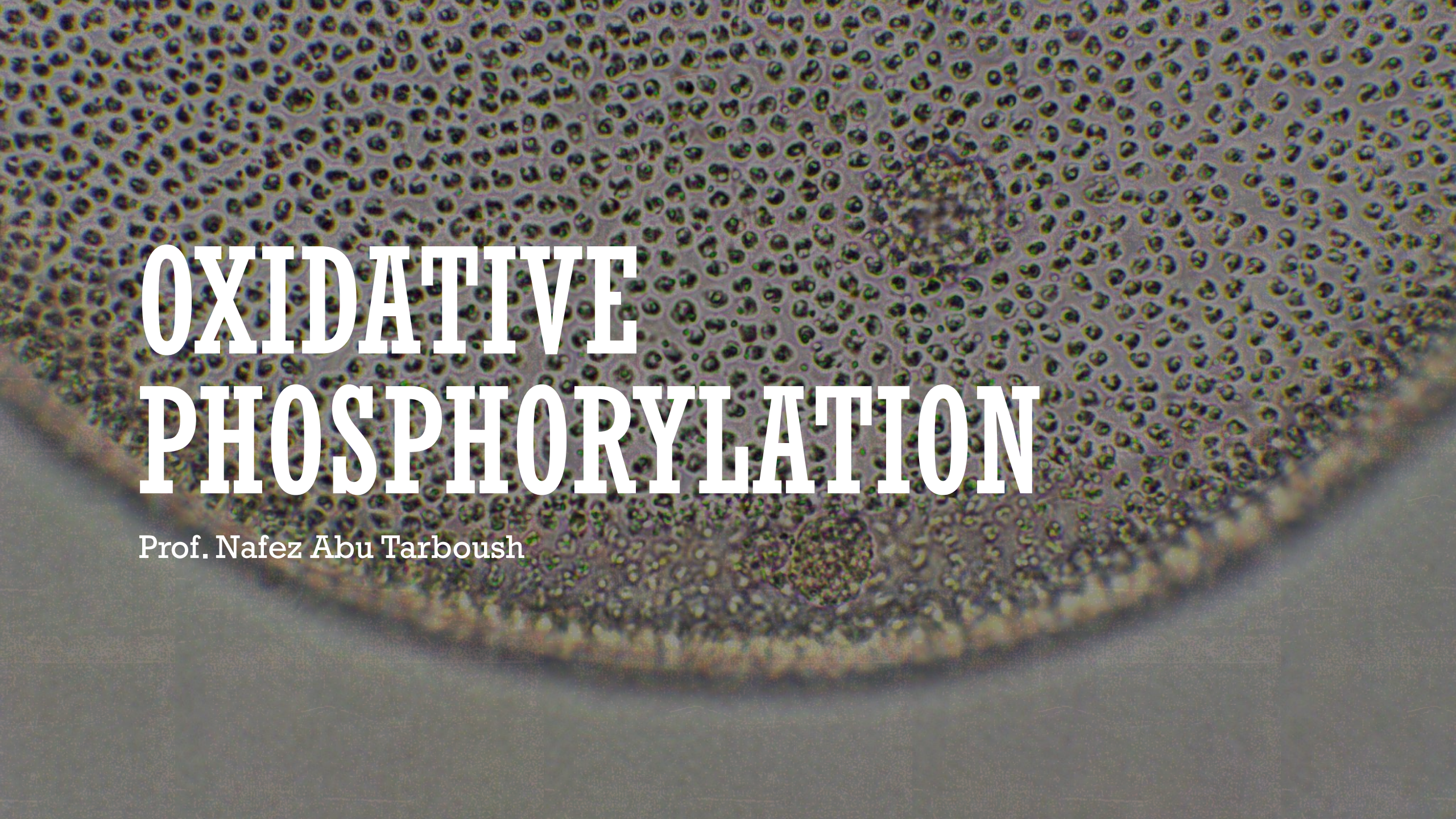
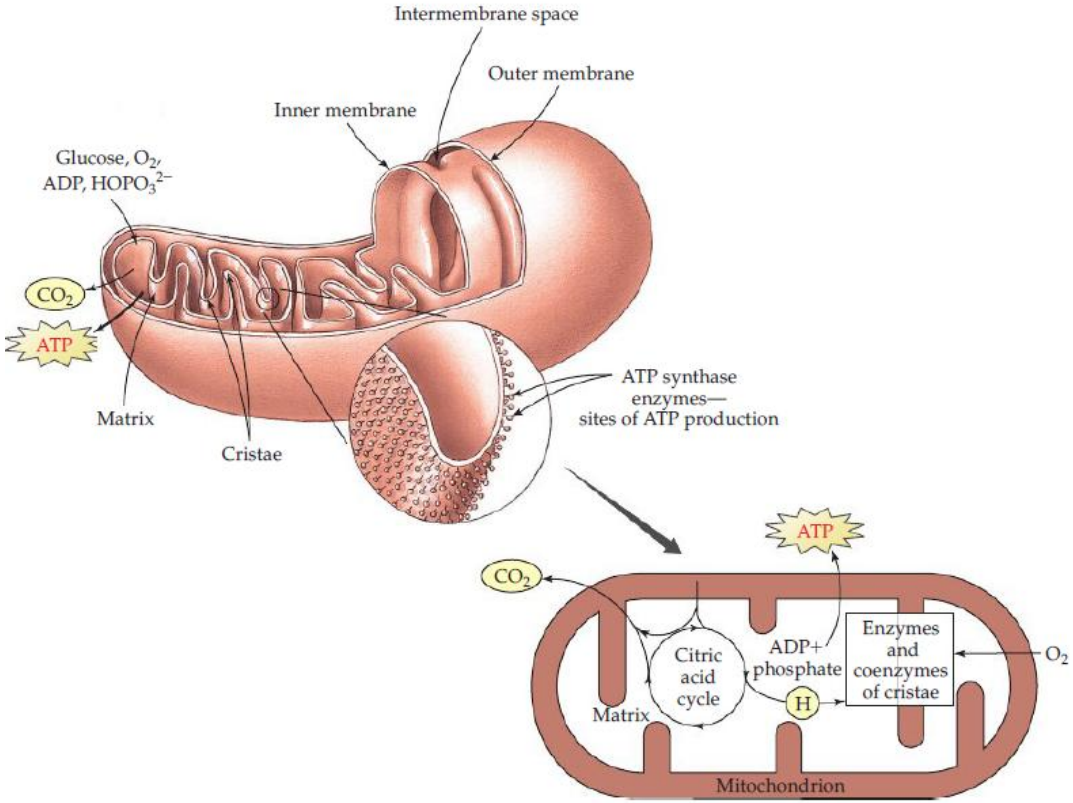


OXIDATIVE PHOSPHORYLATION

Prof. Nafez Abu Tarboush



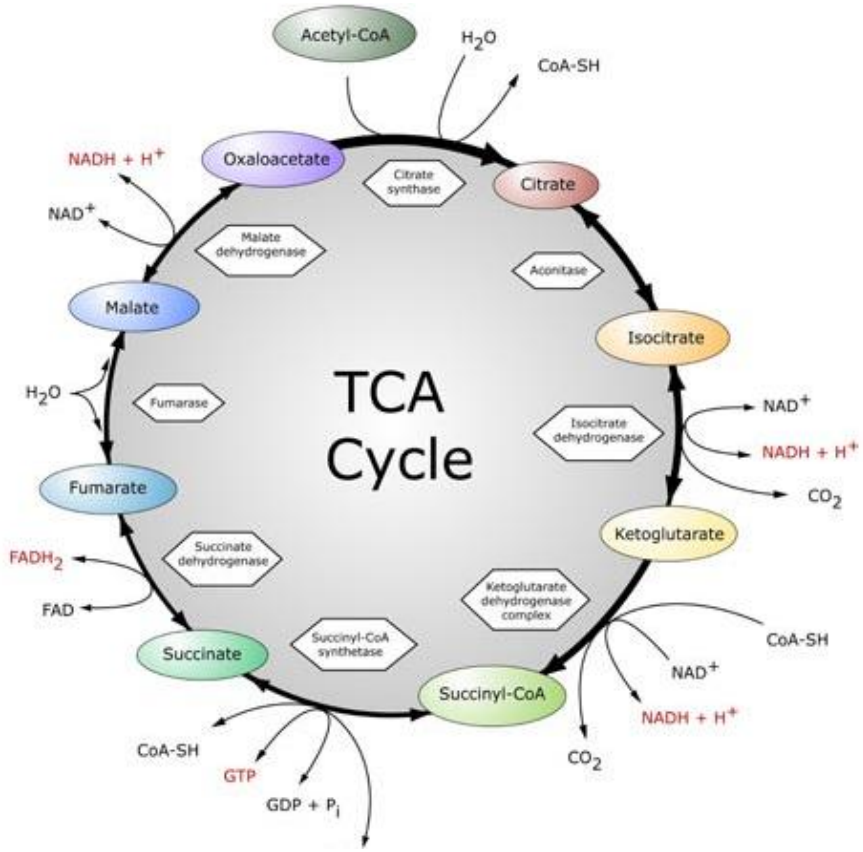
MITOCHONDRIAL ARCHITECTURE

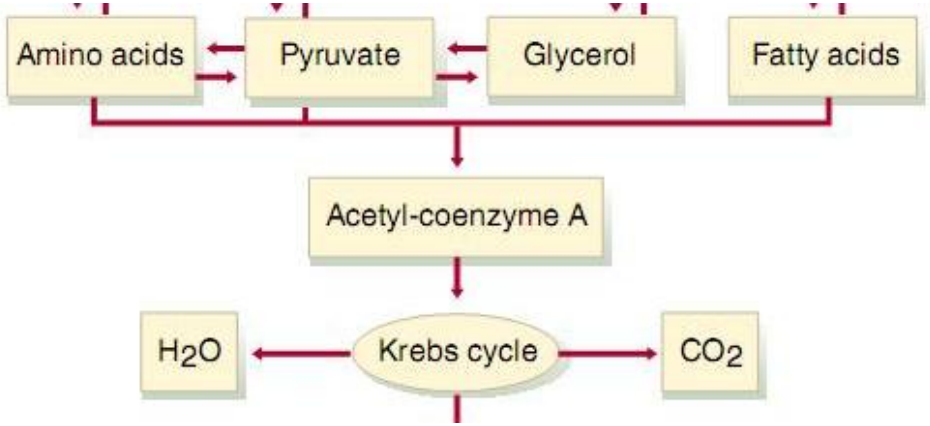
- OMM: permeable (MW<5,000); (transmembrane channels)
- IMM: impermeable (H^+); specific transporters
- IMM: components of respiratory chain and ATP synthase
- Matrix: pyruvate dehydrogenase complex; TCA cycle enzymes, fatty acid β-oxidation pathway, and the pathways of amino acid oxidation
- Matrix contains all pathways of fuel oxidation except glycolysis

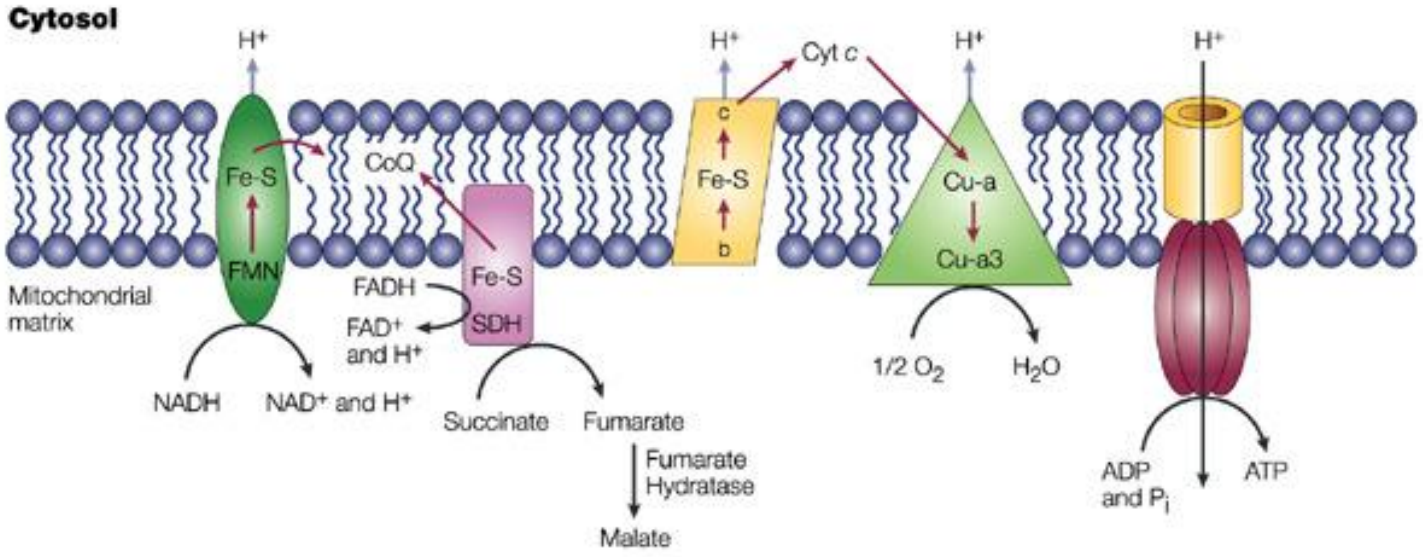
TABLE 20.3: Location of enzymes in mitochondria
Mitochondria, outer membrane:
Monoamino oxidase
Acyl CoA synthetase
Phospholipase A2
In between outer and inner membrane:
Adenylate kinase
Creatine kinase
Inner membrane, outer surface:
Glycerol-3-phosphate dehydrogenase
Inner membrane, inner surface:
Succinate dehydrogenase
Enzymes of respiratory chain
Soluble matrix:
Enzymes of citric acid cycle
Enzymes of beta oxidation of fatty acid

MITOCHONDRIAL MEMBRANES

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





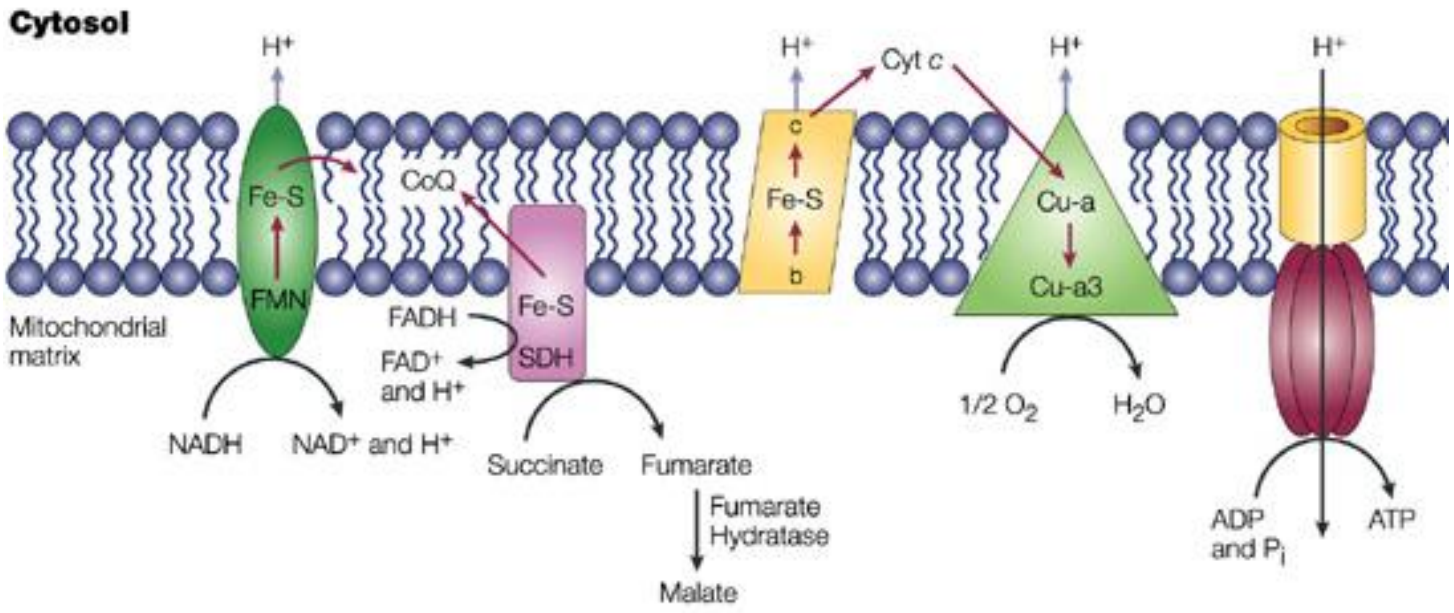


Complex	I	II	III	IV	V
Nuclear gene mutations	*NDU FS1, 2, 4, 7,8 NDU FV1*	*SDHA, B, C, D*	*BCS1L*	*SURF1, SCO1, SCO2, COX10*	
Phenotype	Leigh syndrome Leukodystrophy	Leigh syndrome Paraganglioma	Hepatopathy Encephalopathy	Leigh syndrome Cardioencephalo-	Cardioencephalo- myopathy

