

CHEM 153A Week 10

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March 15, 2025

Citric Acid Cycle Regulation

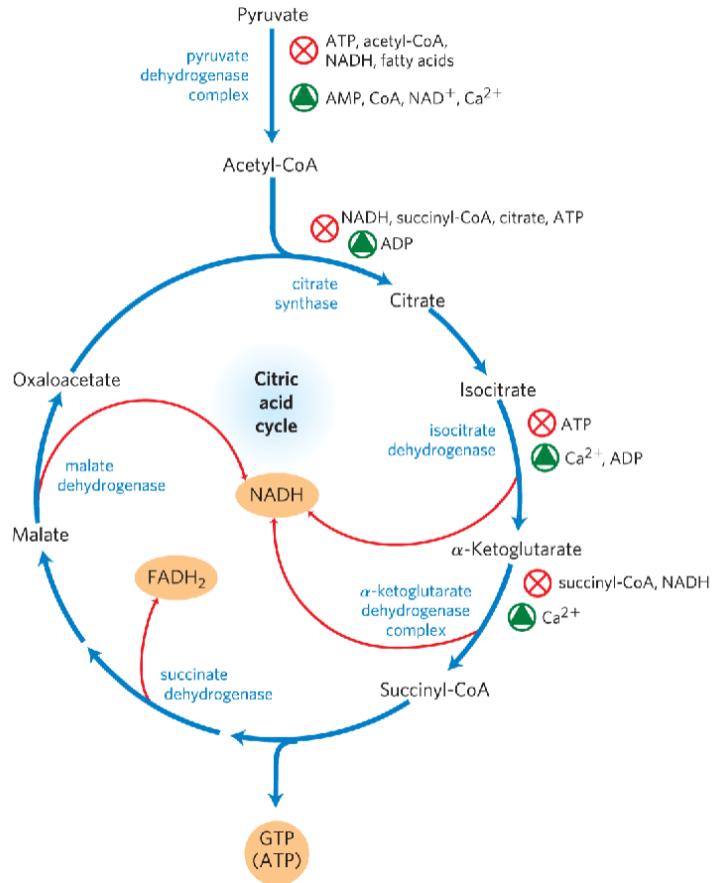
- regulation balances the supply of key intermediates with the demands of energy production and biosynthetic processes
- regulation occurs at several points:
 - PDH complex
 - citrate synthase
 - isocitrate dehydrogenase complex
 - α -ketoglutarate dehydrogenase complex

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

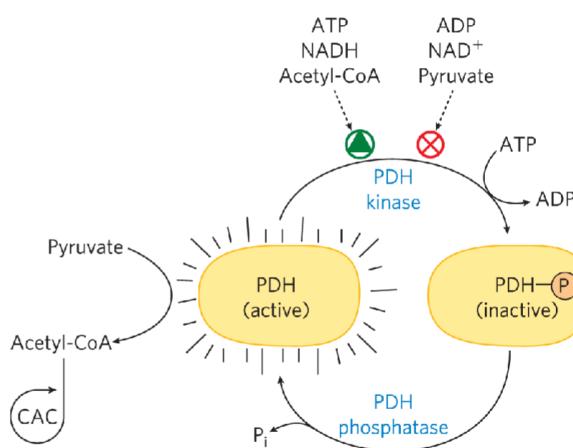
The central role of the citric acid cycle in metabolism requires that it be regulated in coordination with many other pathways. Regulation occurs by both allosteric and covalent mechanisms that overlap and interact to achieve homeostasis.



Nelson & Cox, Lehninger Principles of Biochemistry, 8e, © 2021 W.H. Freeman and Company

