CHEM 153A Week 10

Aidan Jan

March 11, 2025

Citric Acid Cycle Regulation

- regulation balances the supply of key intermediates with the demands of energy production and biosynthetic processes
- regulation occurs at several points:
  - PDH complex
  - citrate synthase
  - isocitrate dehydrogenase complex
  - α-ketoglutarate dehydrogenase complex

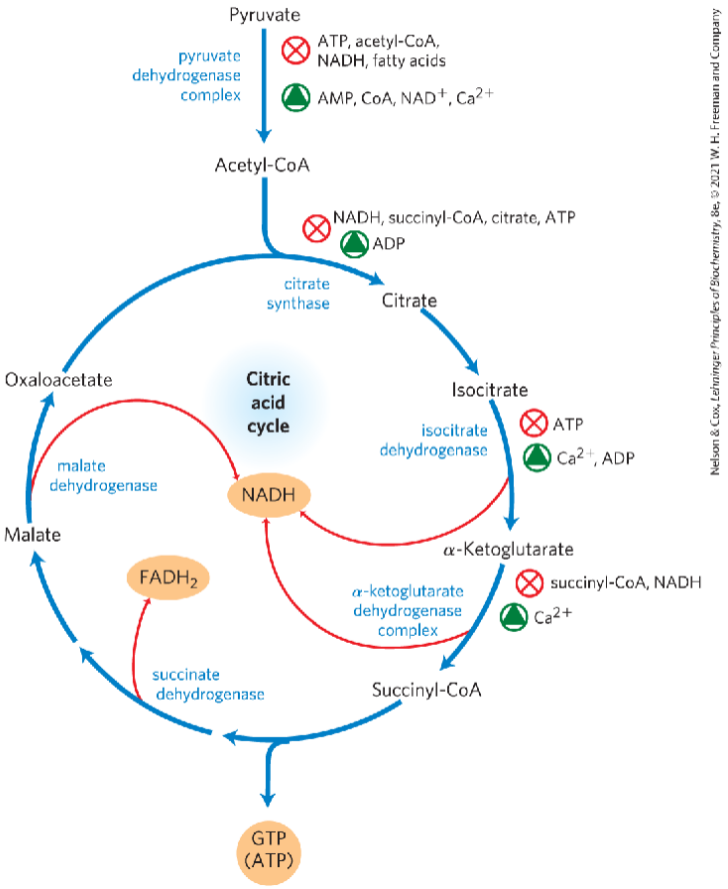
Production of Acetyl-CoA by the PDH Complex is Regulated by Allosteric and Covalent Mechanisms

- PDH complex activity is turned off when:
  - ample fatty acids and acetyl-CoA are available as fuel

  ATP /[ADP] and [NADH]/[NAD^+] ratios are high
- PDH complex activity is turned on when:
  - energy demands are high
  - the cell requires greater flux of acetyl-CoA into the citric acid cycle

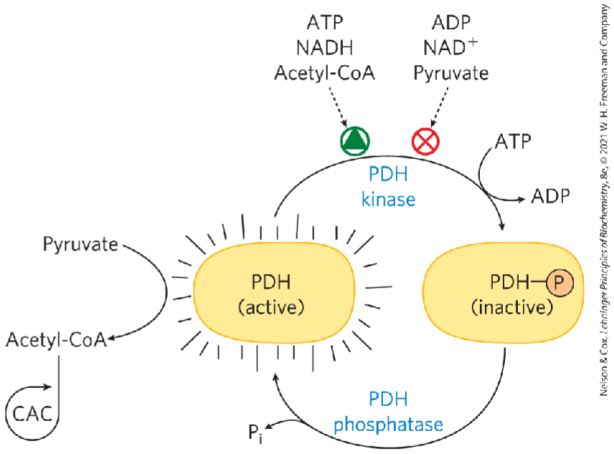
Regulation of Metabolite Flow Through the Citric Acid Cycle

The central role of the citric acid cycle in metabolism requires that it be regulated in coordination with many other pathways. Regulation occurs by both allosteric and covalent mechanisms that overlap and interact to achieve homeostasis.