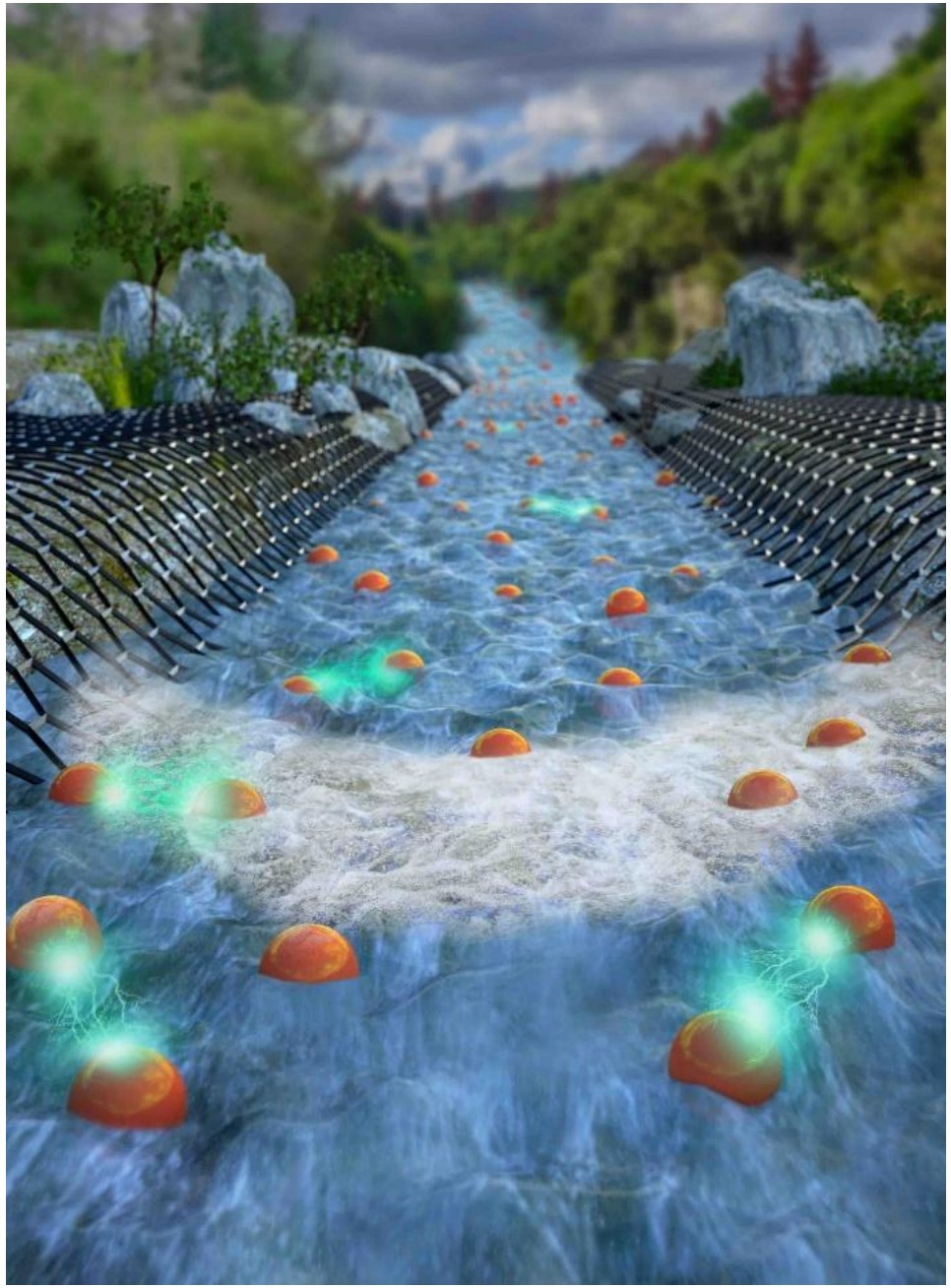
THE OXIDATIVE PHOSPHORYLATION, WHERE ARE WE?

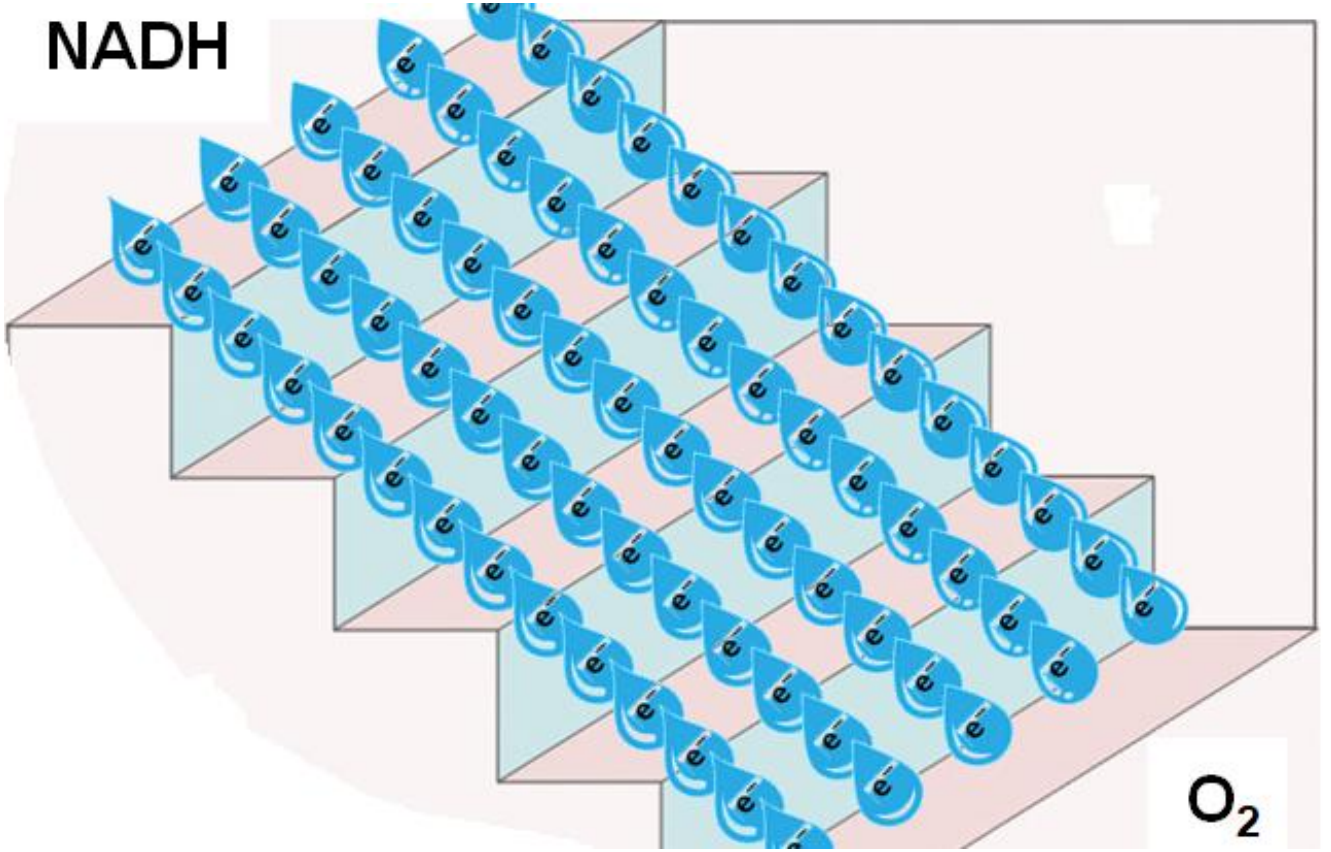
Stages: Digestion; Acetyl-CoA; TCA; OxPhos

(OXPHOS) OXIDATIVE PHOSPHORYLATION

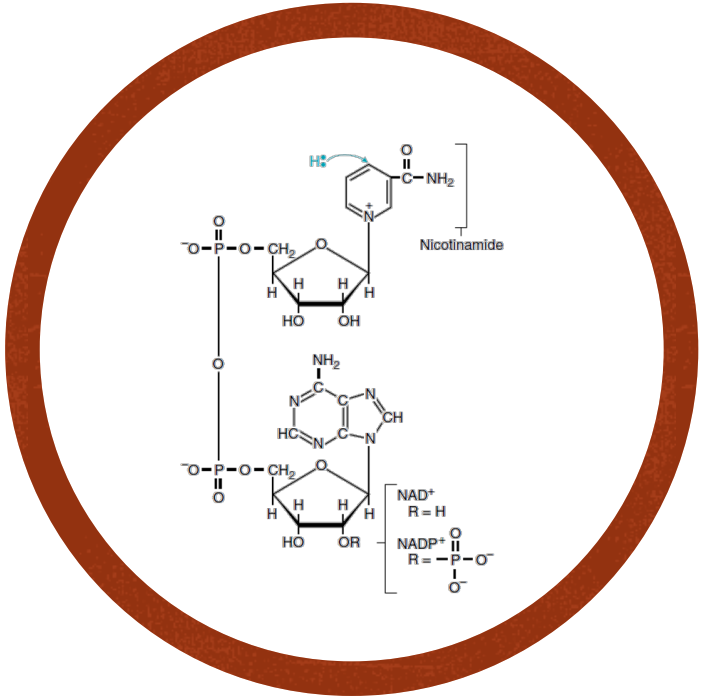


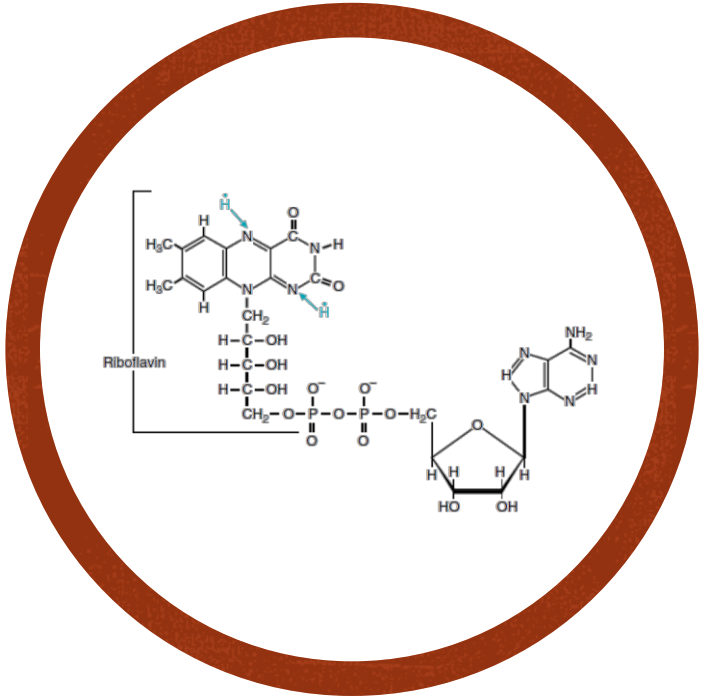
- Generation of ATP aided by O_2 reduction
- Peter Mitchell (1961): the chemiosmotic theory
- 3 major aspects:
  - ✓ (1) Flow of electrons
  - ✓ (2) The free energy available (exergonic) is coupled to transport of protons
  - ✓ (3) Flow of protons provides the free energy for synthesis of ATP (ATP synthase)

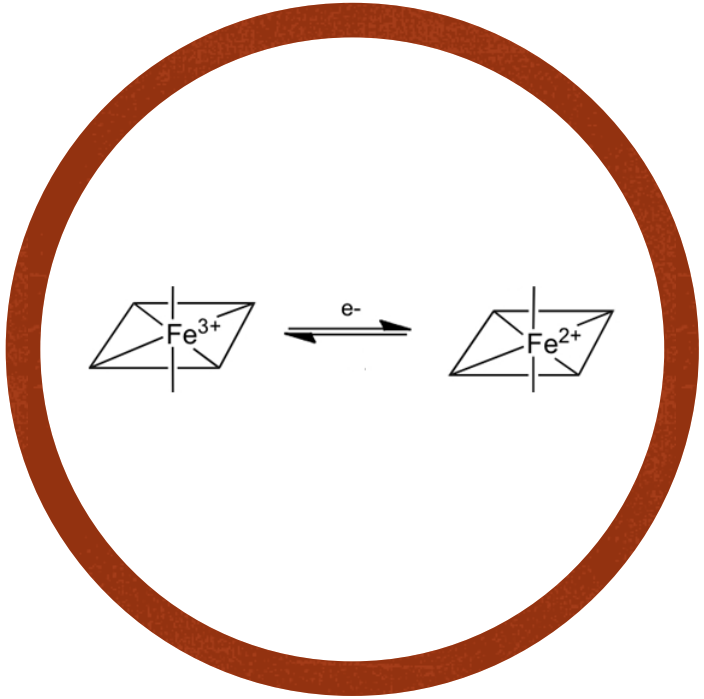




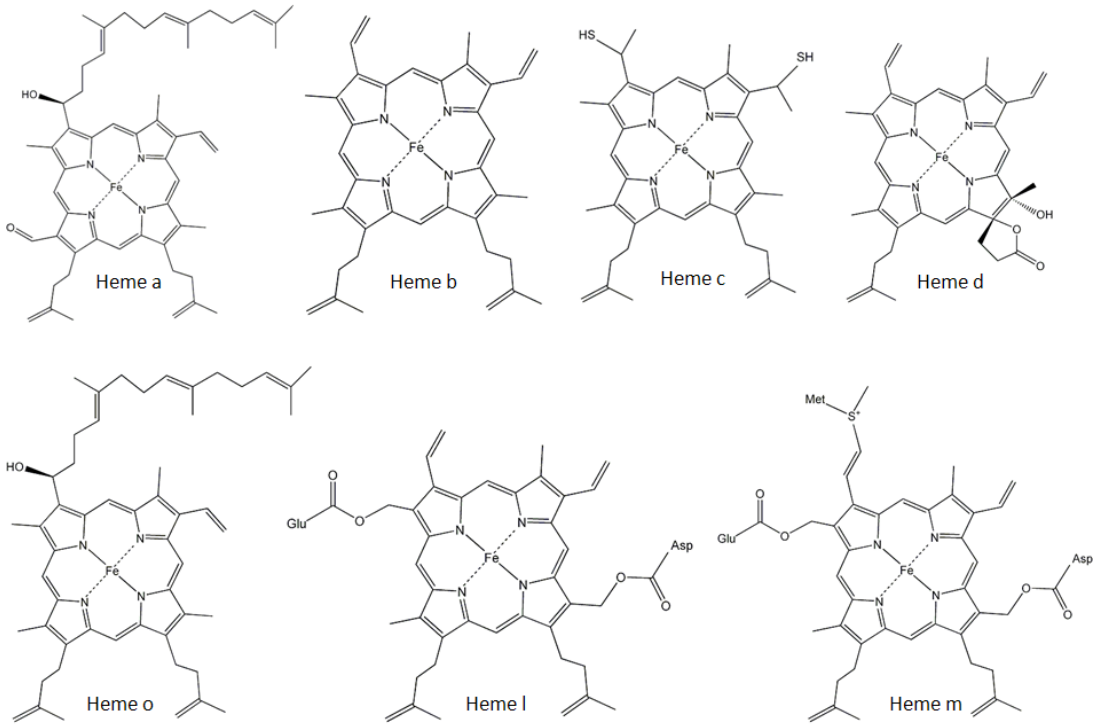
THE ELECTRON TRANSPORT CHAIN: THE RIVER OF ELECTRONS THAT PROVIDES A STEPWISE ENERGY RELEASE

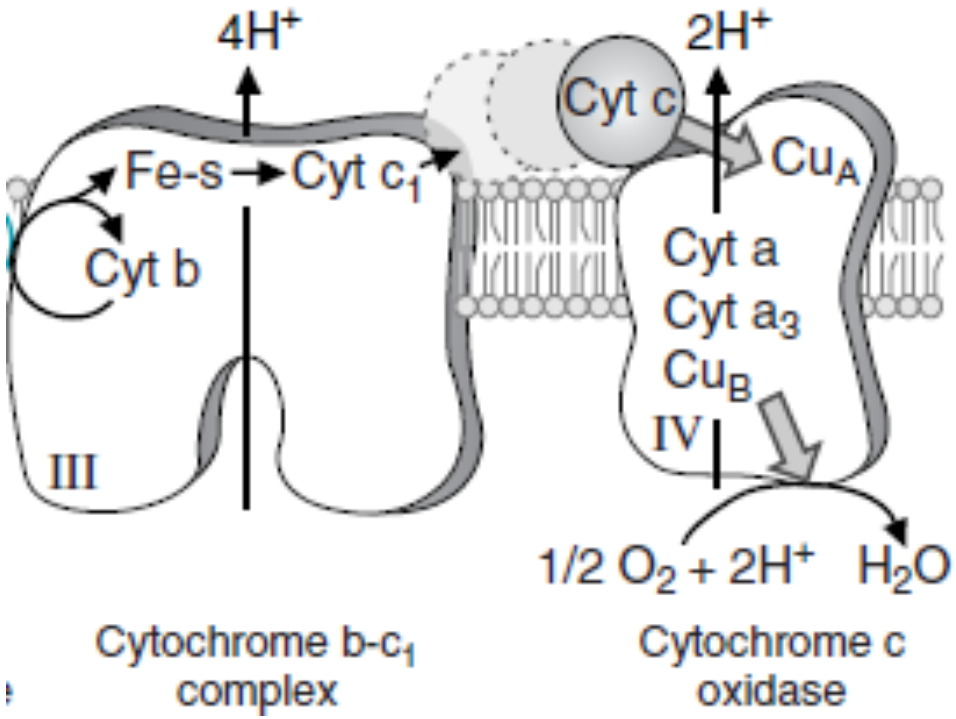






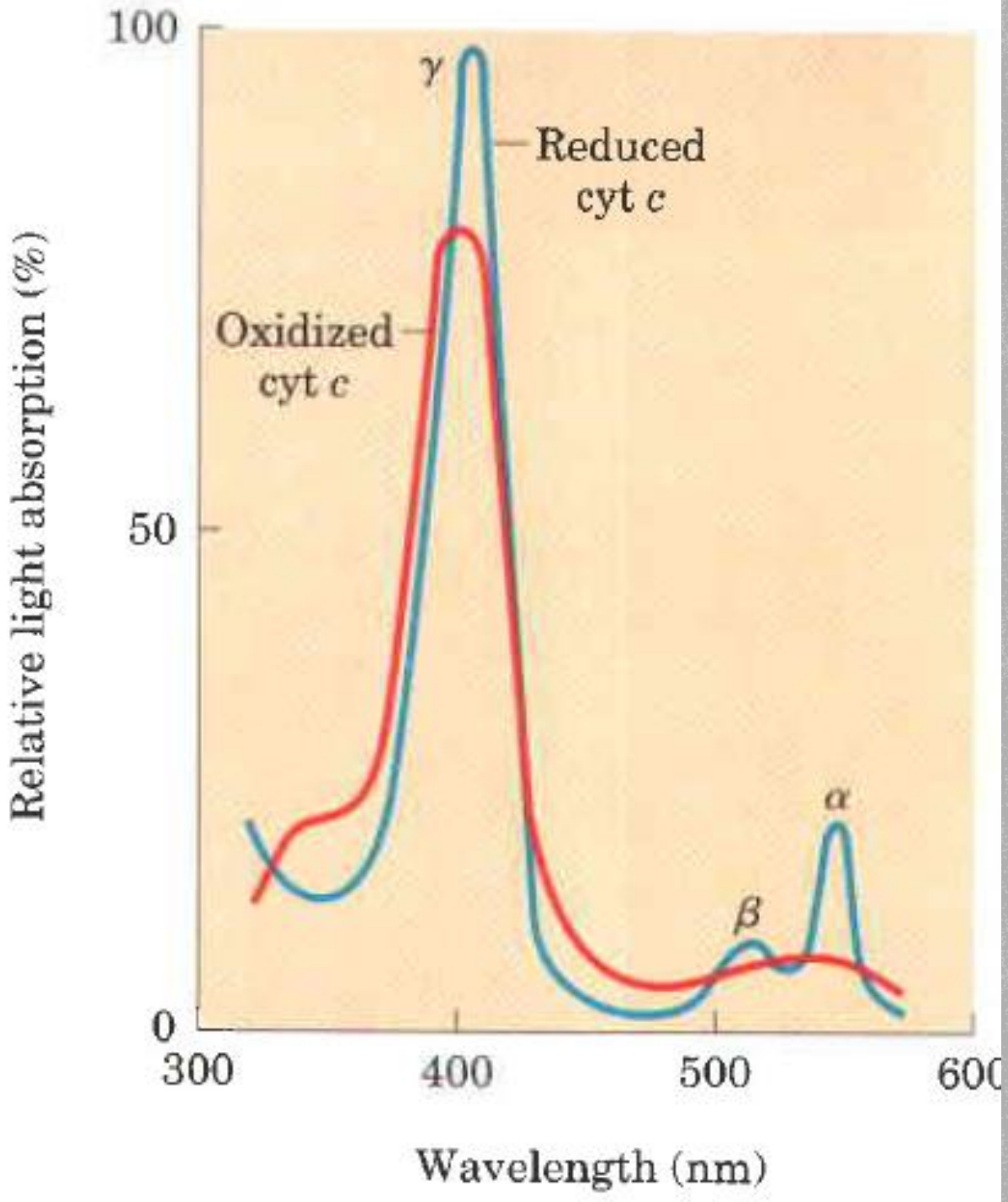
THE CAST OF CHARACTERS: ELECTRON CARRIERS