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

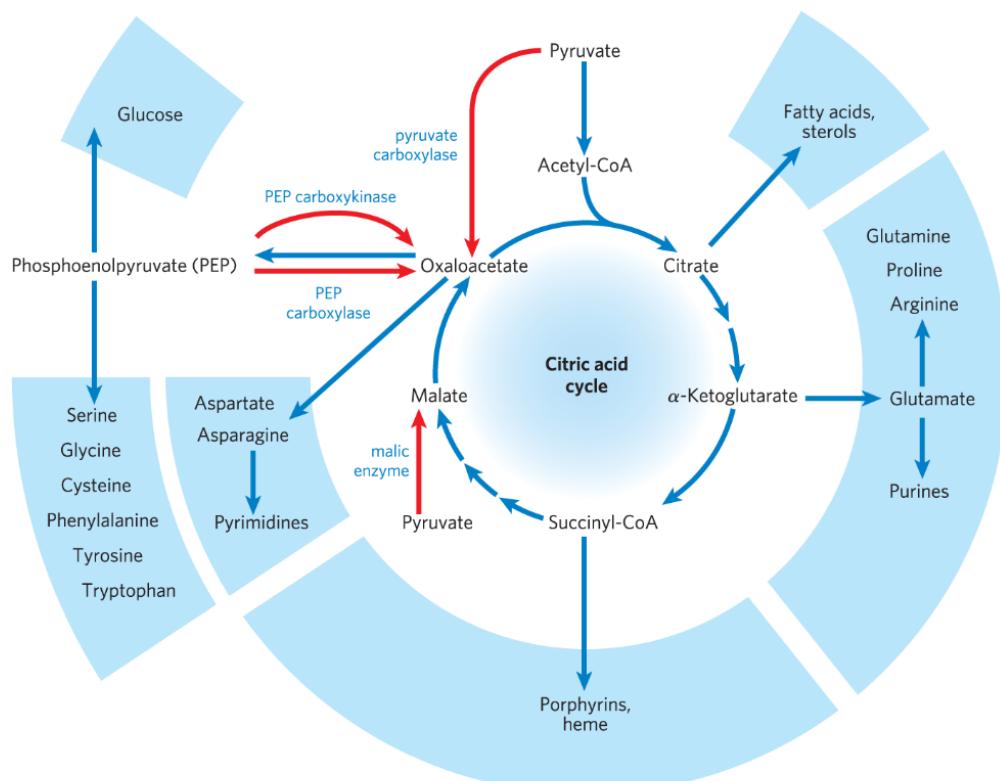


The Citric Acid Cycle is also Regulated at Three Exergonic Steps

- 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

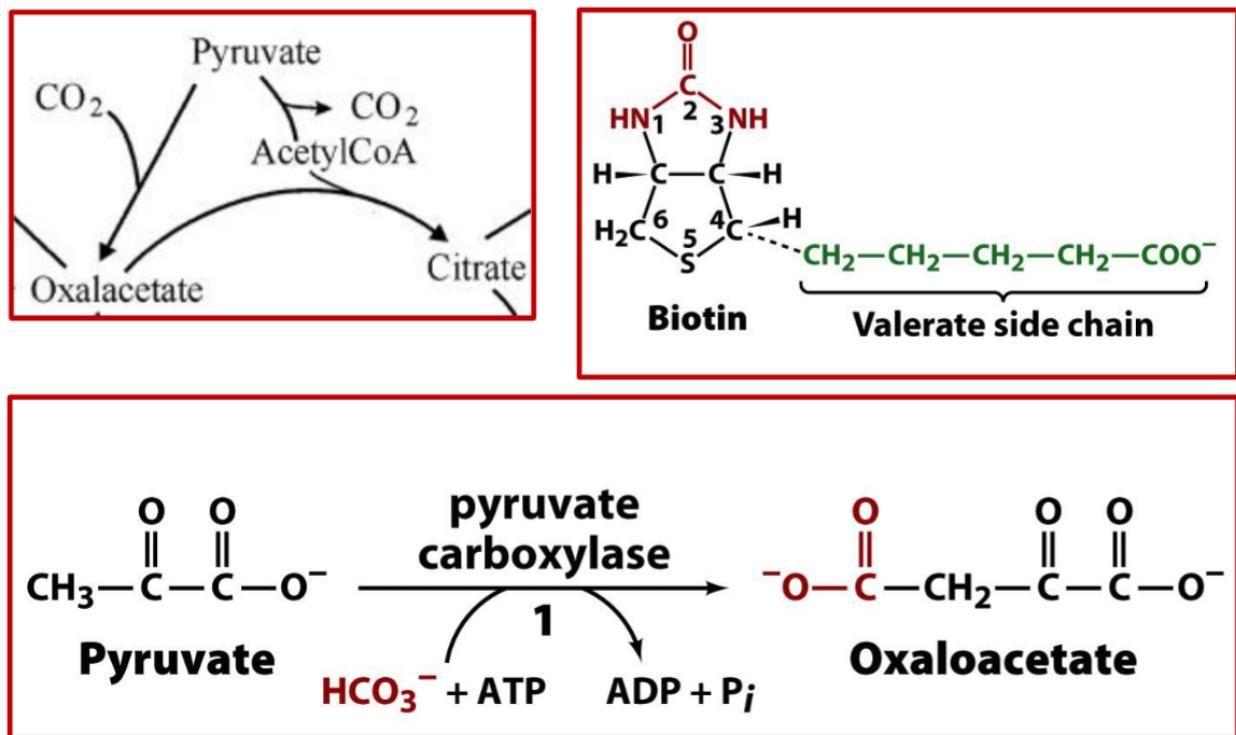
- Cataplerosis** describes the series of enzymatic reactions that draw down pools of metabolic intermediates
- Anaplerosis** describes the series of enzymatic reactions or pathways that replenish pools of metabolic intermediates in the TCA cycle
- As intermediates of the citric acid cycle are removed to serve as biosynthetic 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



