#11: Oxidative Phosphorylation

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Cofactors

- A protein bound, non-protein chemical species to perform a reaction
- Organic molecules (NADH, Coenzyme A)

Types of cofactors:
- Metal ion
- Coenzyme

Types of Coenzymes:
- Cosubstrate
  - Temporarily bound
- Prosthetic Group
  - Permanently bound

Electron Carriers

- Ordered from lowest → highest affinity
- Not absolute
  - Protein environment, structure may affect affinity

Nicotinamide
- NAD, NAD^+ (oxidized)/NADH (reduced)
- Carry 2 e^-
- Transient binding

Flavins
- FAD, FADH
- From riboflavin (vitamin B2)
- Carry 2e^-, 2 protons → are reduced
- Prosthetic groups (not free molecules)

Iron-sulfur centers
- Fe and S covalently bind to proteins via cysteine residues (Fe coordinated by S)
- Each Fe^-S center carries 1 e^- without accompanying proton
- Number of atoms differ (2 Fe 2 S….4 Fe 4S…..but still 1 e^-)

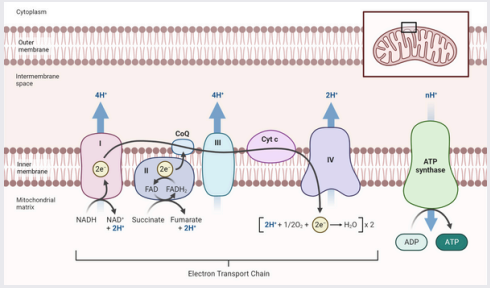
Ubiquinone
- QH (oxidized), QH_2 (fully reduced)
- Predominantly hydrophobic lipid
- Transient binding
- Freely diffuse in hydrophobic phase of inner mitochondrial membrane (temporarily associated with proteins) to gain/lose e^-
- Carry 2 e^-, 2 protons

Cytochromes
- Heme-containing proteins
- Accept 1 e^- (Fe 3+ → Fe 2+)
- Tetra pyruvate ring with coordinated ion in the middle
- Cyt. c = only cytochrome that is a peripheral protein of the IMS
- Others are integral proteins embedded in larger protein complexes

Copper Centers
- Copper ions covalently bonded by histidine
- Each copper accepts 1 e^- (Cu 2+ → Cu+)

Mitochondrial e^- transport System

- 3 large protein complexes in the inner mitochondria membrane transfer O_2
- Enzymes, cofactors catalyze e^- transfer and H^+ across membranes
- Pick up e^- from Krebs cycle → give to proteins → reduce O_2 → H_2O
  - NADH and QH_2 come from the citric acid cycle
  - NAD^+ and Q go to the citric acid cycle
- Energetically **favourable** process
  - H^+ concentration is higher in the IM space than the matrix → makes a H^+ gradient → harness this as potential energy to move H^+ from the matrix to the IM space



Sourced from https://biologynotesonline.com/electron-transport-chain/

Summary:
- NADH give e^- to cofactors → Q (Complex I)
- QH_2 → III → accept e^- → cyt c (Complex III)
- Cyt. c → IV → accept e^- → O_2 → H_2O (Complex IV)
  - All pump H^+ from the matrix → IMS
  - Reduction potential increases from I → III → IV
    - IV has the highest affinity for e^-
- Takes 4 e^- to reduce 1 O_2 → 2 H_2O
- Each NADH carries 2 e^-
- 2 NADH needed to reduce 1 O_2

Complex I: NADH dehydrogenase

Overall Reaction:
NADH + Q + 5 H^+ (matrix) → NAD^+ + QH_2 + 4 H^+ (IM Space)

- Protein contains flavin and Fe/S centres
- Reaction catalyzed by NADH dehydrogenase
- Accept 2 e^- from NADH (matrix)
- NADH → NAD^+ (for the krebs cycle)
  - Occurs at the flavin center
- e^- passed from flavin/FAD → Fe^-S centers → Q
  - Q → QH_2 (gains 2 H^+ at the matrix)
- QH_2 diffuses in the mitochondria inner membrane → pass e^- to Complex III
- As e^- pass, a conformational change in the protein complex occurs, pumping 4 H^+ from the matrix into the IM space