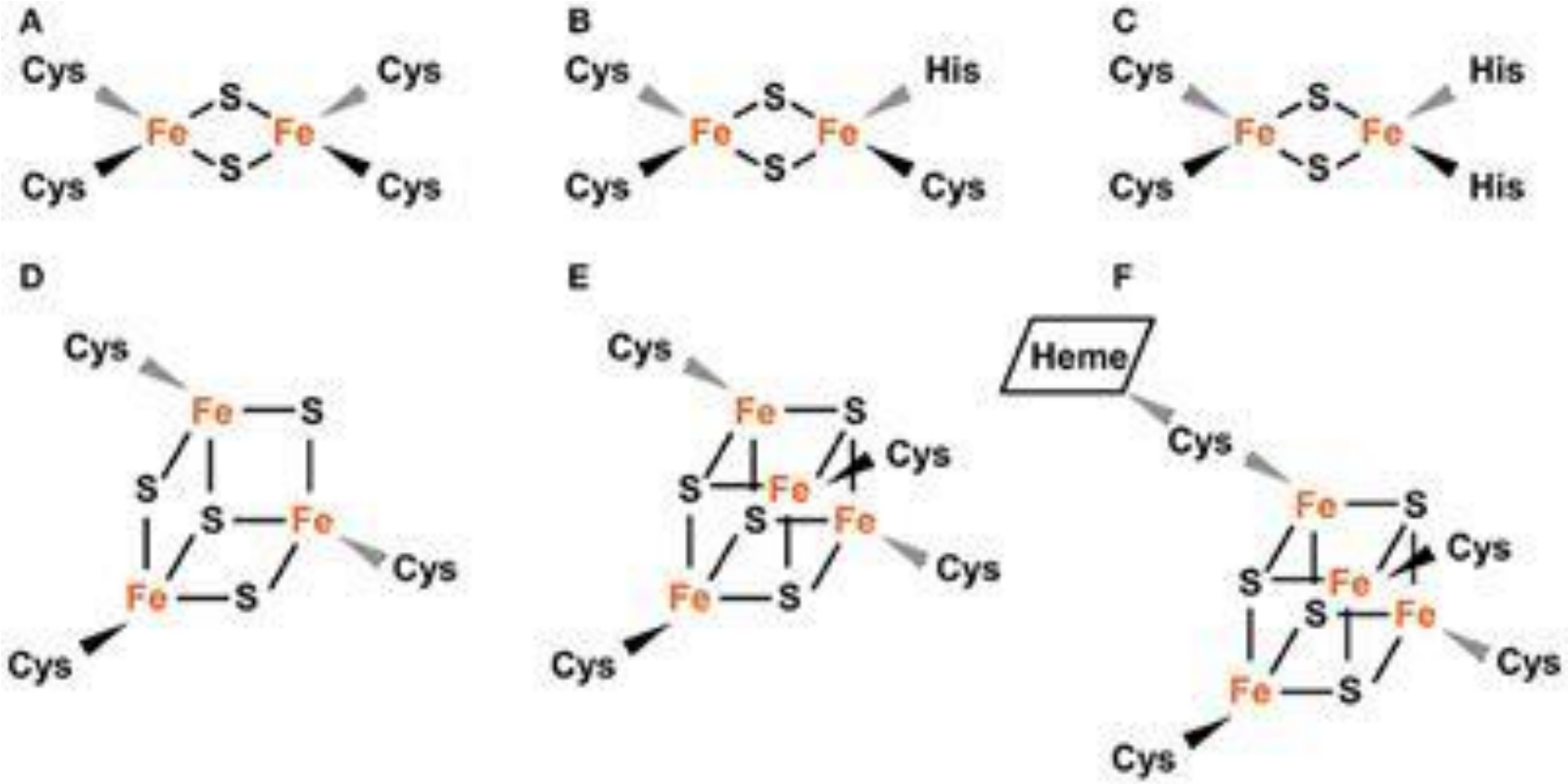
OTHER ELECTRON-CARRYING MOLECULES "CYTOCHROMES"

Mitochondria contain three classes o f cytochromes (a , b, & c)

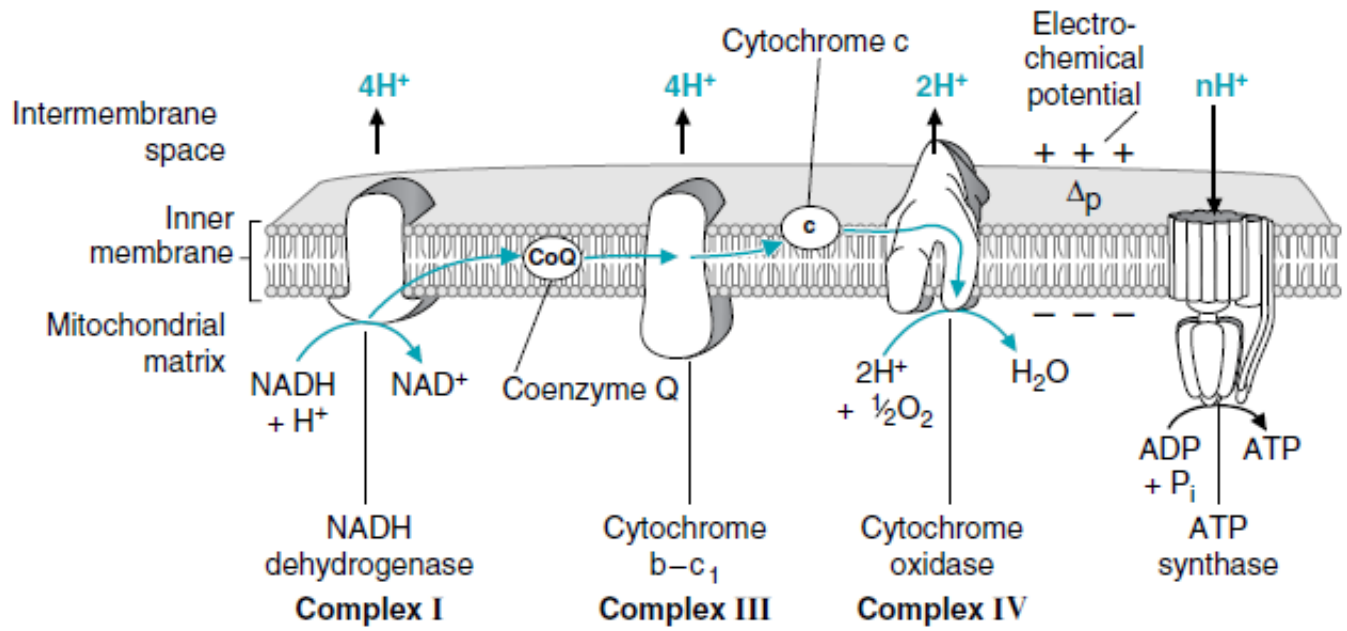


OTHER ELECTRON-CARRYING MOLECULES: "CYTOCHROMES"

- Light absorption: α band (~600 nm in type a; ~ 560 nm in type b; ~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
  - ΔE^{o} depends on the protein

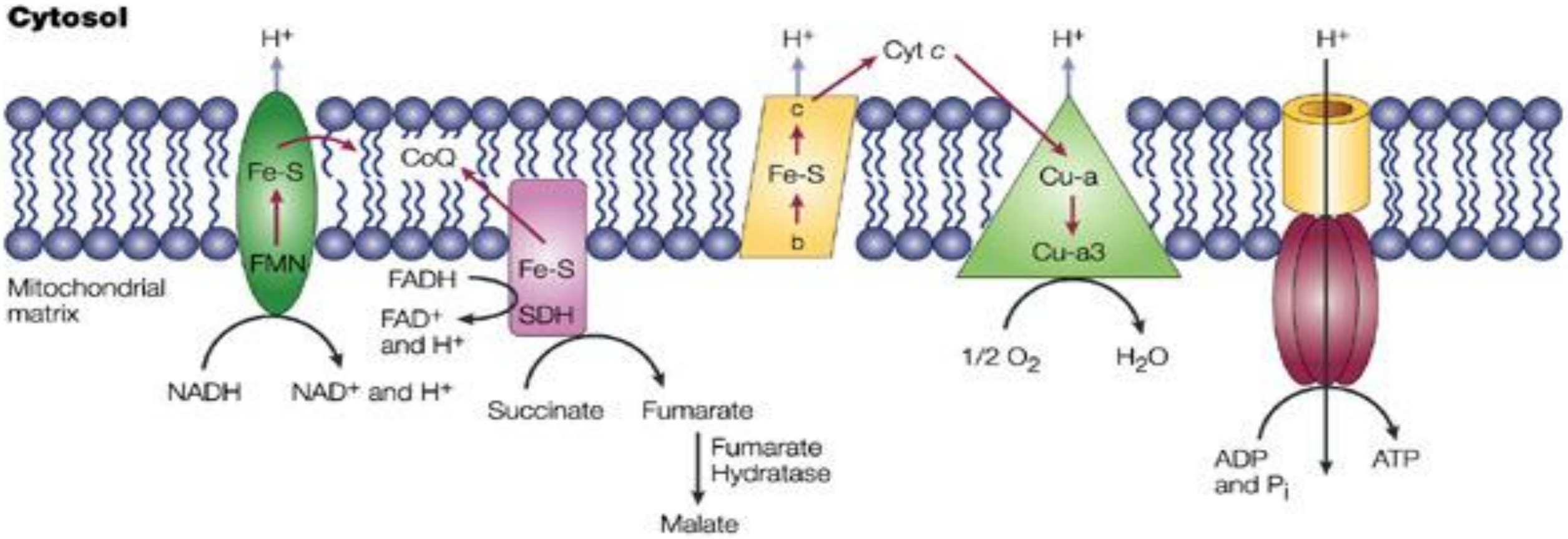


OTHER ELECTRON-CARRYING MOLECULES: IRON-SULFUR CLUSTERS



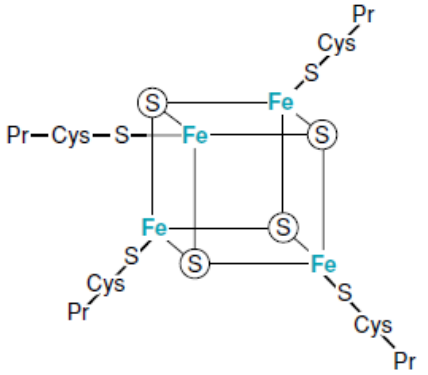
REQUIREMENTS OF OXPHOS

- Electrons' donor (NADH or FADH2) & acceptor (O_2)
- ETC of proteins
- ATP synthase
- An intact IMM

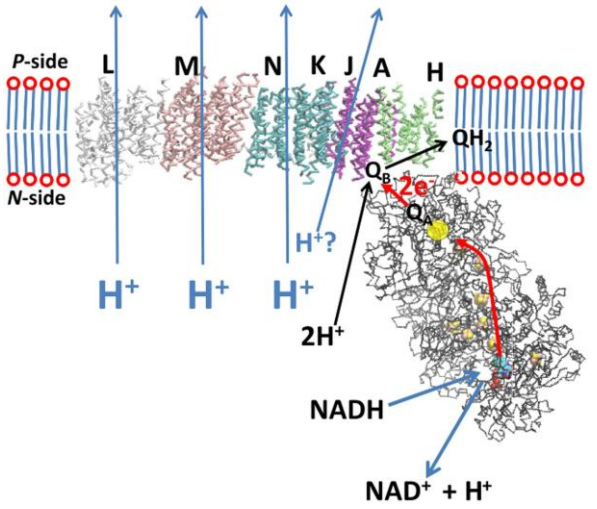


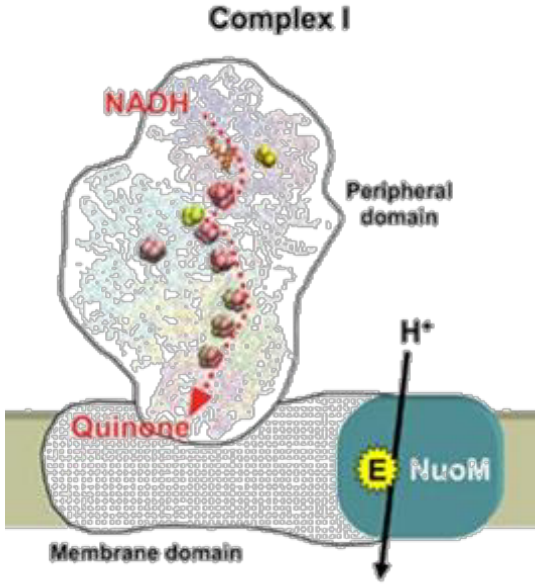
OXIDATIVE PHOSPHORYLATION (OXPHOS)

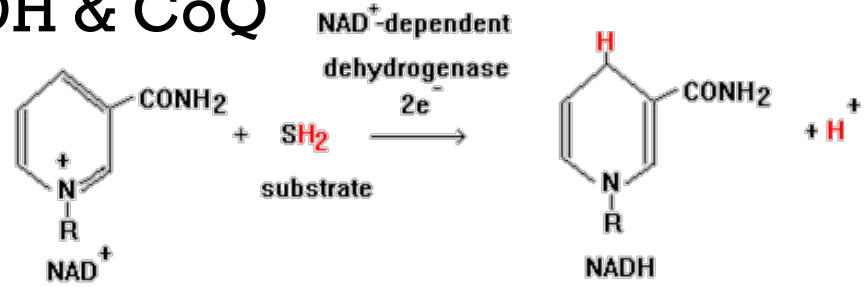
OXI–RED COMPONENTS OF THE ETC: THE NADH DEHYDROGENASE GIANT - COMPLEX I

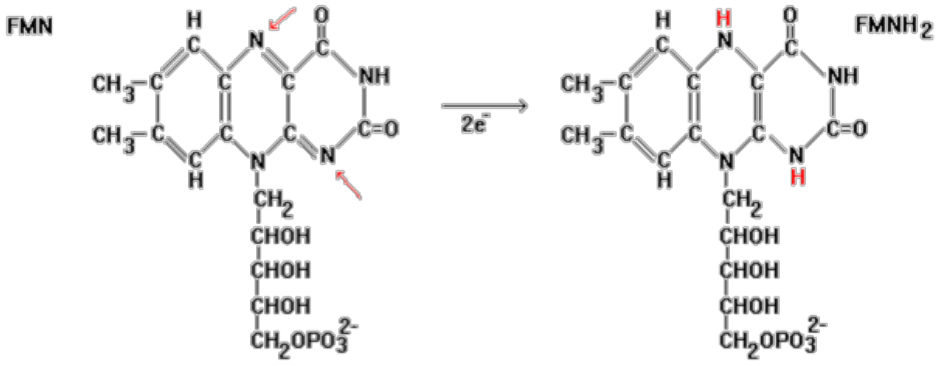


- NADH-Q oxidoreductase
- More than 25 polypeptide chain
- A huge flavoprotein membrane-spanning complex
- FMN is tightly bound
- 7-9 Fe-S centers of at least two different types
- Drop in energy ≈ 13 to 14 kcal
- Binds NADH & CoQ
- 4 H^+