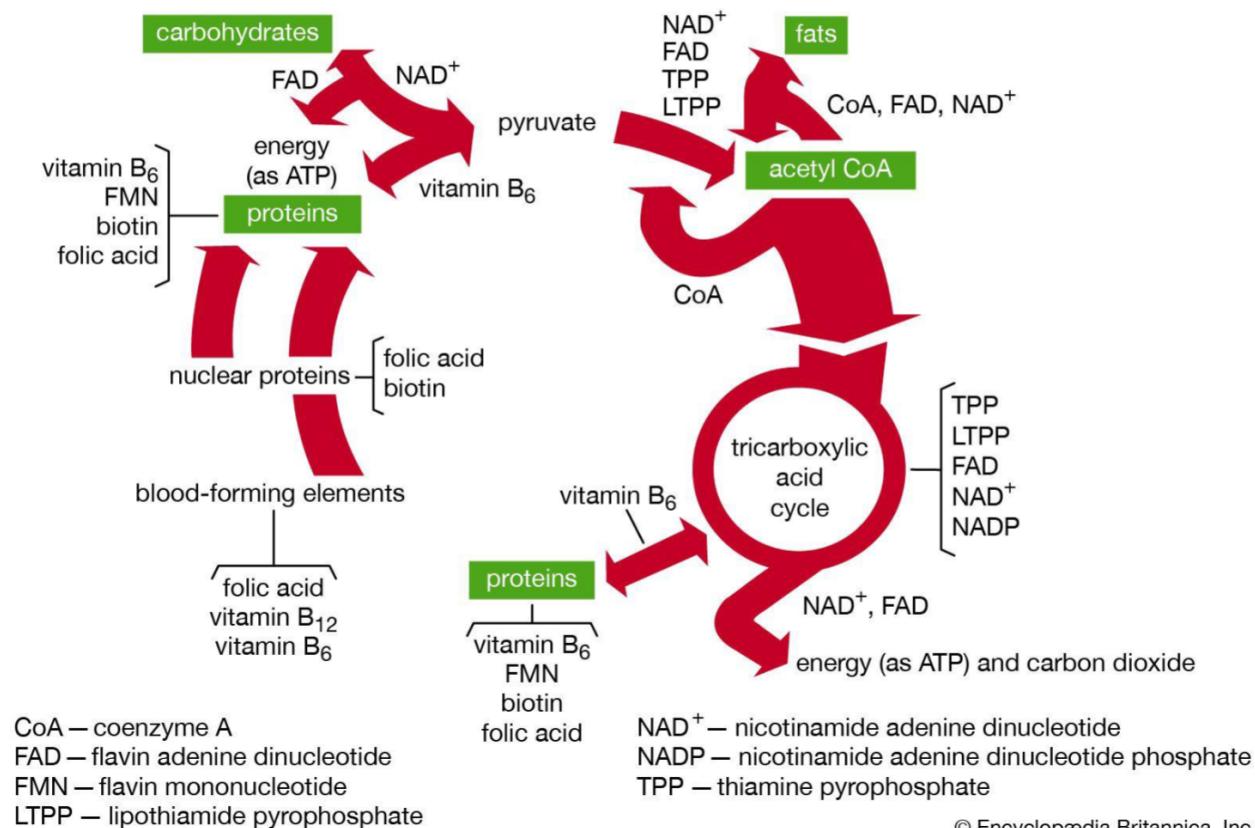
Structures of the B Vitamins along with their Role in Cells and the Disease Caused by their Deficiency