Complex III: Cytochrome b-C1 complex

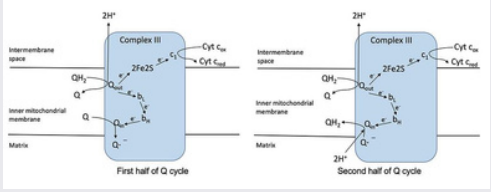
Overall Reaction:
QH_2 + 2 cyt c (ox) + 2 H^+ (matrix) → Q + 2 C cyt c (red) + 4 H^+ (IM space)

- 3 heme groups, 1 Fe^-S center used
- Accept 2 e^- from QH_2 → cyt c (Q cycle)
  - 2 cyt c move to IV (reduced)
- Q returns to inner membrane
- 2 H^+ removed from matrix
- 4 H^+ removed (from QH_2 → Q)

Q cycle

- Complex III has two coenzyme Q binding sites
- Heme bH, heme bL
  - Heme bH near matrix → higher affinity for Q
  - Heme bL near IM space → higher affinity for QH_2
- IM has mix of oxidized + reduced coenzyme Q
  - 3 coenzyme Q molecules (2 reduced 1 oxidized)

Overall Reaction:
QH_2 + 2 cyt c (ox) + 2 H^+ (matrix) → Q + 2 cyt c (red) + 4 H^+ (IM Space)



Sourced from https://www.stemside.co.uk/post/an-overview-of-the-q-cycle

Q cycle Process

Round 1
- 2 molecules: Q and QH_2 bind to their binding sites
  - Based on affinity
- QH_2 loses:
  - 1 e^- → forms Q•- → Fe^-S → cyt → cyt c (reduced)
  - 2H^+ → released into IM space
  - Last e^- → transferred to heme bL → bH → Q
- QH_2 oxidized to Q
- Leaves, replaced by QH_2 from IMS

Round 2
- New QH_2 loses e^- in the same way and forms Q•-
- 2H^+ moved into IM space
- e^- lost → Fe^-S → heme c1 → reduces second cyt c
  - QH_2 –(lose 2H^+ and 1 e^-)→ Q•- (lose 1 e^-) → Q
- Afterwards, Q gains 2H^+ from the matrix → become QH_2

Aftermath
- Both Q and QH_2 no longer match the binding preferences of the 2 sites → dissociate
- Cyt c (x2) move to complex IV

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Complex IV: Cytochrome Oxidase Complex

Overall Reaction:
4 cyt. c (red) + O_2 + 8 H^+ (matrix) → 4 cyt. c (ox) + 2H_2O + 4H^+ (IM Space)

- Oxidize cyt. c by accepting e^-
- 3 sites
  - e^- accepting site with 2 copper ions
  - Site with heme
  - Site with heme + copper ion
- e^- flow through, reduce O_2 → H_2O
- 4 H^+ pumped out of matrix
- Per O_2 reduced, 4 cyt. c give up e^- one at a time, 2 H_2O are formed

Complex II: Succinate Dehydrogenase

Overall Reaction:
Succinate + Q → Fumarate + QH_2

- No H^+ pumped
- Redox reaction that reduces Q → QH_2
  - 2 e^- come from the succinate to fumarate conversion → to FAD/FADH embedded in II (2 H^+ released) → 2 e^- and 2 H^+ used to convert Q → QH_2
- II is an enzyme that catalyzes the krebs cycle by succinate → fumarate conversion
- Gives e^- to III eventually → IV → O_2
- Unlike other krebs cycle enzymes, II is embedded in the inner mitochondrial membrane (transiently produced)

Net ETC Reaction: NADH

10H^+ moved per NADH
20 H^+ moved into IM per 2 NADH
NADH made in cytosol (glycolysis)

Overall Reaction:
2 NADH + O_2 + 22 H^+ (matrix) → 2 NAD + 2H_2O + 20 H^+ (IM Space)

Net ETC reaction: QH_2

1 QH_2 pumps 6H^+

Overall Reaction:
2 QH_2 + O_2 + 12 H^+ (matrix) → 2 Q + 2H_2O + 12 H^+ (IM Space)

e^- Transport Summary

- e^- passed from low → high affinity carriers
- Form H^+ gradient across inner mitochondrial membrane
- Oxidation of 1 NADH pumps 10 H^+
- Oxidation of 1 QH_2 pumps 6 H^+

NADH → I → Q → III → cyt c → IV → O_2
e^- from succinate → II → Q → III → cyt c → IV → O_2