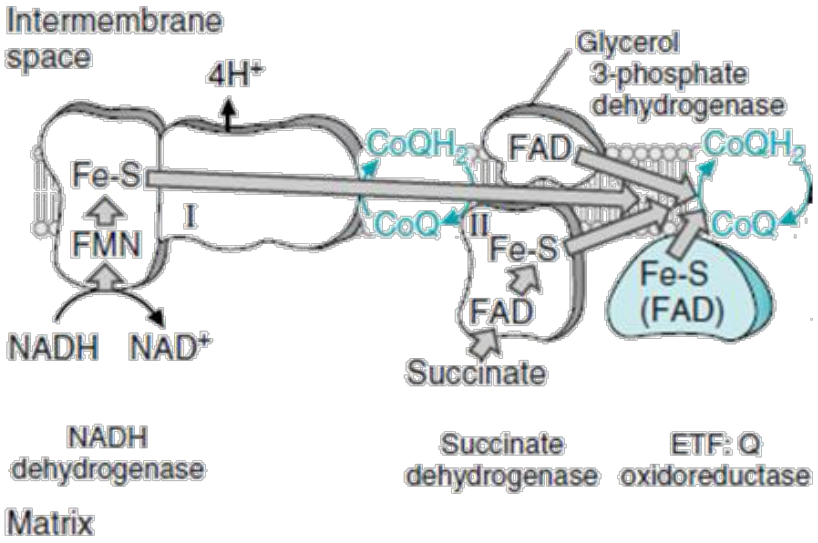


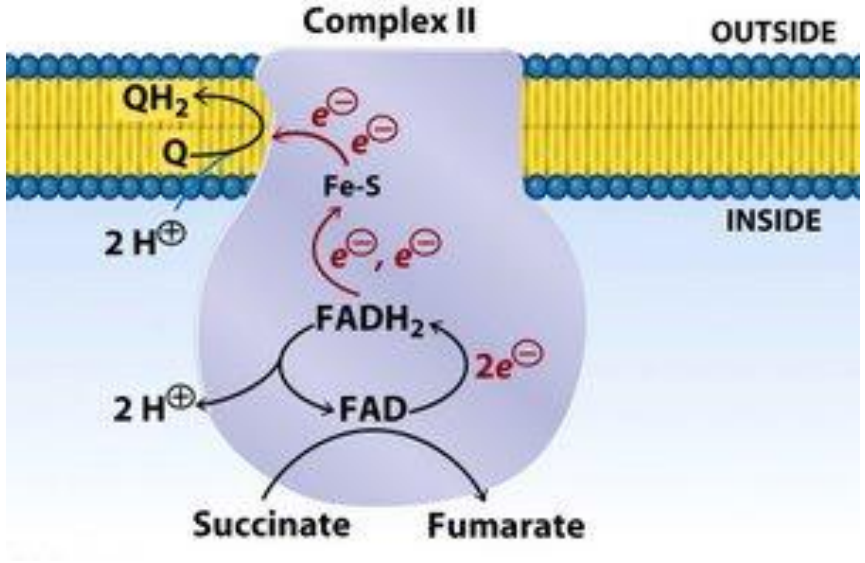




OXI–RED COMPONENTS OF THE ETC: "SUCCINATE DEHYDROGENASE" – COMPLEX II; THE CITRIC ACID CYCLE LINK

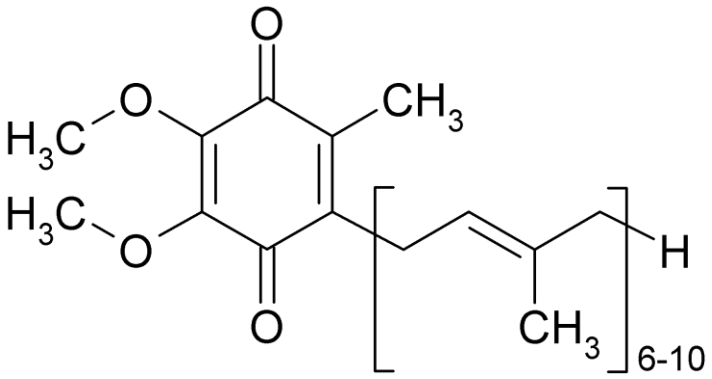
- Succinate Dehydrogenase & other flavoproteins
- TCA cycle
  - ✓ETF-CoQ oxidoreductase (ex. fatty acid oxidation); Fatty acyl CoA dehydrogenase; Mitochondrial glycerol phosphate dehydrogenase
    - ≈ 0 Kcal, H^+?

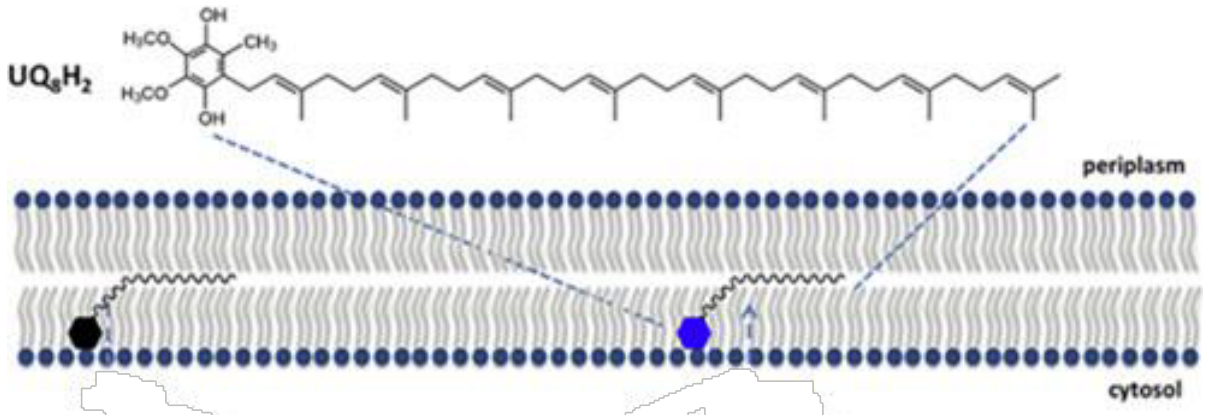


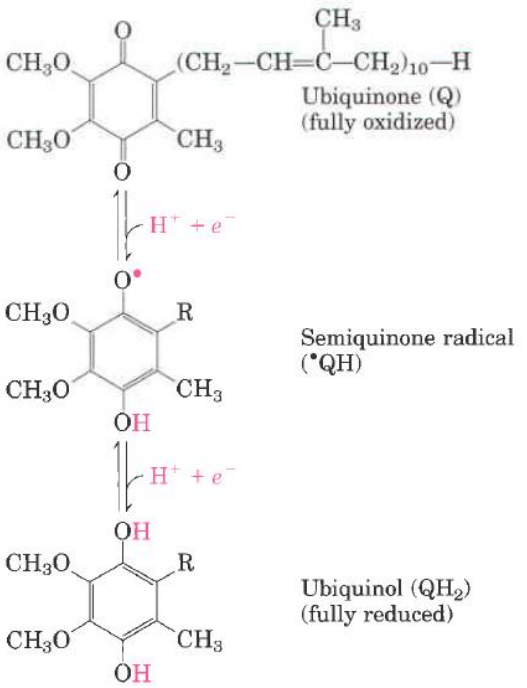


OTHER ELECTRON-CARRYING MOLECULES: "UBIQUINONE" (COENZYME Q): THE MOBILE SHUTTLE

- Lipid-soluble benzoquinone with a long isoprenoid side chain
- Small & hydrophobic (freely diffusible)
- Act at the junction between a 2(e^-) donor and a 1 (e^-) acceptor
- Sometimes prescribed for recovering MI patients

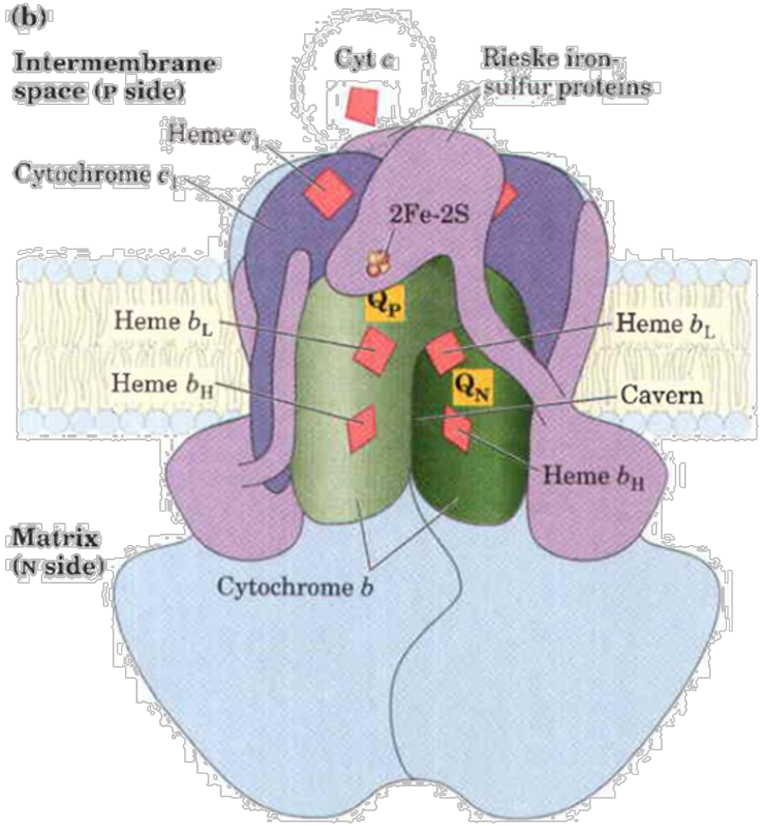


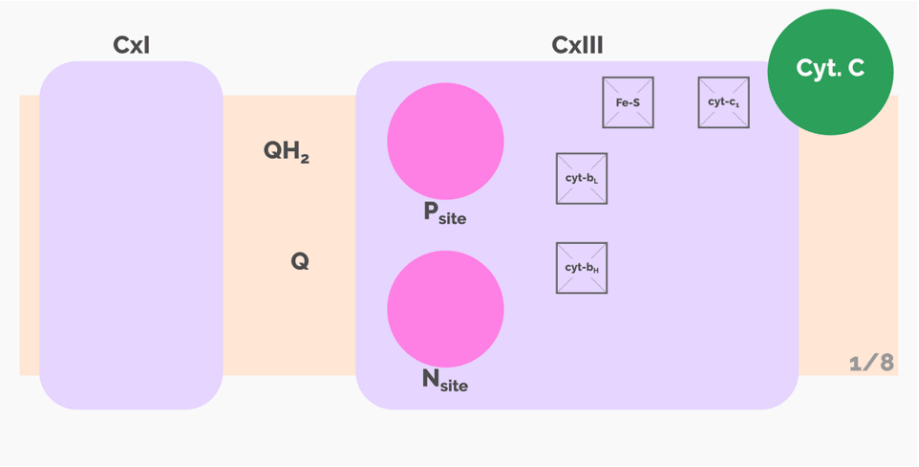


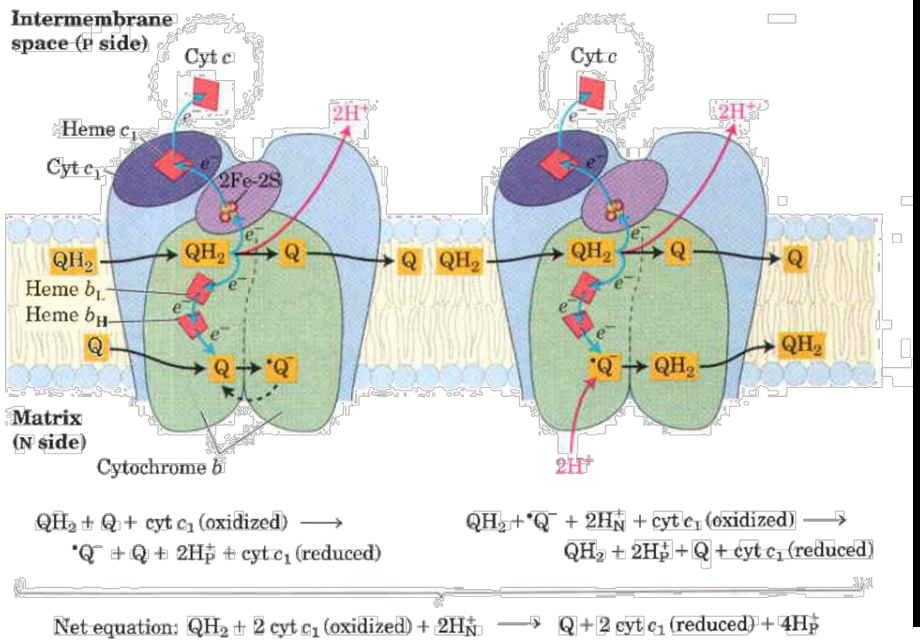


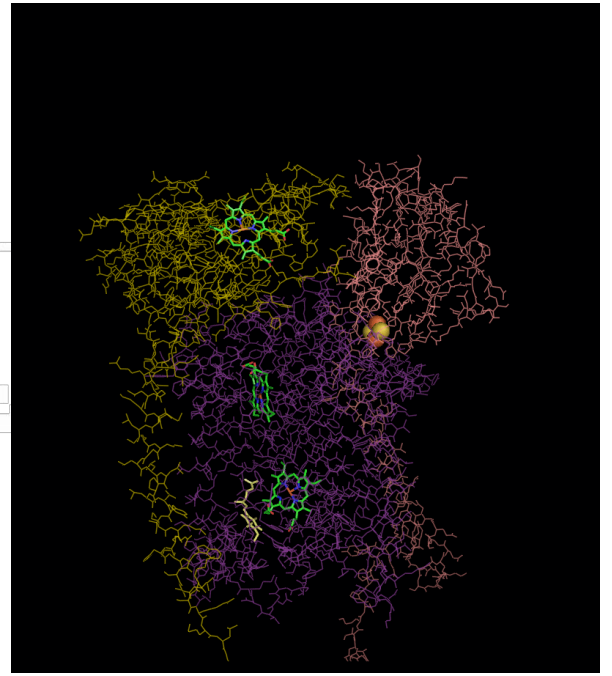
OXI–RED COMPONENTS OF THE ETC: "CYTOCHROME BC_1" – COMPLEX III

- Q-cytochrome c Oxidoreductase
- 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 c_1
- Contain two CoQ binding sites
- 4H+