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

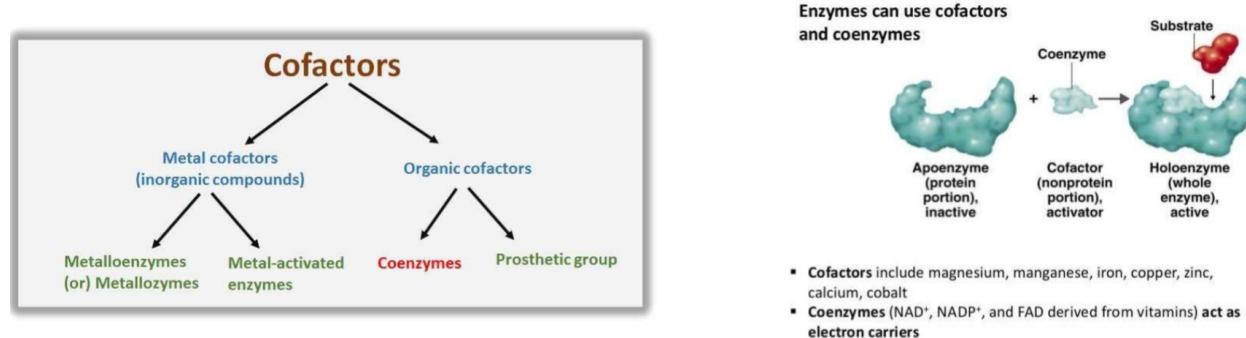
B₁ thiamine formation of the coenzyme thiamine pyrophosphate (TPP) beri-beri		B₇ biotin prosthetic group when covalently attached to a carrier protein none identified	
B₂ riboflavin formation of the coenzymes FMN and FAD riboflavinosis		B₉ folic acid formation of the coenzyme tetrahydrofolate (THF) birth defects	
B₃ niacin formation of the coenzymes NAD and NADP pellagra		B₁₂ cyanocobalamin formation of the coenzymes adenosylcobalamin and methycobalamin pernicious anemia	
B₅ pantothenic acid formation of coenzyme A paresthesia			
B₆ pyridoxine formation of the coenzyme pyridoxal phosphate (PLP) various symptoms			

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, manganese, iron, copper, zinc, calcium, cobalt) 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

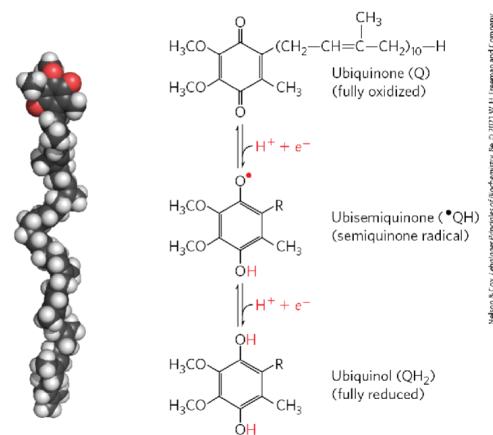
- Three types of electron transfers occur in oxidative phosphorylation:
 - direct transfer of electrons
 - transfer as a hydrogen atom ($\text{H}^+ + e^-$)
 - transfer as a hydride ion ($:\text{H}^-$)
- **reducing equivalent** = a single electron equivalent transferred in an oxidation-reduction reaction