Covalent Modification of the PDH Complex

- **PDH Kinase** - inhibits the PDH complex by phosphorylation
  - Allosterically activated by products of the complex
  - Inhibited by substrates of the complex
- **PDH phosphatase** = reverses the inhibition by PDH kinase

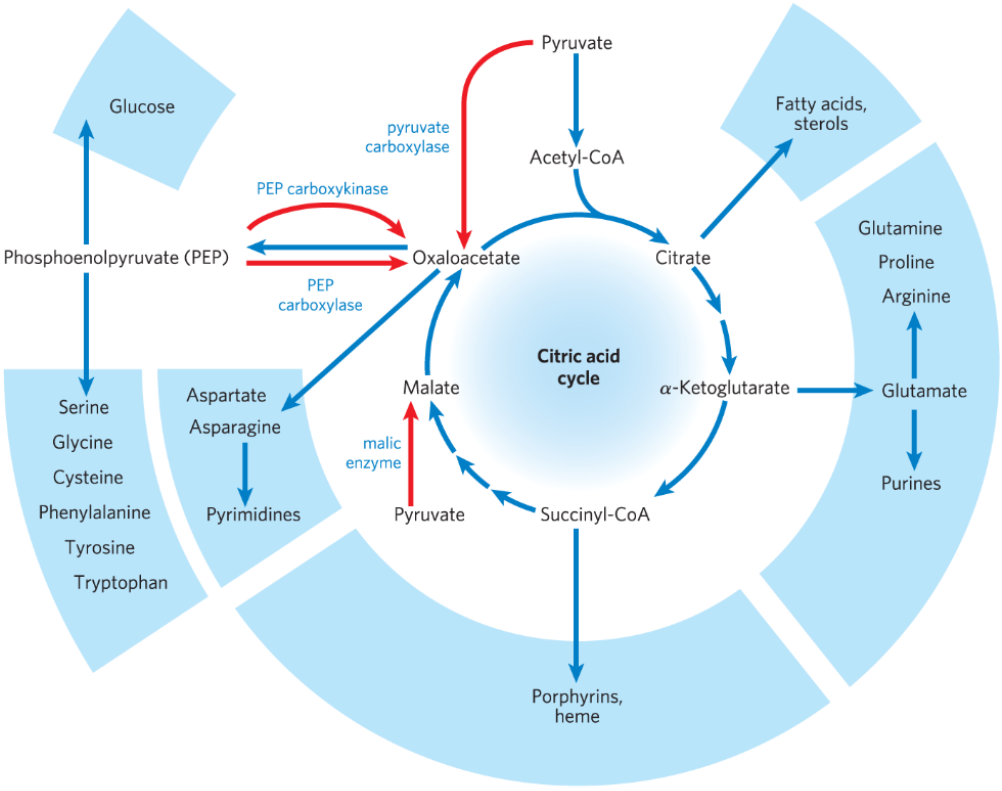


The Citric Acid Cycle is also Regulated at Three Exergonic Steps

- regulation occurs at strongly exergonic steps catalyzed by:
  - citrate synthase
  - isocitrate dehydrogenase complex
  - α-ketoglutarate dehydrogenase complex
- fluxes are affected by the concentrations of substrates and products:
  - end products ATP and NADH are inhibitory
  - $NAD^+$and ADP are stimulatory
  - long-chain fatty acids are inhibitory

Role of the Citric Acid Cycle in Anabolism

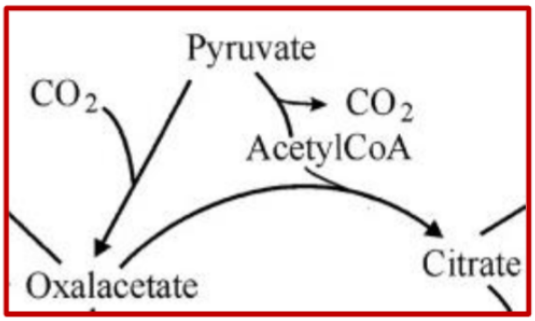
- **Cataplerosis** describes the series of enzymatic reactions that draw down pools of metabolic intermediates
- **Anaplerosis** describes the series of enzymatic reactions or pathways that replenish pools of metabolic intermediates in the TCA cycle
- As intermediates of the citric acid cycle are removed to serve as biosynthesic precursors, they are replenished by **anaplerotic reactions**