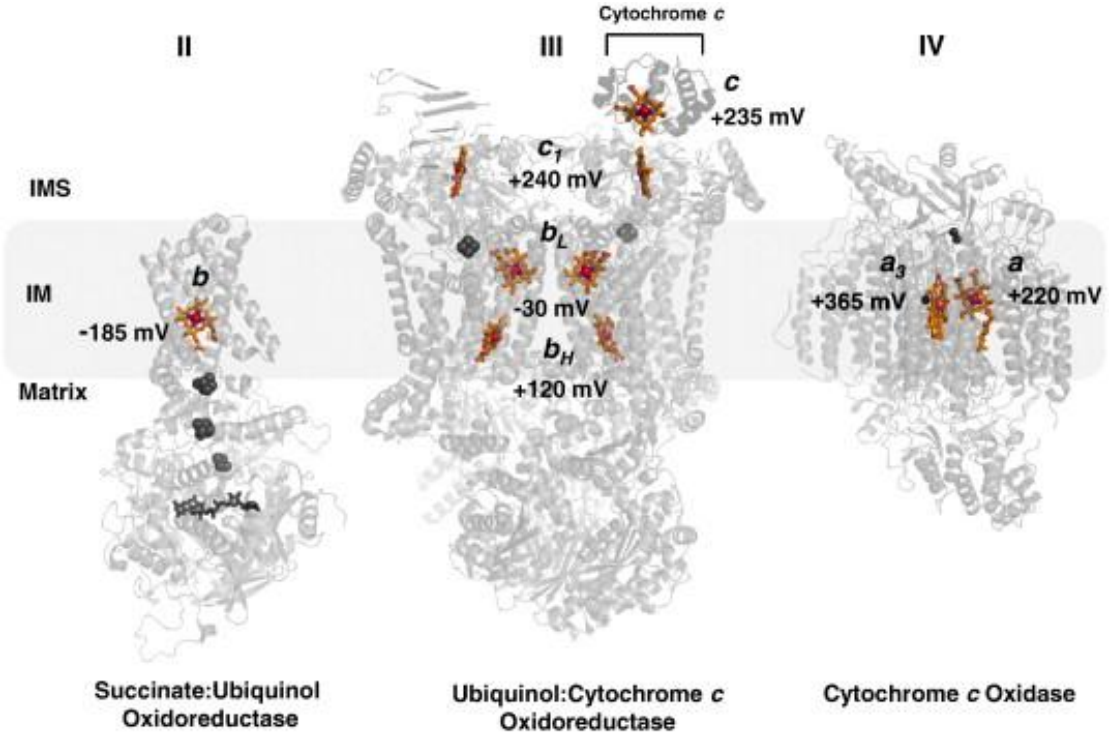


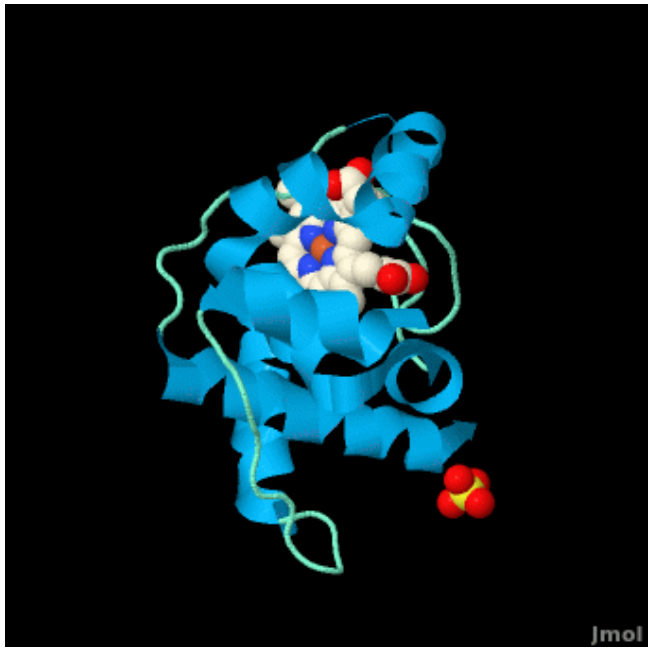


THE Q-CYCLE

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

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

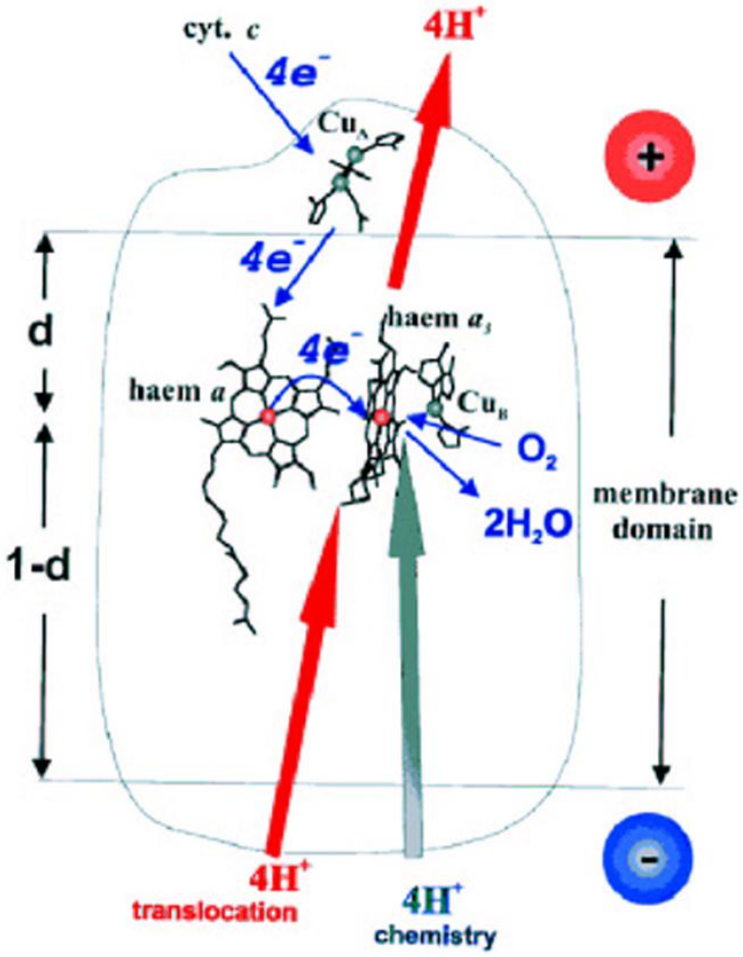




CYTOCHROME C: THE SURFACE MESSENGER

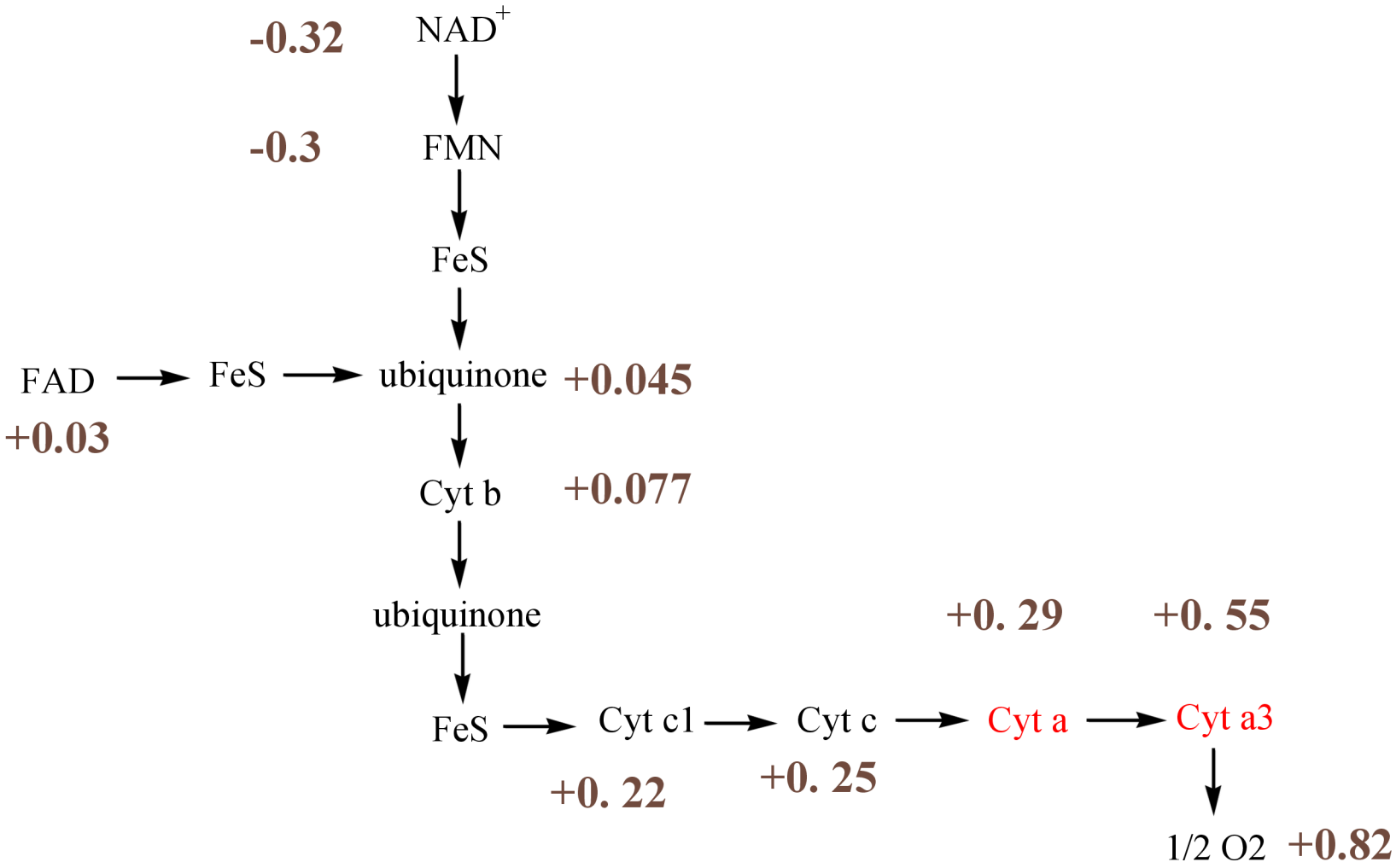
OXI–RED COMPONENTS OF THE ETC: "CYTOCHROME C OXIDASE" – COMPLEX IV

- Contains cytochrome a and a_3
- Contains two copper sites
- Contains oxygen binding sites
- O_2 must accept 4 electrons to be reduced to 2 H_2O ($2H^+/2e$-)
- Cytochrome c is one electron carrier
  - Cyt $c_{red} + 4H^+ + O_2 \rightarrow$ Cyt $c_{ox} + 2H_2O$
- Cytochrome oxidase has a much lower Km for O_2 than myoglobin (hemoglobin, myoglobin, complex IV)
- Partial reduction of O_2 is hazardous



THE RIGHT ARRANGEMENT: HOW CAN WE PROVE IT?

1. MEASURING THE STANDARD REDUCTION POTENTIALS (REAL?)



THE RIGHT ARRANGEMENT: HOW CAN WE PROVE IT?

1. MEASURING THE STANDARD REDUCTION POTENTIALS (REAL?)

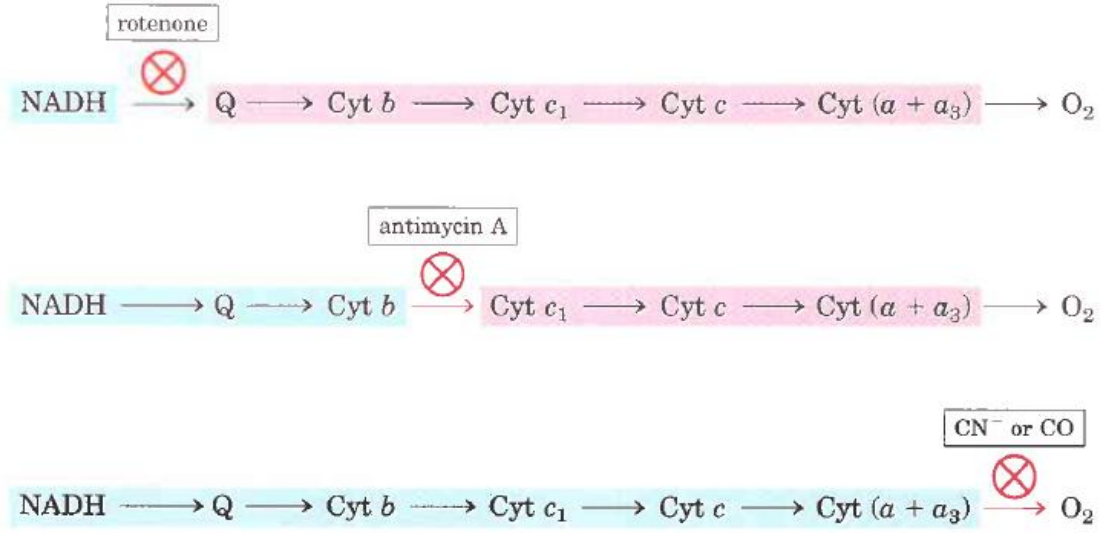
Redox reaction (half-reaction)	E'° (V)
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ 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

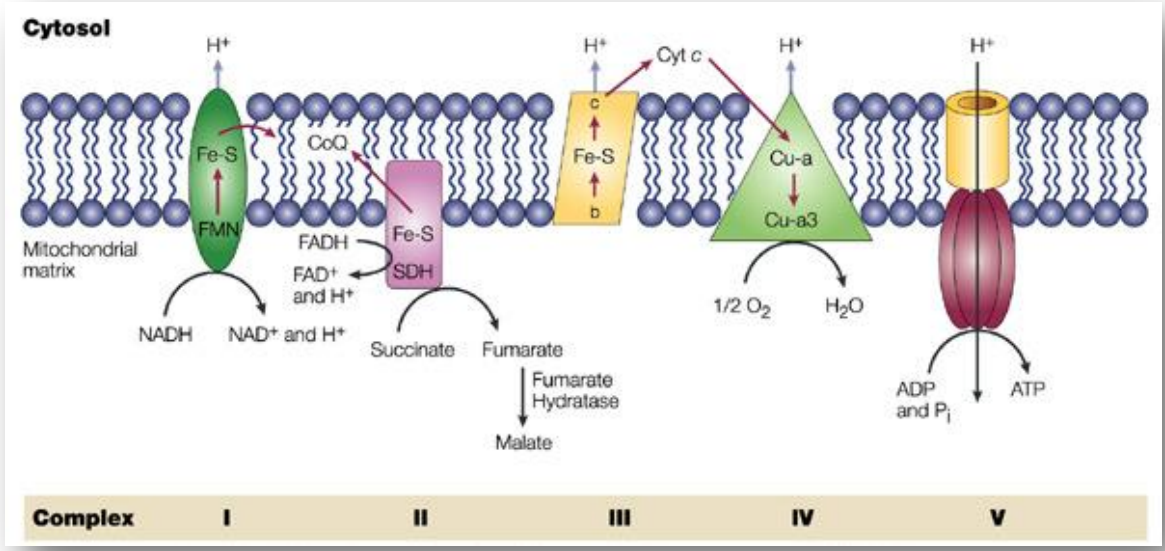
NADH → Q → cytochrome b → cytochrome c1 → cytochrome c → cytochrome a → cytochrome a3 → O2

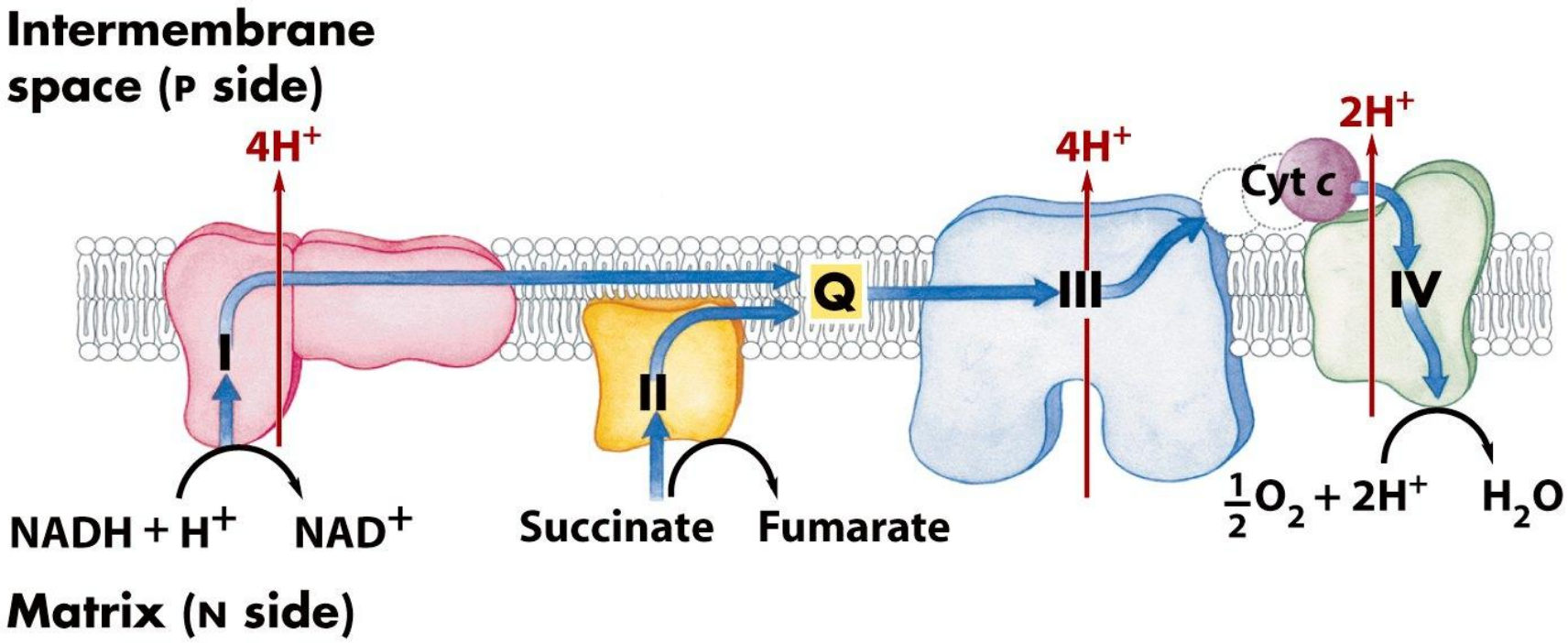
THE RIGHT ARRANGEMENT: HOW CAN WE PROVE IT?

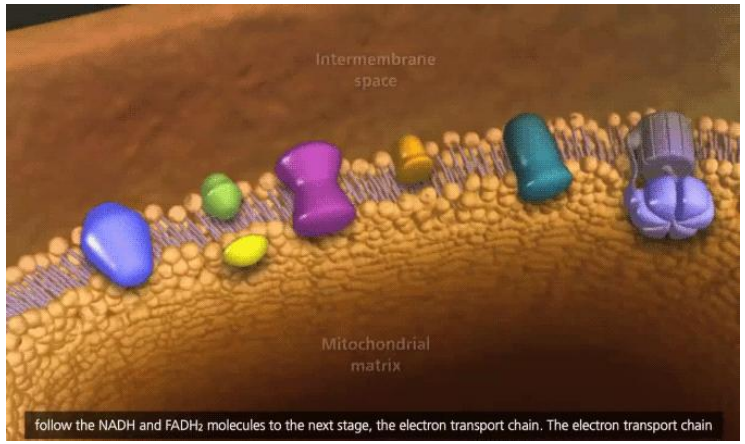
NADH → Q → cytochrome b → cytochrome c1 → cytochrome c → cytochrome a → cytochrome a3 → O2

- 2. Reduction of the entire ETC with no O_2
- 3. Addition of inhibitors









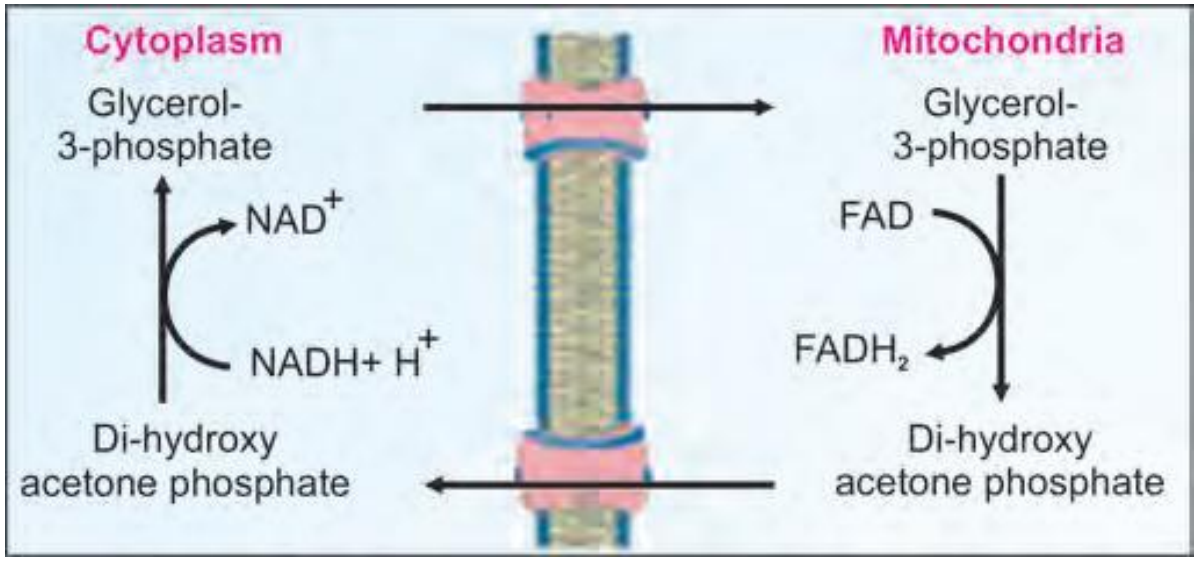
PUMPING OF PROTONS

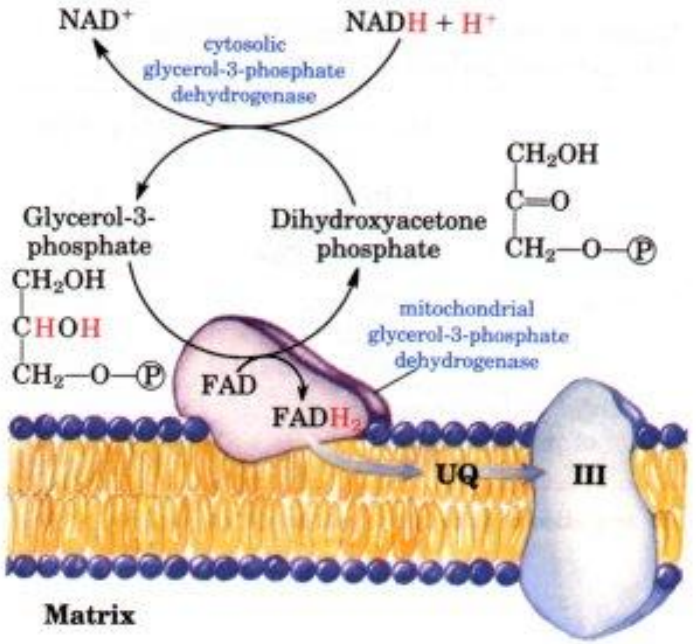
- For every 2 electrons passing:
- $4H^+$ (complex I); $0H^+$ (complex II); $4H^+$ (complex III), $2H^+$ (complex IV)