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

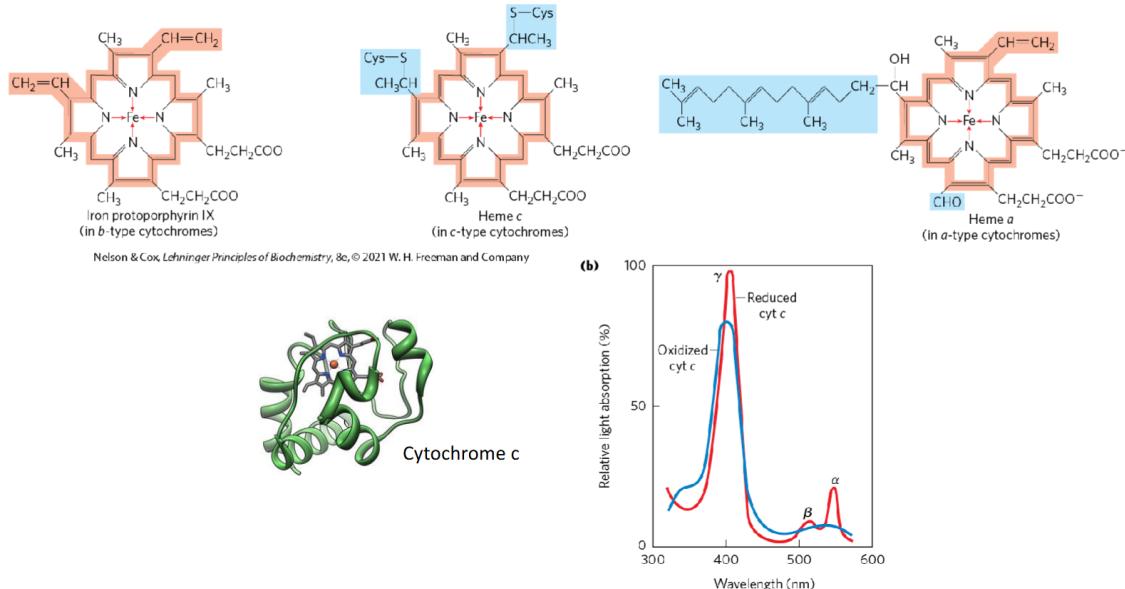
- Ubiquinone (coenzyme Q) = a lipid-soluble benzoquinone with a long isoprenoid side chain
 - Can accept one or two electrons
 - Freely diffusible within the inner mitochondrial membrane
 - Plays a central role in coupling electron flow to proton movement