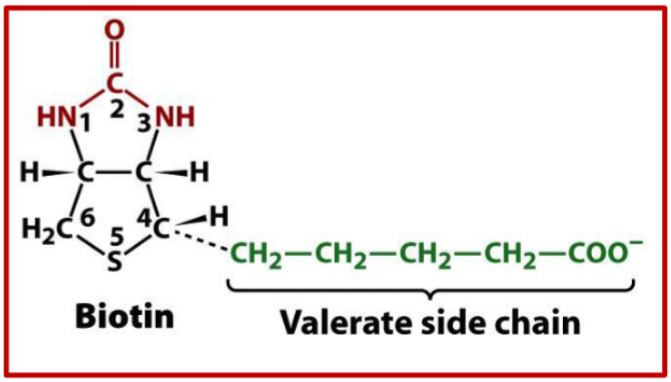


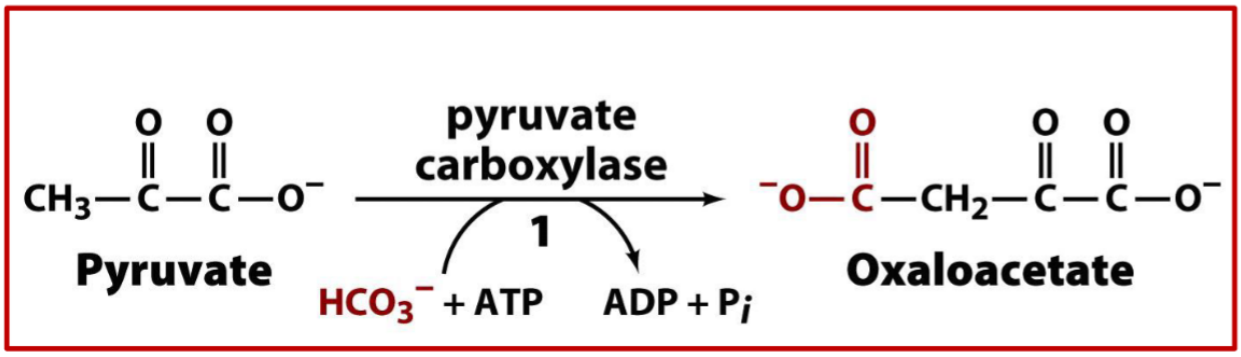
Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways. Shown in red are four anaplerotic rea

Pyruvate Carboxylase

- Catalyzes the first step of **gluconeogenesis**
- Also replenishes oxaloacetate allowing TCA to continue
- Allosterically activated by acetyl-CoA
  - Fate determination for pyruvate
- Uses interesting cofactor called **biotin** that allows for carbon-carbon bond formation



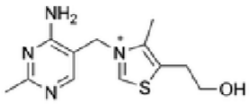




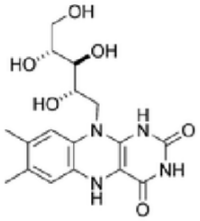
Structures of the B Vitamins along with their Role in Cells and the Disease Caused by their Deficiency

Vitamins are organic compounds required in small amounts for human health, distinct from essential amino acids, fatty acids, and elements.

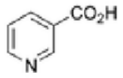
B_1 thiamine
formation of the coenzyme thiamine pyrophosphate (TPP)
beri-beri



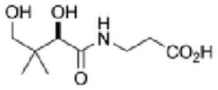
B_2 riboflavin
formation of the coenzymes FMN and FAD
ariboflavinosis



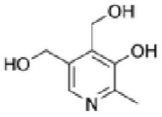
B_3 niacin
formation of the coenzymes NAD and NADP
pellagra



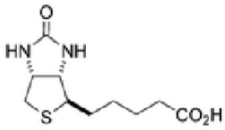
B_5 pantothenic acid
formation of coenzyme A
paresthesia



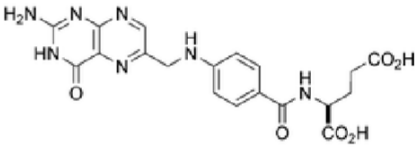
B_6 pyridoxine
formation of the coenzyme pyridoxal phosphate (PLP)
various symptoms



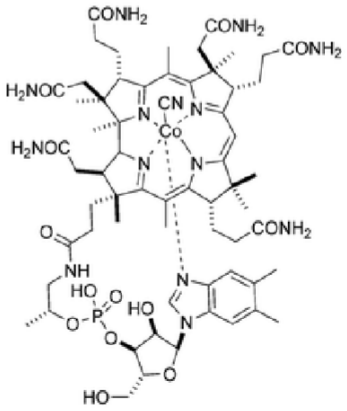
B_7 biotin
prosthetic groupwhen covalently attached to a carrier protein
none identified