SOME 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)



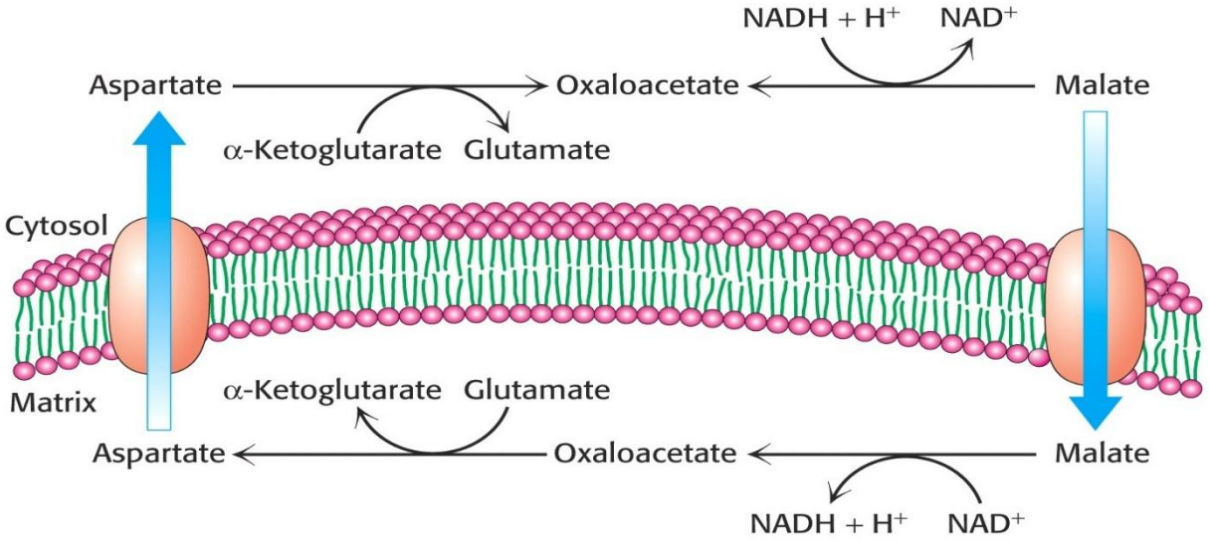


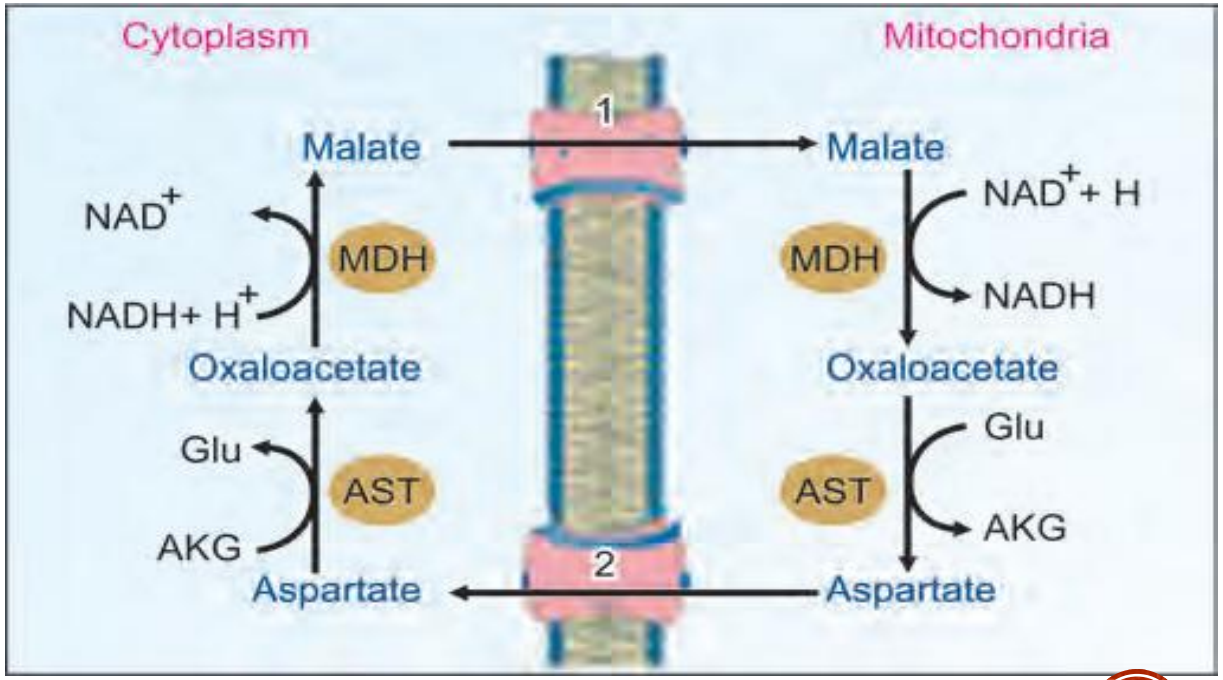
MITOCHONDRIAL SHUTTLING SYSTEMS "CYTOSOLIC NADH"

- Glycerol 3-phosphate shuttle
- In skeletal muscle and brain
- Glycolytic pathway as an example
- How NADH passes?
- ATP yield?

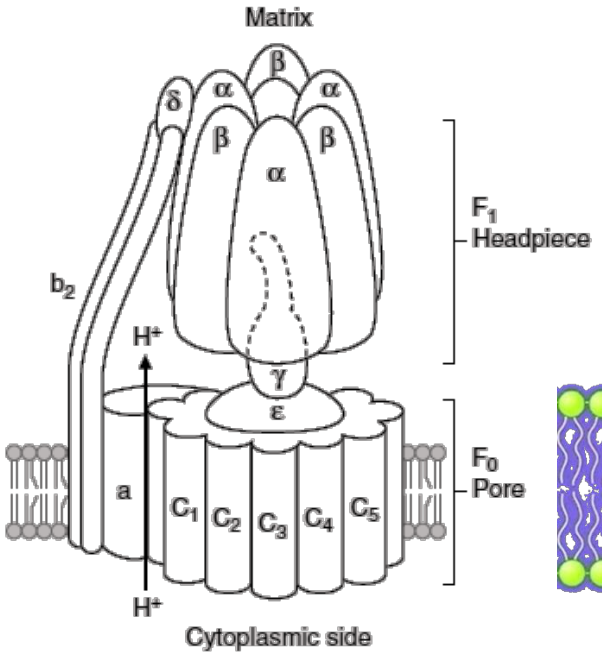
MITOCHONDRIAL SHUTTLING SYSTEMS - "CYTOSOLIC NADH"

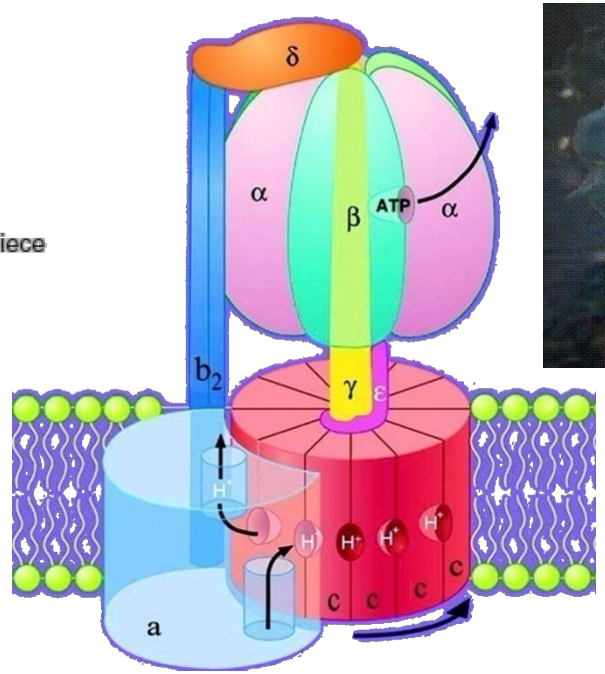
- Malate-Aspartate shuttle
- operates mainly in liver, kidney
- 2 membrane carriers & 4 enzymes
- Readily reversible (vs. Glycerol 3-phosphate shuttle)
- NADH can be transferred only if the NADH/NAD+ ratio is higher in the cytosol than in the mitochondrial matrix
- Exchange of key intermediates between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

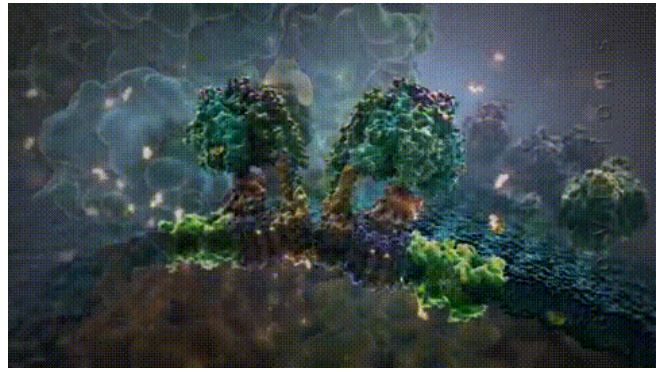


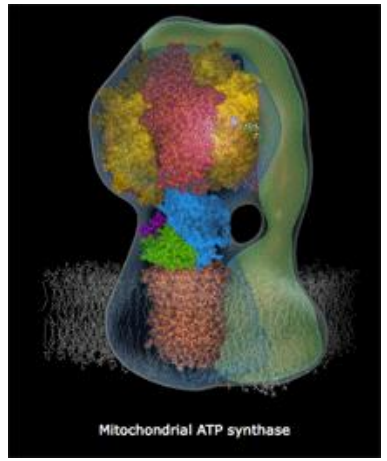


ATP SYNTHASE

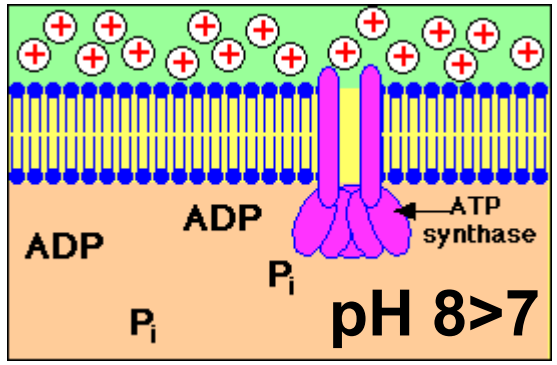


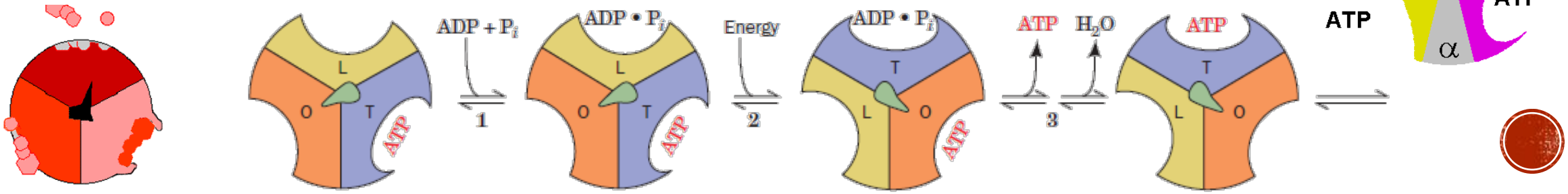


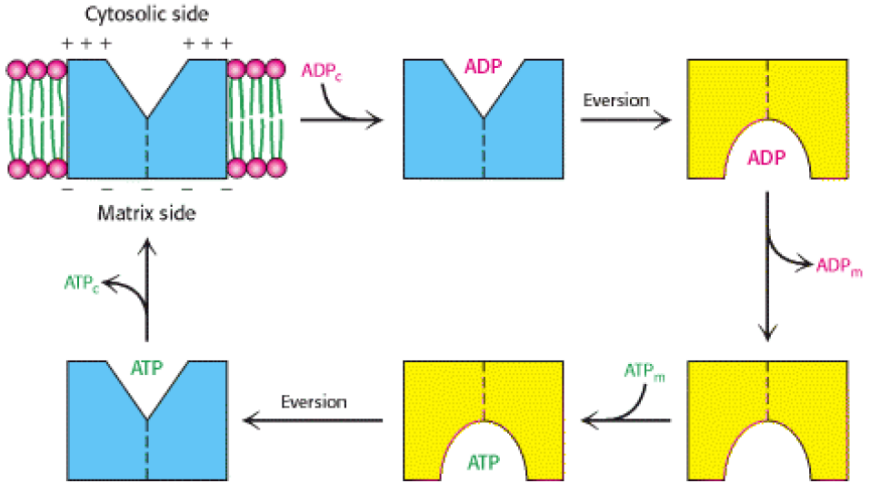


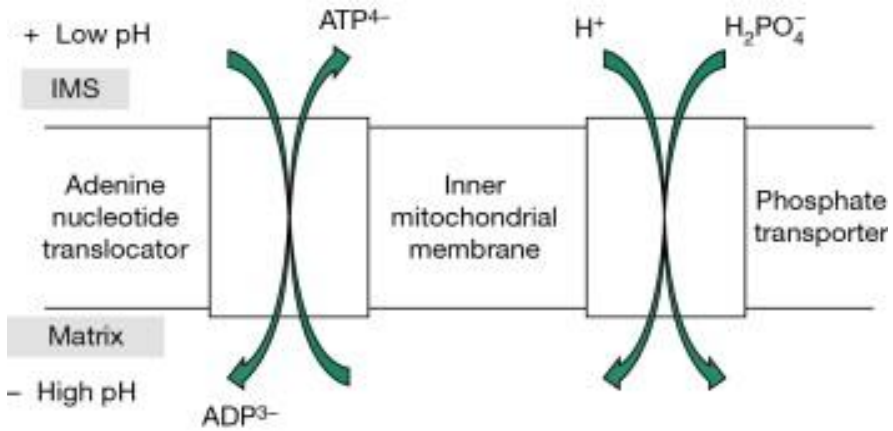


- F1:
  - "γ" subunit: rotates
  - "β" subunit: binds
  - "α" subunit: structural
  - 3 conformations: tight (T), loose (L), open (O)
- F0:
  - "a" subunit: point of entry & exit
  - "c" subunit rotates
  - 4H+/ATP
- Can run backwards