Proton Gradient from e^- Transport

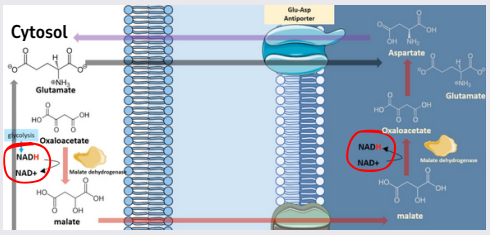
- IM space pH = 7.2 (more acidic) = more H^+ present
- Matrix pH = 7.9

Proton Motive Force: Generates ATP

1. Electrical potential
   - + → -
   - IM → matrix
   - H^+ are attracted
2. H^+ concentration difference
   - High → low
   - IM → matrix

Malate-Aspartate shuttle

- Moves e^- into matrix
- Liver, kidney, heart muscle cells
- NADH pass through pores in outer mito. membrane
  - Carried by **malate** → pass to NAD^+ in matrix
- Malate carbons return to cytosol to repeat
- **Oxaloacetate** = produced a result of malate conversion occurring in matrix → transfer e^- to complex I → ultimately to O_2
- NADH is not directly moved across the membrane, but its e^- are



Modified from https://www.youtube.com/watch?app=desktop&v=b4BFnROcAI0

Matrix

Stoichiometry of ATP synthesis

- Occurs in the heart, muscle, liver, kidney
- 1 NADH allows synthesis and export of **2.5 ATP**
- 1 QH_2 allows synthesis and export of **1.5 ATP**

Glycolysis:
2 NADH → 5 ATP/glucose
2 ATP → 2 ATP/glucose

Pyruvate → Acetyl-CoA (2/glucose):
2 NADH → 5 ATP/glucose

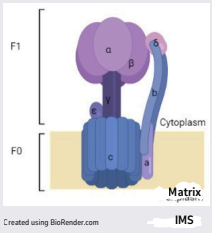
Citric Acid Cycle (2/glucose):
6 NADH → 15 ATP/glucose
2 QH_2 → 3 ATP/glucose
2 GTP → 2 ATP/glucose

Total yield: 32 ATP/glucose

F_1F_0 ATP Synthase

- Transmembrane (F0)
- Peripheral (F1)
  - C subunits and a subunits from half channels through the membrane
- F0: 8 identical subunits
  - Allow H^+ to move across the membrane, down electrochemical gradient
  - Each C subunit binds 1 H^+
  - Movement rotates subunits
- Central Stalk (F1 subunits) also rotates
- 3 alpha 3 beta hexamer of F1 joined to C subunits by a gamma subunits
  - 2 long, curved alpha helices from central stalk extend into hexamer
  - Rotation of gamma/central stalk changes conformation of active sites on beta subunits
    - Contain catalytic site for ATP synthesis
  - Peripheral stalk prevents hexamer from rotating (b subunit)
- F1 contains sites for ATP
- F0 slow H^+ movement across the membrane
  - H^+ → bind to C → rotate to second channel → released from C → matrix
- 2 ⅔ protons per ATP per 1 rotation of C

Overall Reaction (Pumps 2 2/3 protons/ATP):
3 ADP + 3 Pi + 8 H^+ (IM space) → 3 ATP + 3H_2O + 8 H^+ (matrix)



Movement of ADP, Pi and ATP

- Movement driven by electrochemical gradient
- ATP must leave the matrix
- ADP + Pi enter matrix
  - Occurs via an antiporter on the inner membrane
  - Δ change in matrix = +1
  - Δ H^+ in matrix = 0
- H2PO4- and H^+ enter matrix
  - Occurs via a symporter on the inner membrane
  - Δ change in matrix = 0
  - Δ H^+ in matrix = +1
- **Overall: energy from 1 H^+ used**

"Electro"
- ATP more negative than ADP (has phosphate)
  - One negative charge removed from matrix (relatively negatively charge) = favorable
- Phosphate movement net charge = 0
  - Phosphate = negative
  - H^+ = positive

"Chemical"
- Phosphate driven by H^+ movement
  - H^+ high → low concentration = favorable
- During ATP and ADP exchange, no H^+ are exchanged = pH is unaffected

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Note: Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin 24.4.1, Chemaxon (https://www.chemaxon.com)

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