B_9 folic acid
formation of the coenzyme tetrahydrofolate (THF)
birth defects

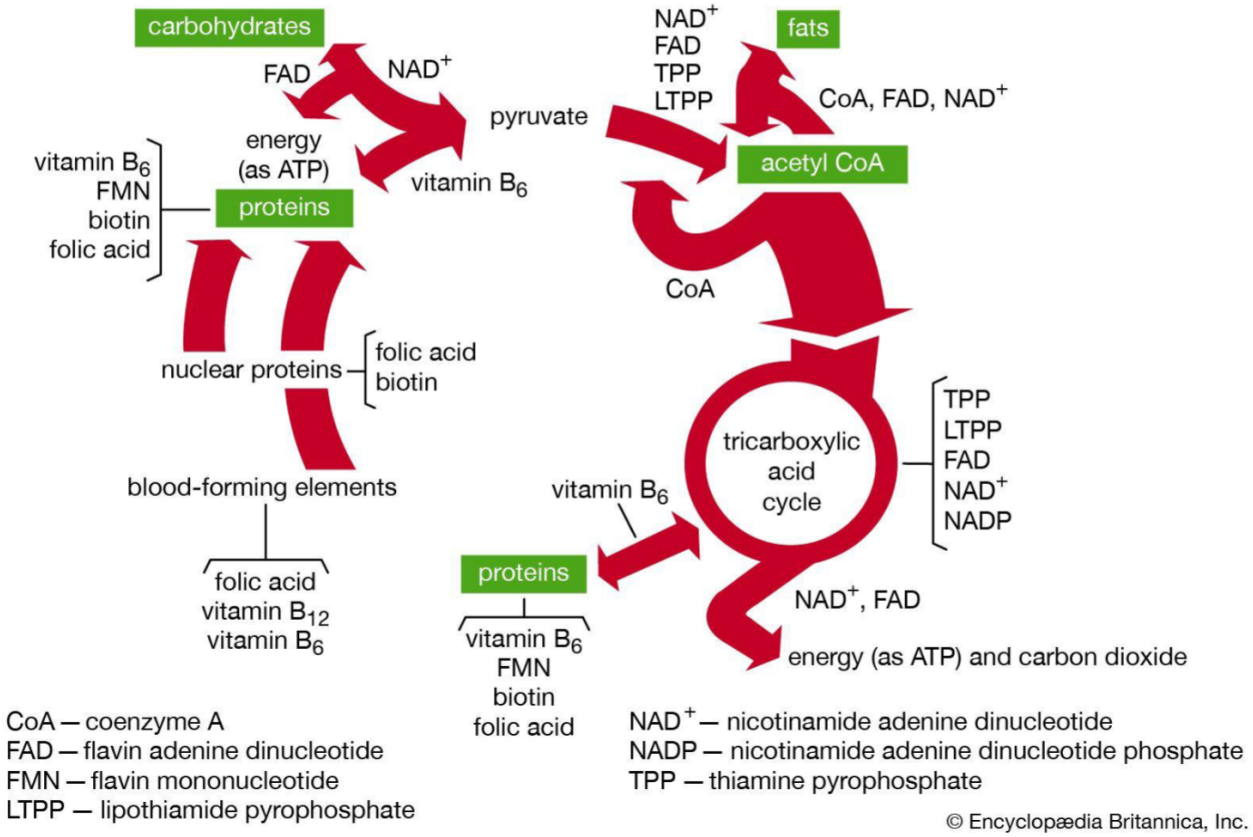


B_{12} cyanocobalamin
formation of the coenzymes adenosylcobalamin and methycobalamin
pernicious anemia

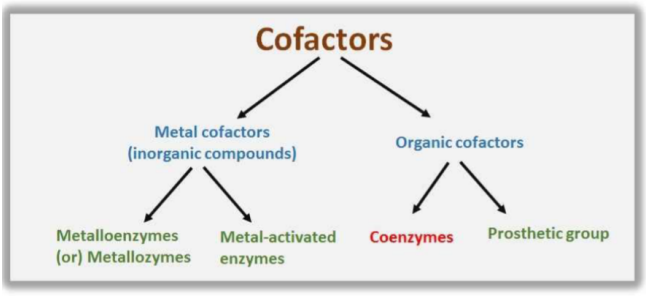


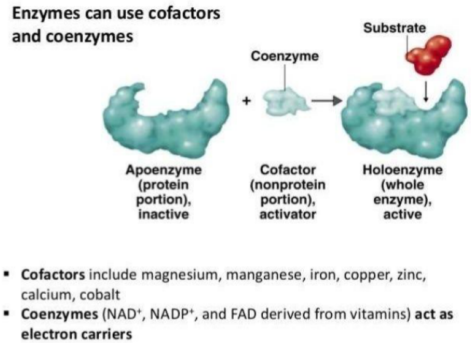
All B vitamins indeed act as precursors for **coenzymes** or are directly involved in enzymatic reactions. Evolutionarily, animals lost the ability to biosynthesize vitamins, relying on dietary intake instead.