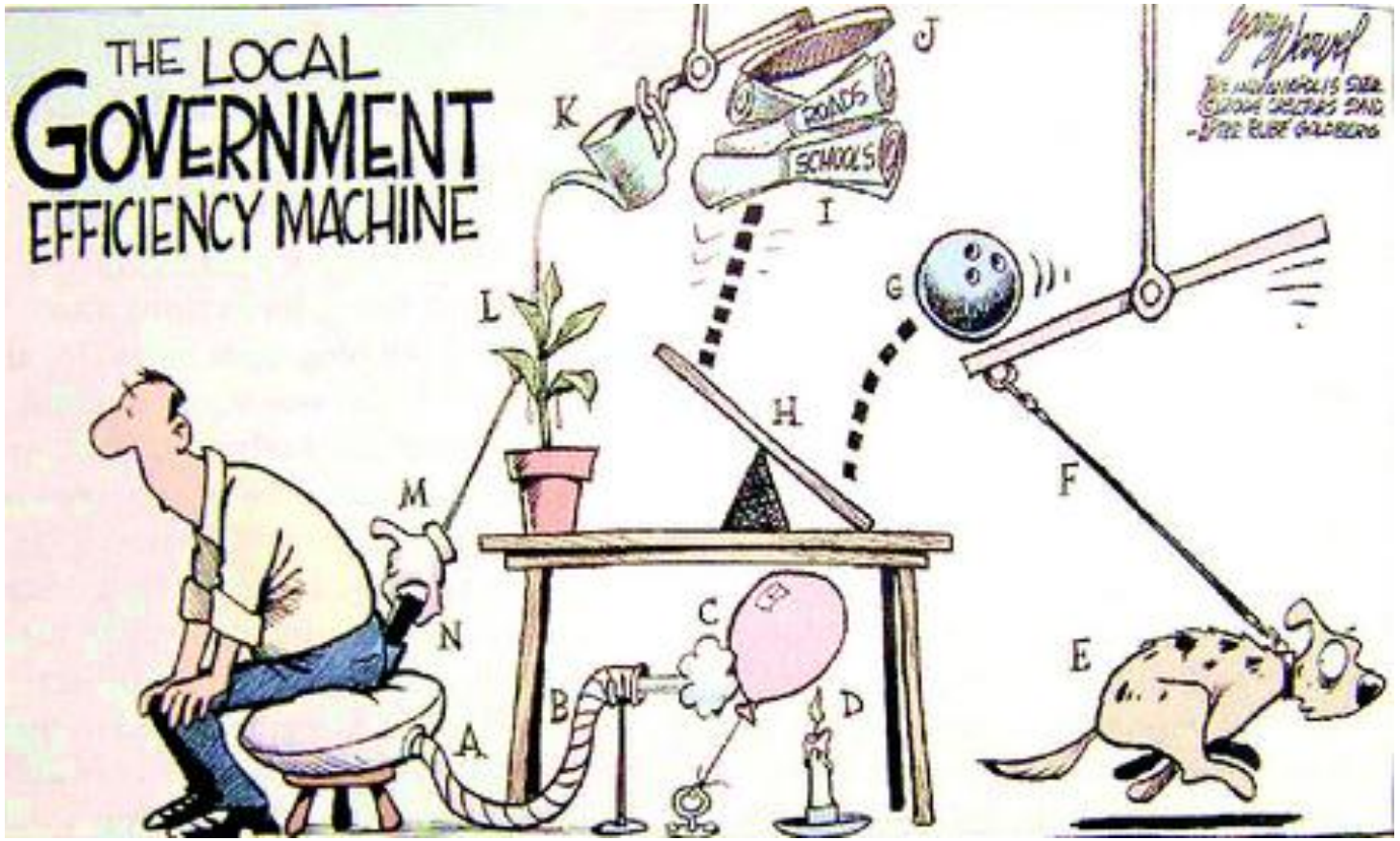






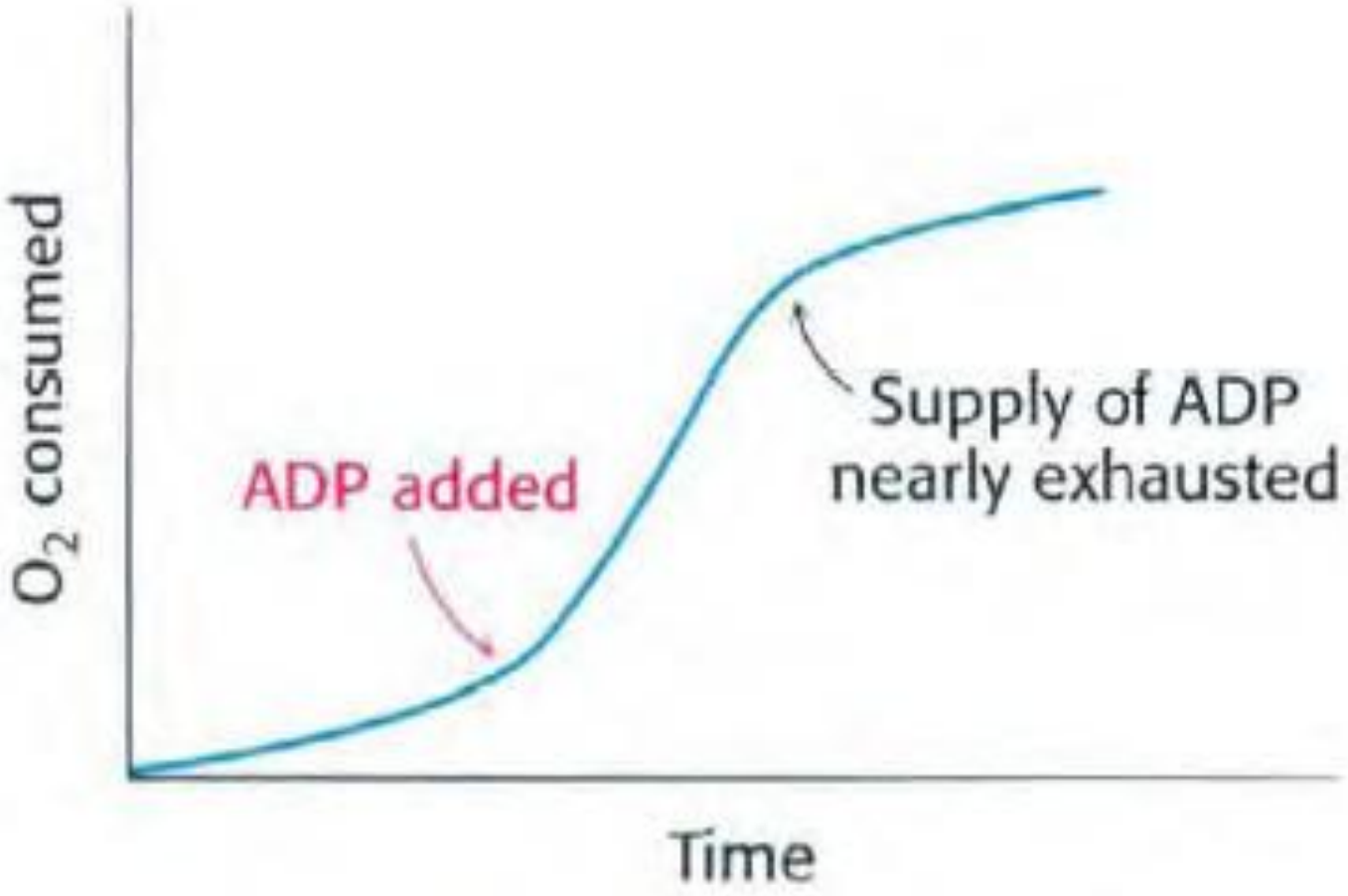
MITOCHONDRIAL SHUTTLING SYSTEMS "ATP/ADP"

- 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



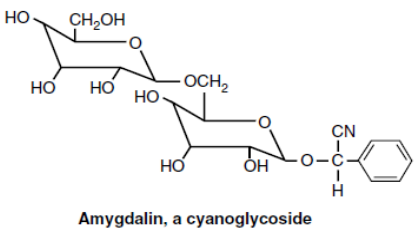
ENERGY YIELD FROM THE ETC

- NADH, -53 kcal, ATP?
- FADH2, -41 kcal, ATP?
- $\Delta G^{o'}$ is so negative, never reversible
- ATP machine efficiency, (anions, Ca^{+2}, heat, phosphate, substrates)



Regulation – the need for ATP

- 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 (<u>respiratory control</u>)

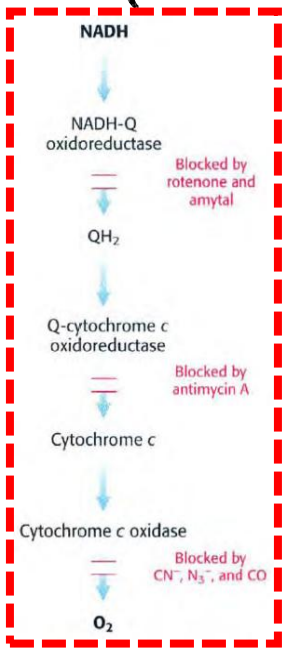


Amygdalin, a cyanoglycoside

Anit-cancerous drug

REGULATION – INHIBITION (COUPLING)

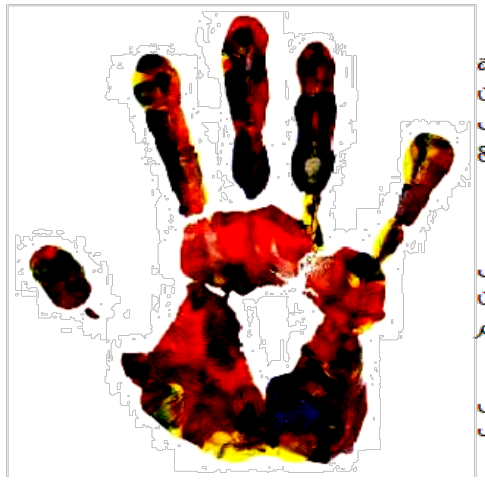
- **Specific inhibitors:**
  - ✓ **Cyanoglycosides such as amygdalin are present in edible plant pits**



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 -

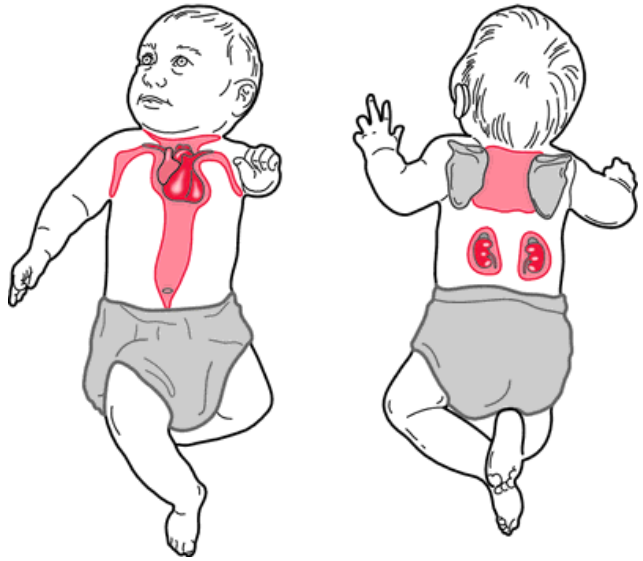
جراسا -تعرض فيما يلي قائمة بأشهر جرائم القتل العائلية التي حدثت في الاردن خلال السنوات الماضية ، والتي كان لكل منها وقع الصدمة حين وقوعها لما تمثله من فعل غريب على المجتمع وأعرافه ، فضلا عن مخالفتها الشرائع السماوية والقوانين الناقذة والطبيعة الإنسانية بعامة.

قضية السيانيد

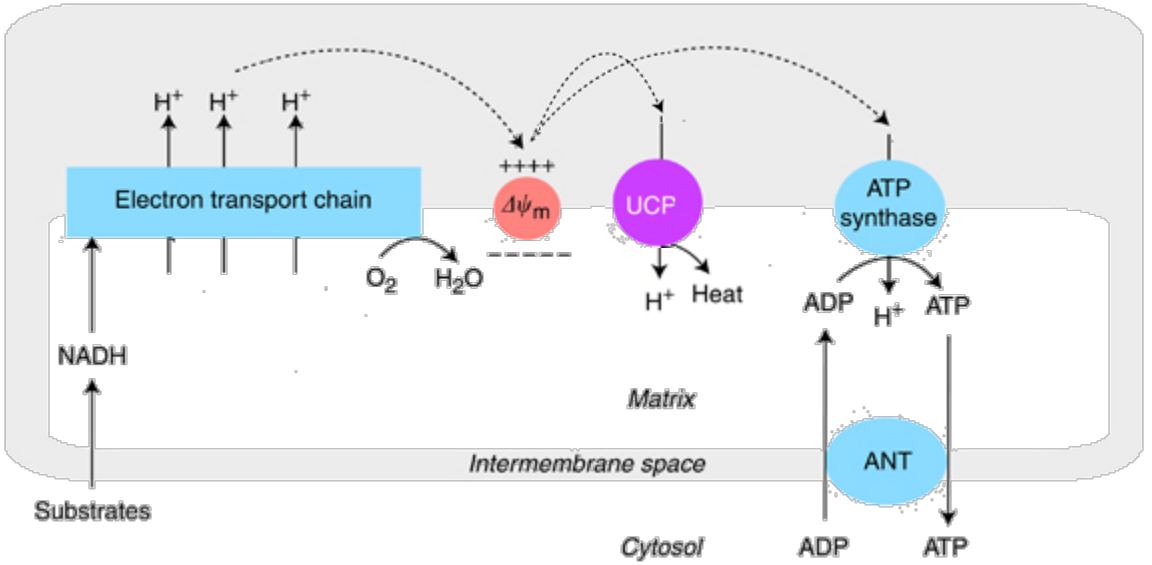
أول جريمة من نوعها يرتكبها أب ضد ولديه ، اذ قام الاب بوضع مادة السيانيد في كأس الحليب وطلب من طفليه ان يشربا منه ، حيث فارقا الحياة بعد 10 دقائق من مغادرة الام المنزل لتعود وتجدهما جثتين هامدتين.

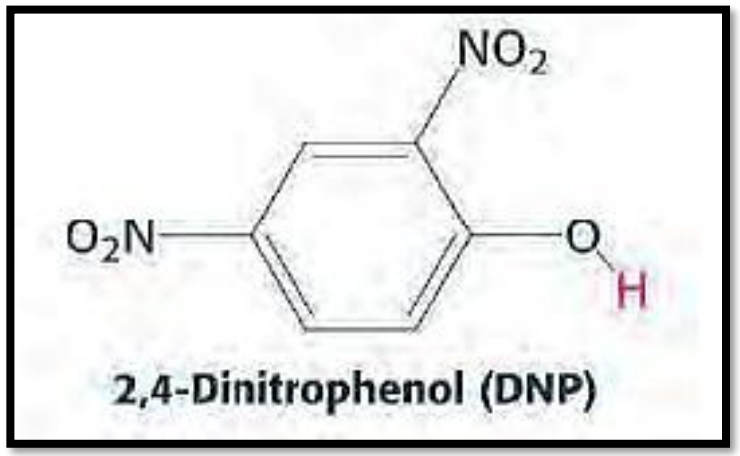
وقد ادين الاب بعقوبة الاعدام شنقا الا ان والده اسقط الحق الشخصي كونه وليا عن الطفلين وحكم عليه بالاشغال المؤبدة.

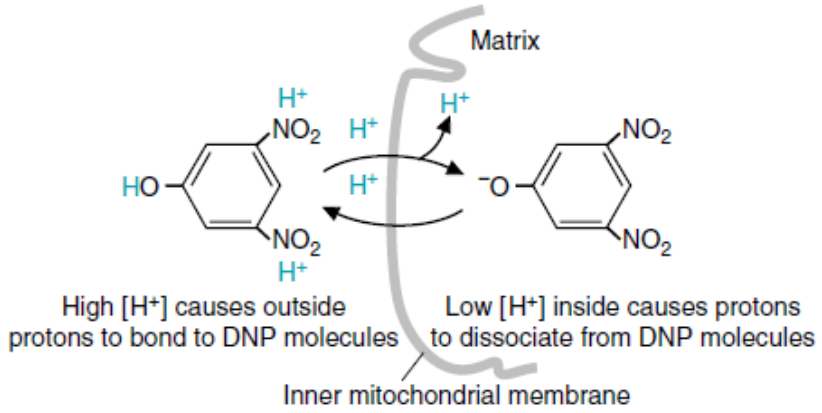
REGULATION – UNCOUPLING
REGULATED – UNCOUPLING PROTEINS (UCPS)



- 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

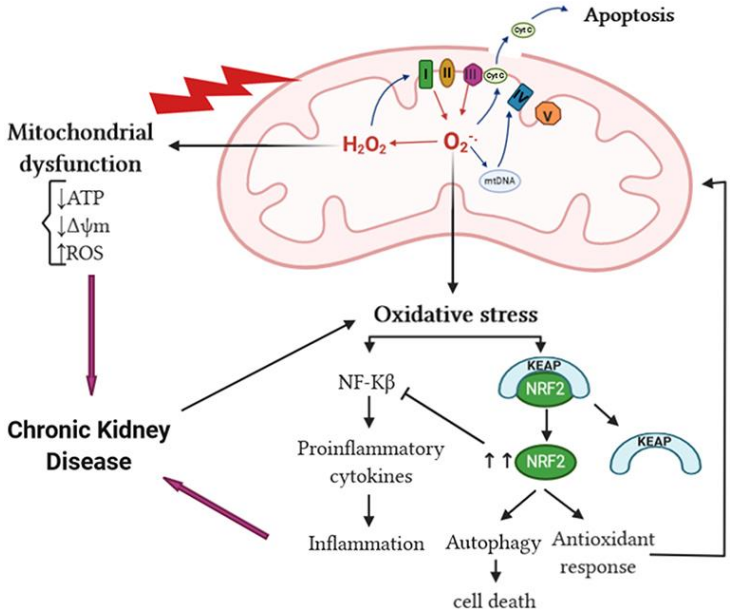


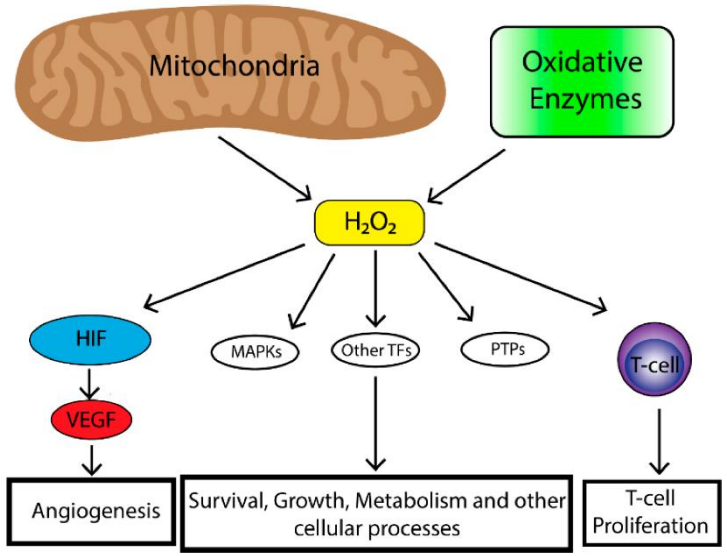




REGULATION – UNCOUPLING
UNREGULATED – CHEMICAL UNCOUPLERS

- 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)