Functions of B-vitamin Coenzymes in Metabolism



Cofactors and Their Role in Enzyme Function





Cofactors are essential non-protein components that assist enzymes in catalyzing reactions. They are classified into **metal cofactors** (e.g., magnesium, zinc, and iron) and **organic cofactors** (e.g., coenzymes like NAD^+, FAD, and prosthetic groups). Metal cofactors can activate enzymes directly, while coenzymes often act as electron carriers. Together, cofactors and the protein portion of an enzyme (apoenzyme) form an active holoenzyme capable of binding substrates and catalyzing reactions effectively

The Mitochondrial Respiratory Chain

Electrons Are Funneled to Universal Electron Acceptors

- **respiratory chain** = series of electron carriers
- dehydrogenases collect electrons from catabolic pathways and funnel them into universal electron acceptors:
  - nicotinamide nucleotides (NAD^+or $NADP^+$)
  - flavin nucleotides (FMN or FAD)

Electrons Pass through a Series of Membrane-Bound Carriers

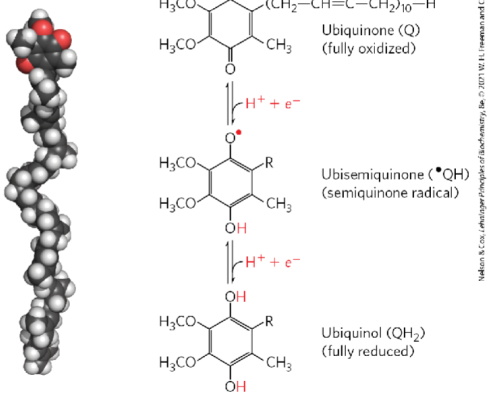
- Three types of electron transfers occur in oxidative phosphorylation:
  - direct transfer of electrons
  - transfer as a hydrogen atom ($H^+ + e^-$)
  - transfer as a hydride ion ($:H^-$)
- **reducing equivalent** = a single electron equivalent transferred in an oxidation-reduction reaction

Electron-Carrying Molecules in the Respiratory Chain

- Five types of electron-carrying molecules:
  - NAD
  - flavoproteins
  - **ubiquinone (coenzyme Q or Q)**
  - **cytochromes**
  - **Iron-sulfur Proteins**

Ubiquinone

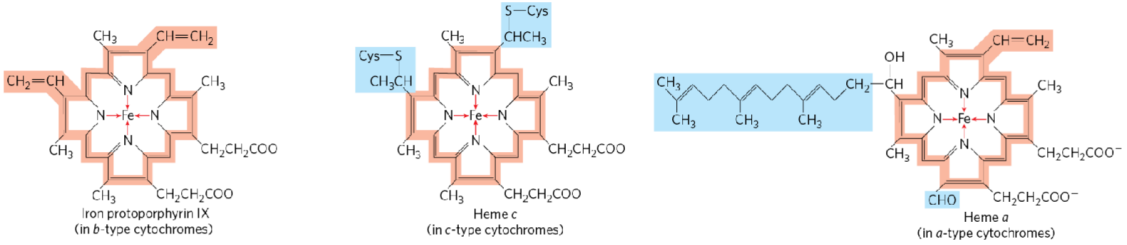
- Ubiquinone (coenzyme **Q**) = a lipid-soluble benzoquinone with a long isoprenoid side chain
  - Can accept one or two electrons
  - Freely diffusible within the inner mitochondrial membrane
  - Plays a central role in coupling electron flow to proton movement

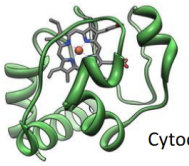


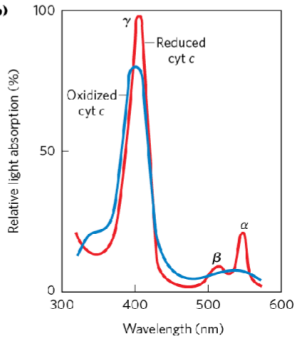
Cytochromes

- **cytochromes** = proteins with characteristic strong absorption of visible light due to their iron-containing heme prosthetic groups
  - one-electron carriers
  - 3 classes in mitochondria: a, b, and c
    - hemes of a and b are not covalently bound to associated proteins
    - c is covalently attached through Cys residues

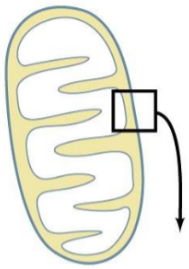
Prosthetic Groups of Cytochromes



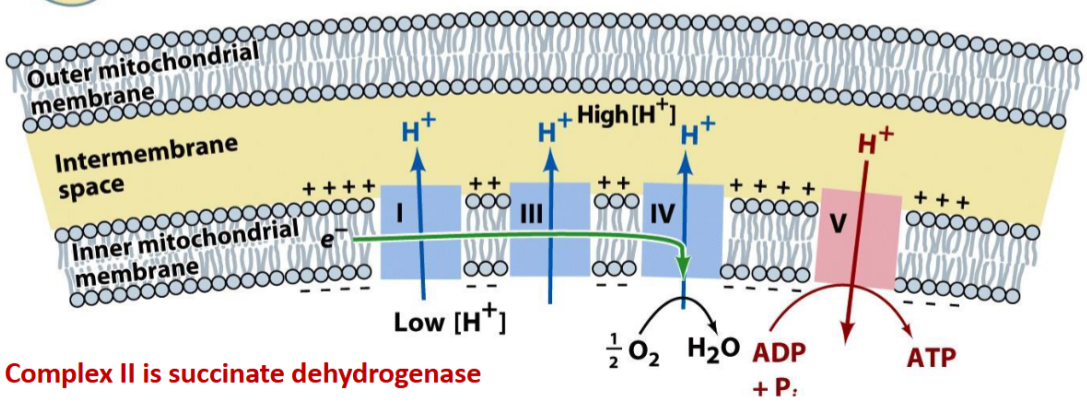




Spatial Context 1

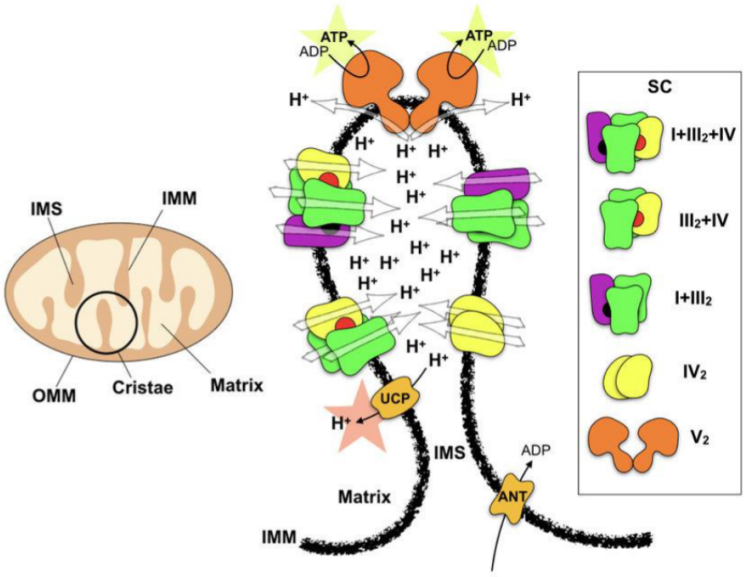


tend to appear on cristae



Complex II is succinate dehydrogenase

Spatial Context 2



The ETC complexes and accessory proteins are organized into individual **complexes** and **supercomplexes** embedded in the IMM. These complexes pump **protons (H^+)** from the **matrix** into the **intermembrane space (IMS)**, creating an **electrochemical gradient**. The electrochemical gradient drives **Complex V (ATP synthase)** to produce ATP (lime-colored stars) from ADP

Cristae structure does **two** things:

- Higher surface area allows for more ETC subunits (more ATP production)
- Allows for higher localized proton density, creating stronger gradient

Reduction Potential

- **Standard reduction potential** (E°) is a measure of the tendency for a chemical species to be reduced
  - The more positive the potential, the more favorable the reduction
  - Completely proportional to ΔG

    $$\Delta G^\circ_{cell} = -nFE^\circ_{cell}$$

    Connects Gibbs free energy change (ΔG°_{cell}) with the reduction potential (E°_{cell}) where:
    - ΔG°_{cell} is the standard Gibbs free energy change
    - n is the number of electrons transferred
    - F is Faraday's constant (96485 C/mol)
    - E°_{cell} is the standard cell potential

    A **positive E°_{cell} results in a negative ΔG°_{cell}** indicating a spontaneous reaction, while a **negative E°_{cell} leads to a positive ΔG°_{cell}**, meaning the reaction is non-spontaneous
- This can help us predict which direction redox reactions will flow naturally
  - The less favorable reduction will flip to become an oxidation

$X^+ + e^- \longrightarrow X$	less favorable (lower Eo)
$Y^+ + e^- \longrightarrow Y$	more favorable (higher Eo)
$Y^+ + X \longrightarrow Y + X^+$	net reaction
	Y^+ is reduced, and X is oxidized.