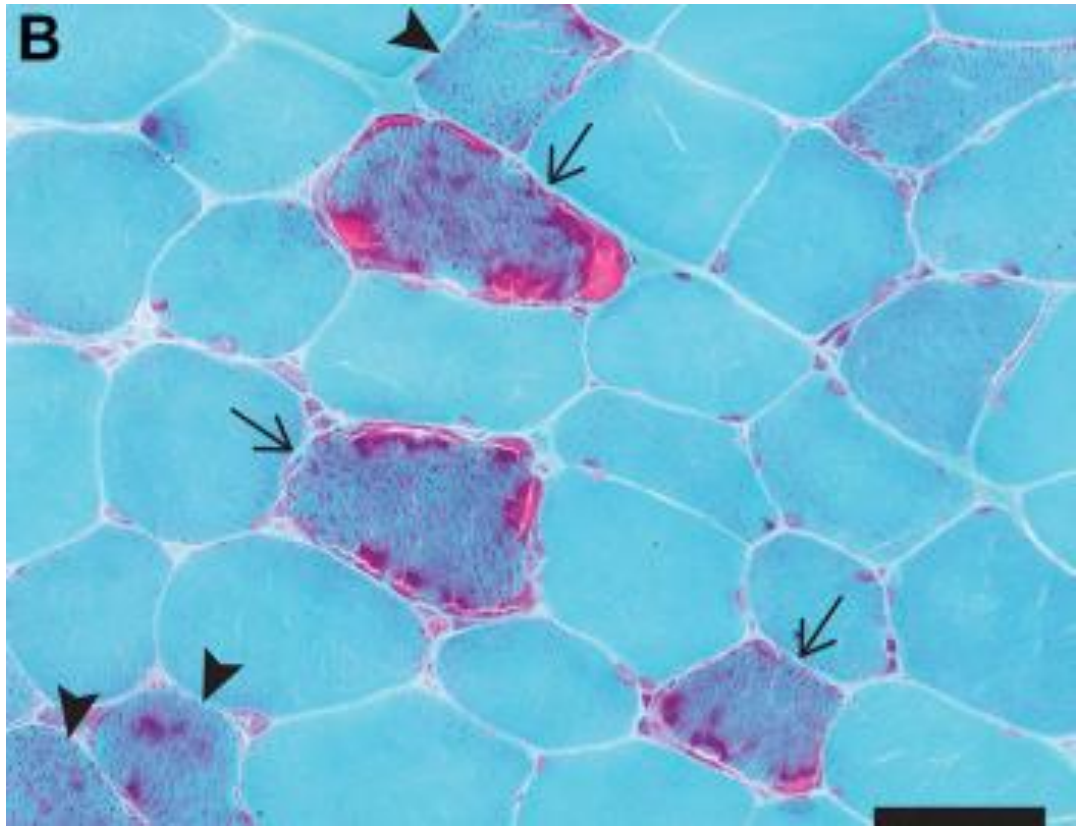




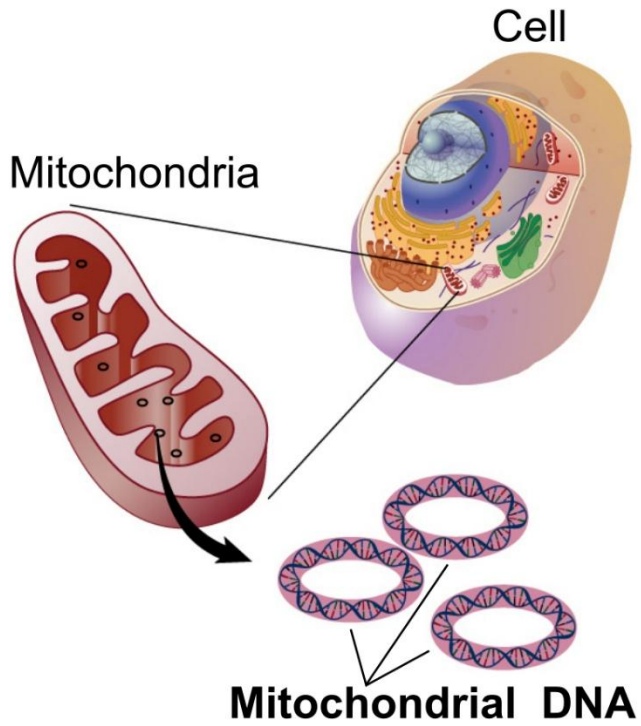
THE DARK SIDE: REACTIVE OXYGEN SPECIES (ROS)

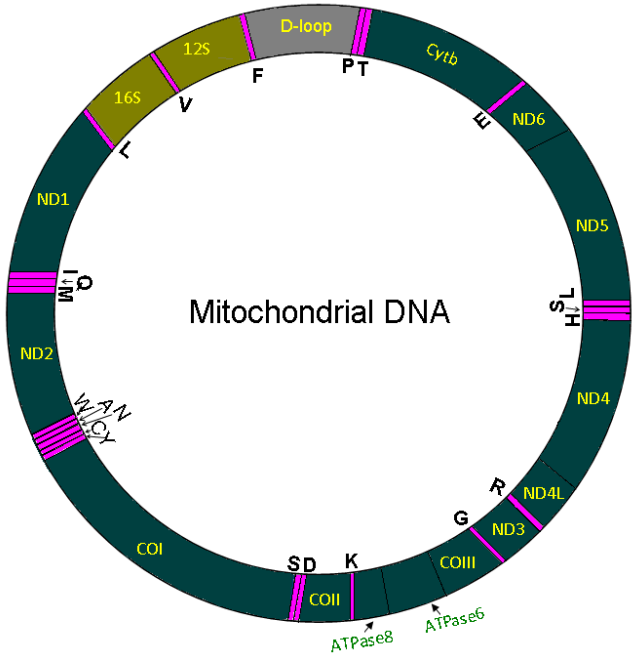
- E.g., FMN, semiquinone in Q cycle
- Single electrons can leak and reduce O_2 to superoxide ($O_2^{\bullet-}$)
- **Pathophysiology:**
  - **Primary ROS:** Superoxide ($O_2^{\bullet-}$). Converted by Superoxide Dismutase (SOD) to H_2O_2
  - **Damage:** lipids (peroxidation), proteins (carbonylation), and DNA (strand breaks)
  - **Role in Disease:** Contributes to aging, neurodegenerative diseases (Parkinson's, Alzheimer's), ischemia-reperfusion injury, and inflammatory damage

MITOCHONDRIAL DISEASES: GENETICS AND PRINCIPLES



- A muscle biopsy with "ragged red fibers" (Gomori trichrome stain)
- Etiology:
- nDNA or mtDNA
- mtDNA Inheritance: Maternal. High mutation rate, no histones, poor repair.
- Heteroplasmy: A mixture of wild-type and mutant mtDNA within a cell.
- Symptoms appear when the mutant load exceeds a threshold.
- Tissues Affected: High-energy demand tissues: CNS, muscle, heart, liver, kidney.



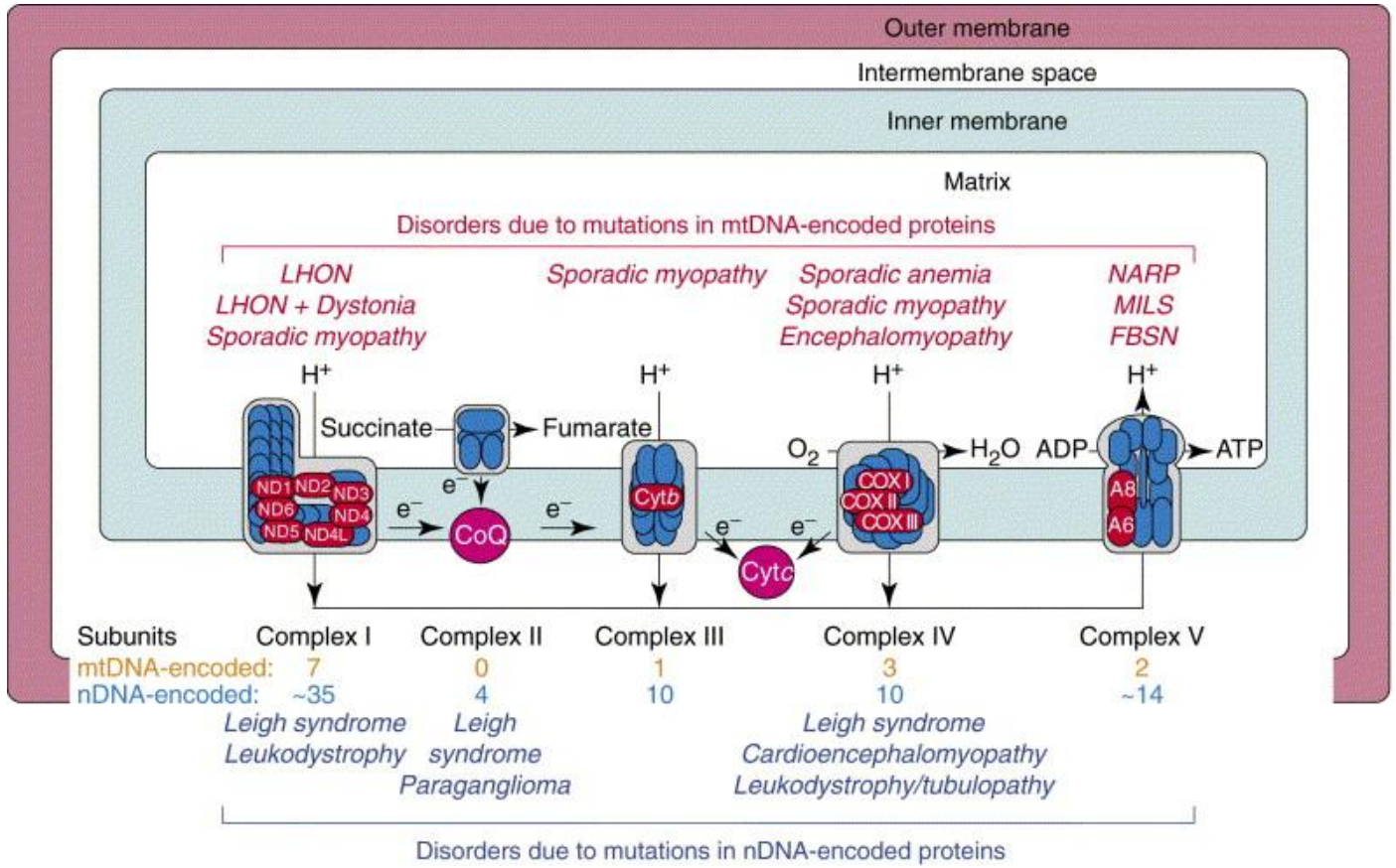


OXPHOS DISEASES – 1 (GENETIC)

- 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 and heteroplasmy
  - Accumulation of somatic mutations with age
  - Highest ATP demands: CNS, heart, skeletal muscle, and kidney, liver

OXPHOS DISEASES – 2 (GENETIC)

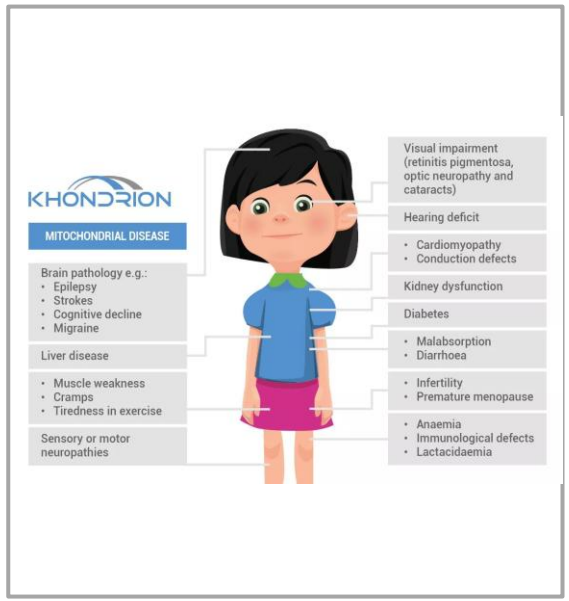
- Nuclear Genetic Disorders of Oxidative Phosphorylation
  - 1,000 proteins
  - Usually autosomal recessive
  - Expressed in all tissues
  - Phenotypic expression with high ATP demand

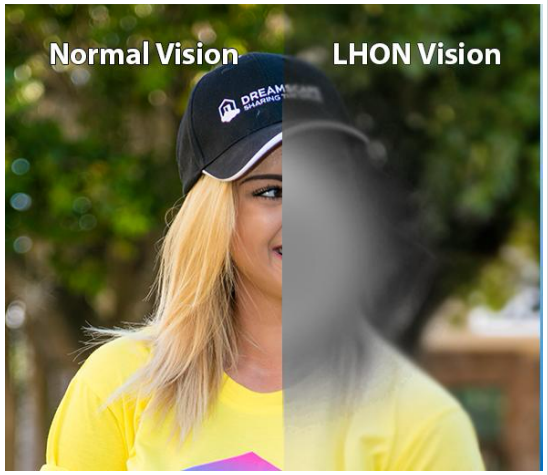


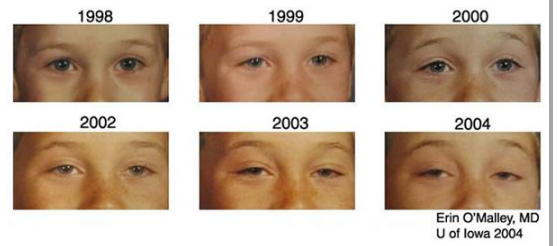
OXPHOS DISEASES (GENETIC)

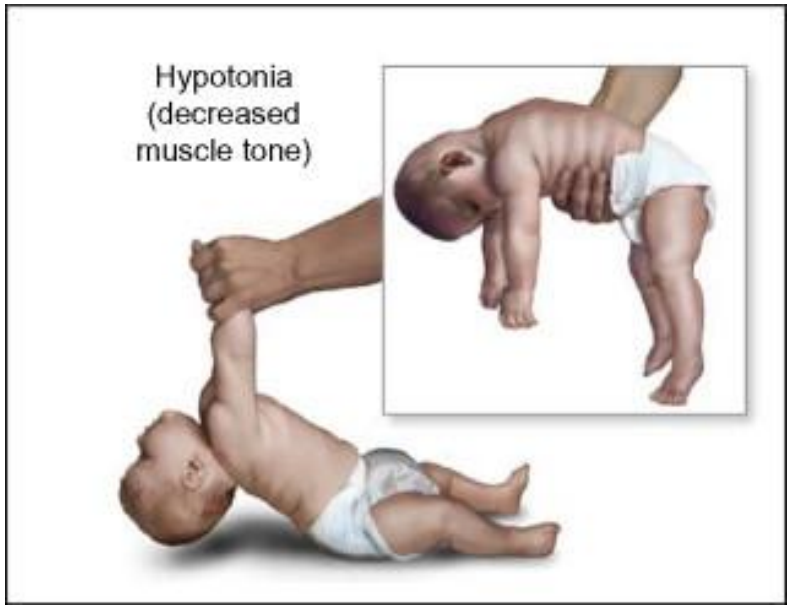
Mitochondrial Diseases 2: Clinical Examples

- **Examples:**
  - **Leber's Hereditary Optic Neuropathy (LHON):** mutation in Complex I. Causes sudden, painless bilateral vision loss in young adults.
  - **MELAS:** Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes. Caused by mtDNA point mutation.
  - **Chronic Progressive External Ophthalmoplegia (CPEO):** ptosis and inability to move the eyes. Often associated with large-scale mtDNA deletions.



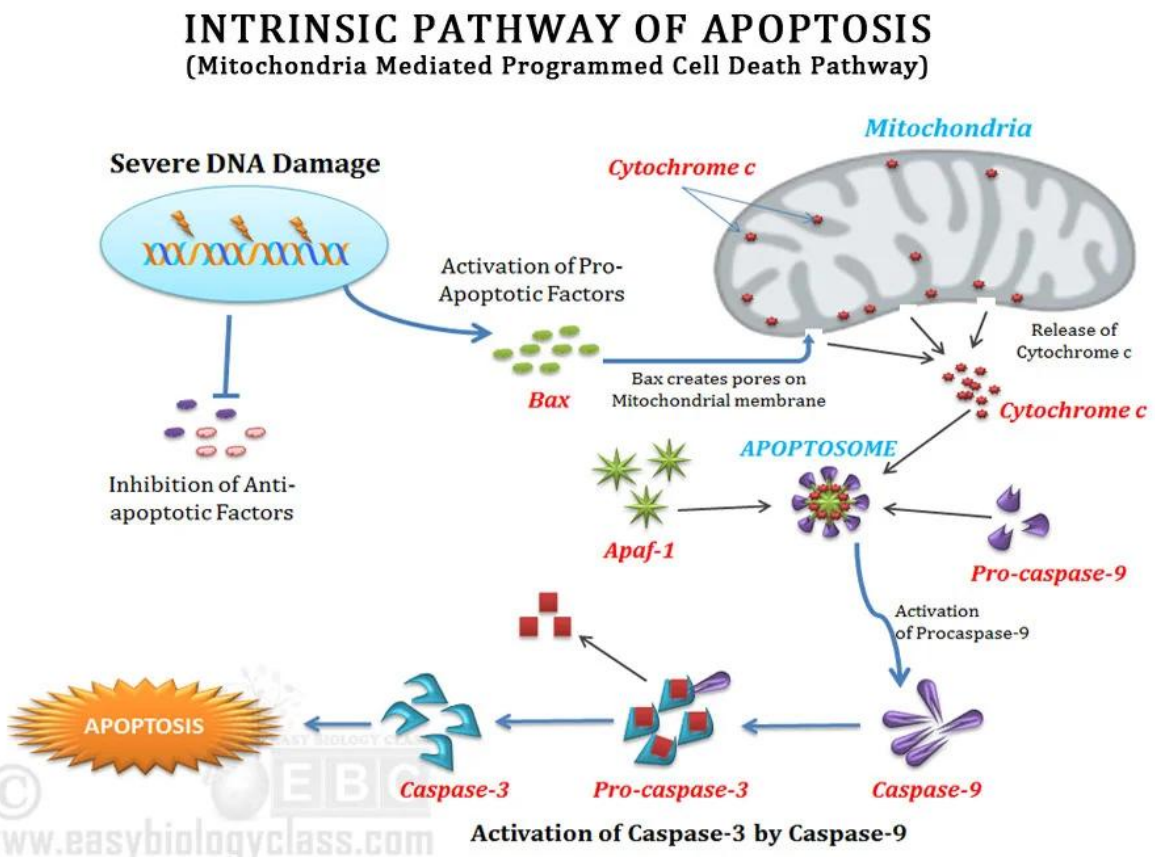






INTERACTIVE CASE STUDY
CLINICAL CORRELATION: A DIAGNOSTIC PUZZLE

- **Presentation:**
  - A 4-year-old boy presents with progressive muscle weakness, exercise intolerance, and developmental delay.
  - Lab tests: profound **lactic acidosis**.
  - Muscle biopsy: **ragged red fibers**.
- **Questions:**
  - What is the most likely category of disease?
    - **(Mitochondrial Myopathy)**
  - What is the biochemical basis for lactic acidosis?
    - **(Impaired OXPHOS → anaerobic glycolysis)**
  - What molecular tests could be ordered?
    - **(mtDNA sequencing, nuclear gene panel for OXPHOS proteins)**



BEYOND ENERGY: MITOCHONDRIA IN APOPTOSIS (CELL DEATH) AND AGING

- **Apoptosis:** In response to severe stress, mitochondria release Cytochrome c and other proteins, which activate caspases and trigger programmed cell death.
- **The Mitochondrial Theory of Aging:** Accumulation of mtDNA mutations over a lifetime → progressive decline in OXPHOS capacity → increased ROS production → further damage → tissue dysfunction and aging.