The Electron Transport Chain

ETC Redox Overview

Remember: these values indicate the tendency of each molecule to gain electrons (be reduced).

[FILL 27]

- The flow of electrons in the ETC is "downhill" energetically, moving from molecules with lower Eo values (e.g., NADH at -0.315) to those with higher Eo values (e.g., O_2 at 0.815V)
- For example, Q at +0.045V to Cyt b at +0.077V

Electron Transport Chain: Complexes I to IV

[FILL 29, full]

Protein Components of the Mitochondrial Respiratory Chain

[FILL 30, full]

* generalization from organisms where it has already been studied. Remember that there are always exceptions.

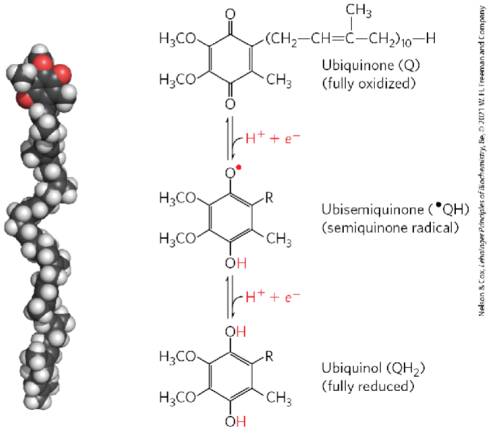
Complex I: NADH Oxidoreductase

- Also known as NADH oxidoreductase or NADH dehydrogenase
- Large, large L-shaped enzyme with >40 polypeptide chains
- Accepts 2 electrons from NADH and passes them to FMN (Flavin Mononucleotide)
- Then passes electrons through 8+ Fe/S clusters to Ubiquinone **one at a time**
- This complex uses this electrical work to pump 4 H^+ions out of the matrix and into the intermembrane space (likely an induced conformational change)

[FILL 32, edited]

(Review) Ubiquinone

- Ubiquinone, also known as coenzyme Q, is a lipid with a quinone ring structure at the top
- Can be reduced with two electrons, then travels to Complex III: freely diffusible within the inner mitochondrial membrane
- plays a central role in coupling electron flow to proton movement



Complex I Catalyzes Two Simultaneous and Obligately Coupled Processes

Complex I catalyzes:

- the exergonic transfer of a hydride ion (hydrogen atom with two electrons) from NADH and a proton from the matrix to ubiquinone

$$NADH + H^+ + Q \rightarrow NAD^+ + QH_2$$

- the endergonic transfer of 4 protons from the matrix to the intermembrane space

Electron Flow in Complex I

- **N1a** has a unique role compared to other Fe-S clusters: it can accept electrons from FMN but **does not always participate in the main electron transfer chain to ubiquinone.** It may act as a reserve or moderate the process.
- **N3** is part of the main pathway and facilitates the transfer of electrons to downstream clusters like **N2** (which transfers electrons to ubiquinone)

[FILL 35]

Complex I Overview

[FILL 36, full]

Proton Wires

Protons are transported by proton "wires" - a series of amino acids that undergo protonation and deprotonation

[FILL 37, right, 38, left, edited]

Proton transfer pathways, outlined by blue arrows. Membrane arm contains the central axis of charged residues, essential for the proton transfer and the coupling

Pumping Protons and Free Energy

[FILL 39]

- Free energy is released on each step, with every reduction. Oxygen is the final electron acceptor, with the lowest free energy and the highest electronegativity.

- To find how much free energy is released between two steps, subtract their values to find ΔE°, then plug into the equation $\Delta G^\circ = -nFE^\circ$.
  - For example, to find the amount of free energy released through Complex I, subtract NADH oxidation ($E^\circ = -0.32$ V) from Ubiquinone reduction ($E^\circ = +0.045$ V).
  - This gives $\Delta E^\circ = +0.36$ V. Now, plug into the equation. $n = 2$ since two electrons are transferred, and $F = 96.5$ kJ/mol (Faraday's constant)
  - The result is -70 kJ/mol

The Coupling of Proton Pumping with Electron Flow

[FILL 46]

Complex II: Succinate Dehydrogenase

- As we've discussed - succinate dehydrogenase oxidizes succinate to fumarate as part of the TCA
  - The two electrons are passed to FAD, forming $FADH_2$
- The electrons are then passed **one at a time** through 3 Fe/S clusters to ubiquinone (Q)
  - Also passes ubiquinol (reduced to Q) to Complex III
  - Effectively works in parallel with Complex I, **ETC can start from either Complex**
  - **Does not transport protons** - This is why $FADH_2$ produces less ATP than NADH (1.5 vs. 2.5)

[FILL 49]

Why Doesn't Complex II Pump Protons?

- Reduction of Fumarate and reduction of Ubiquinone only has a ΔE° of $+0.014$ V! There is not enough energy differential to pump protons.

Complex III: Cytochrome c reductase

- Dimer of 11 subunits (22 in total)
  - Relevance comes from the cavity in the center of the dimer
  - **Cavity has two binding sites for Coenzyme Q molecules**
- Uses two electrons from ubiquinol (CoQ) to reduce two molecules of cytochrome c (does so sequentially)
- **Rieske center**, specialized Fe/S center with two His and Cys residues coordinating
- **Issue:** We no longer have access to Flavin cofactors, making it hard for the protein to hold onto two electrons at the same time
  - **Solution:** Releasing one electron at a time to cytochrome c while pumping the second electron through a secondary pathway called the Q cycle.

[FILL 52 + 53 + 54]

Cytochromes

- Proteins with heme groups that are involved in redox reductions (as opposed to O_2 binding)
- **Oxidation state change in central iron allows for electron carrying**
- Can be embedded in complexes (e.g., cytochrome b, cytochrome c_1) or freely moving between them (cytochrome c)

[FILL 55]

The Q Cycle

- The two cavities in Complex III can bind both reduced **ubiquinol (QH_2)** and **oxidized ubiquinone (Q)**
- When ubiquinol (QH_2) attaches to its cavity, it releases one electron towards cytochrome c (via the Fe-S cluster and cytochrome c_1), but also releases one to cytochrome b chain, **reducing a bound ubiquinone halfway (semiquinone radical: $Q^{\cdot -}$)**
- Same process occurs with a second ubiquinol, generating a second reduced cytochrome c, but **fully reduces one CoQ**
  - This ensures that while two QH_2 molecules are oxidized, one QH_2 is regenerated, maintaining the balance of the ubiquinone pool

[FILL 56]

- One reason for this cycle (besides lack of FAD/FMN) is that H^+ions can be donated directly to the intermembrane space from oxidized QH_2
  - Four protons transported for every two electrons reaching cytochrome c

$$2\ QH_2 + Q + 2\ \text{Cyto}\ c\ (Fe^{3+}) + 2\ H^+_{(\text{matrix})} \rightarrow 2\ Q + QH_2 + 2\ \text{Cyto}\ c\ (Fe^{2+}) + 4\ H^+_{(\text{IMS})}$$
$$QH_2 + 2\ \text{Cyto}\ c\ (Fe^{3+}) + 2\ H^+_{(\text{matrix})} \rightarrow Q + 2\ \text{Cyto}\ c\ (Fe^{2+}) + 4\ H^+_{(\text{IMS})} \quad \underline{\textbf{NET}}$$

$FILL59, full$

Complex III Overview

[FILL 60, full]

Complex IV: Cytochrome c Oxidase

- Two cytochrome c molecules each transfer one electron to Complex IV through two redox-active centers: two cytochrome groups (cyt a and cyt a3) and two copper atom groups (Cu_A and Cu_B)
- One electron is held at the Cu_B center
- The other is held at cytochrome a3
- Once oxygen (O_2) binds to cytochrome a3 and Cu_B, it accepts the two electrons, forming a peroxide bridge

Two additional cytochrome c molecules donate two more electrons to the system. These electrons, along with two protons (H^+), break the peroxide bridge, reducing the oxygen to two water molecules (2 H_2O)

- The complete process involves the oxidation of **four cytochrome c molecules**, transferring four electrons to reduce one molecule of O_2 to two molecules of H_2O. This process requires the uptake of four protons from the matrix, contributing to the proton gradient necessary for ATP synthesis

[FILL 62, 63, 64]

Path of Electron Through Complex IV

[FILL 65]

Complex IV Overview

[FILL 66]

Complex V: ATP Synthase

[FILL 31]