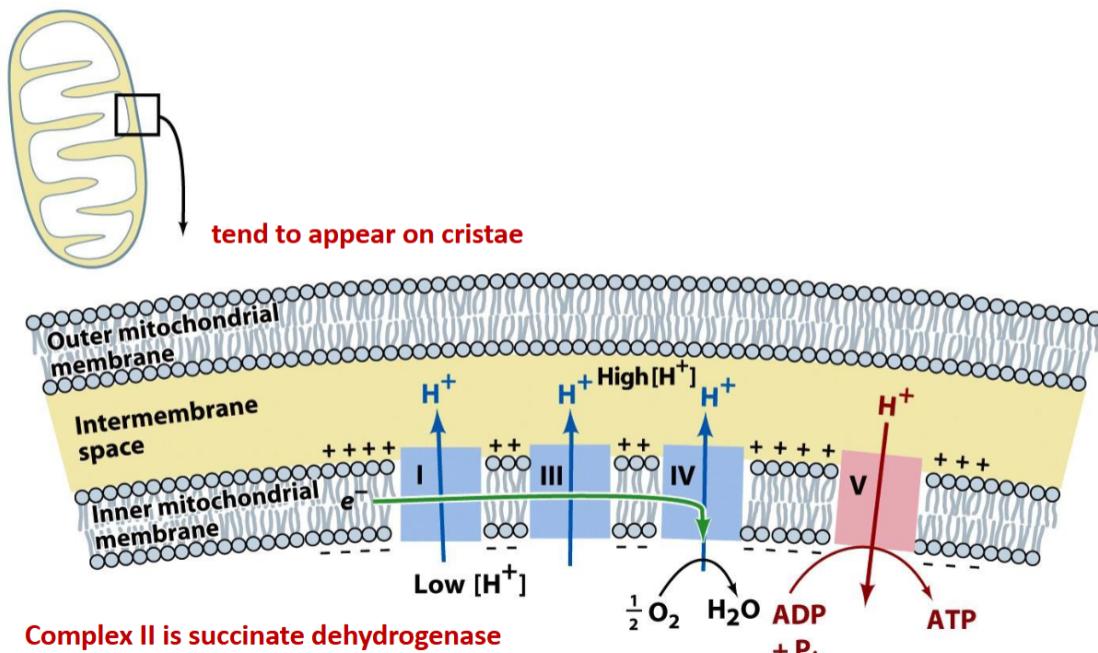
Cytochromes

- **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

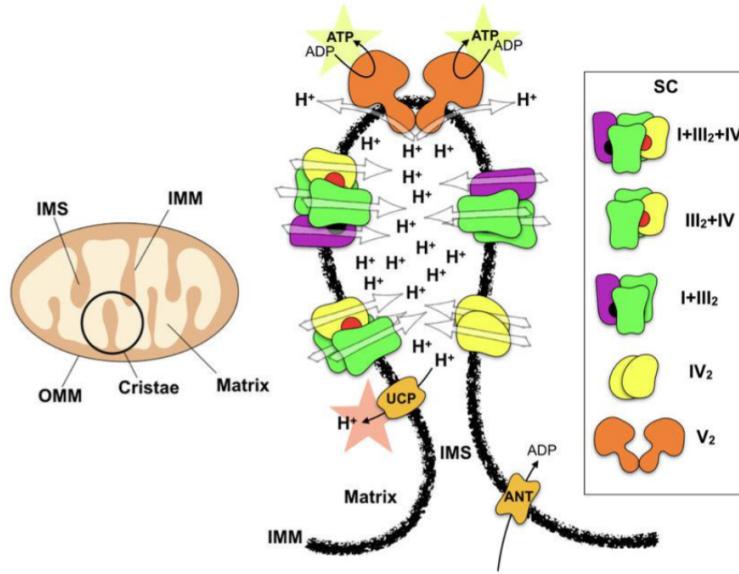
Prosthetic Groups of Cytochromes



Spatial Context 1



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

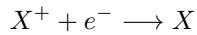
$$\Delta G_{cell}^\circ = -nFE_{cell}^\circ$$

Connects Gibbs free energy change (ΔG_{cell}°) with the reduction potential (E_{cell}°) where:

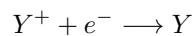
- * ΔG_{cell}° is the standard Gibbs free energy change
- * n is the number of electrons transferred
- * F is Faraday's constant (96485 C/mol)
- * E_{cell}° is the standard cell potential

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

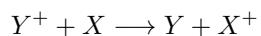
- This can help us predict which direction redox reactions will flow naturally
 - The less favorable reduction will flip to become an oxidation



less favorable (lower E_o)



more favorable (higher E_o)



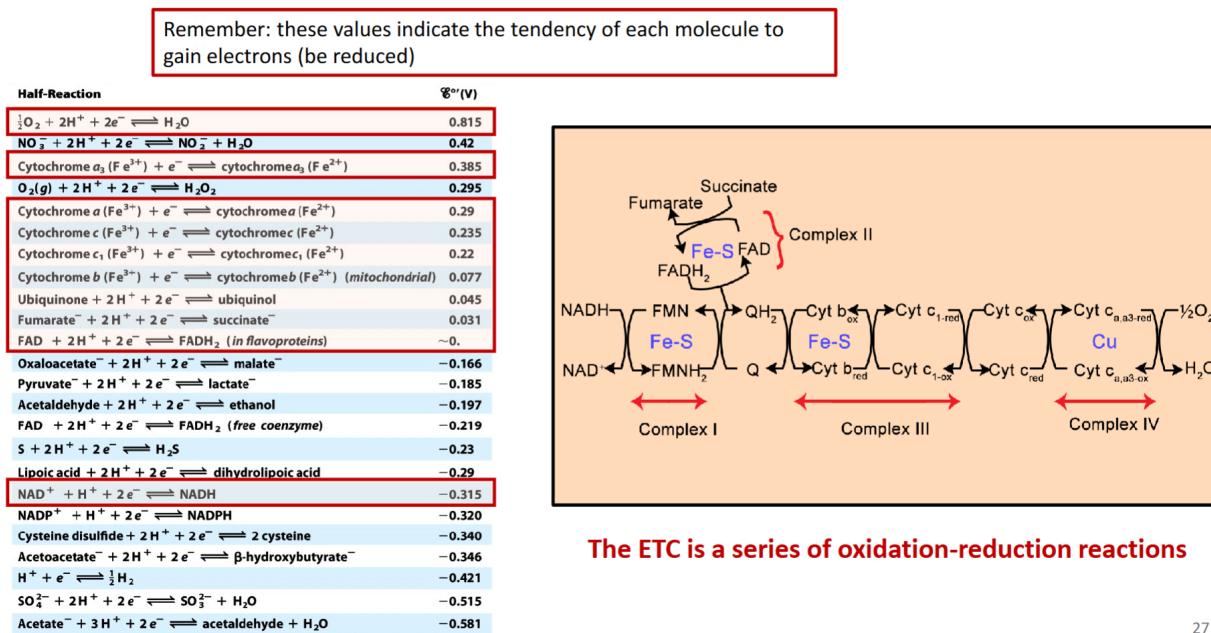
net reaction

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).

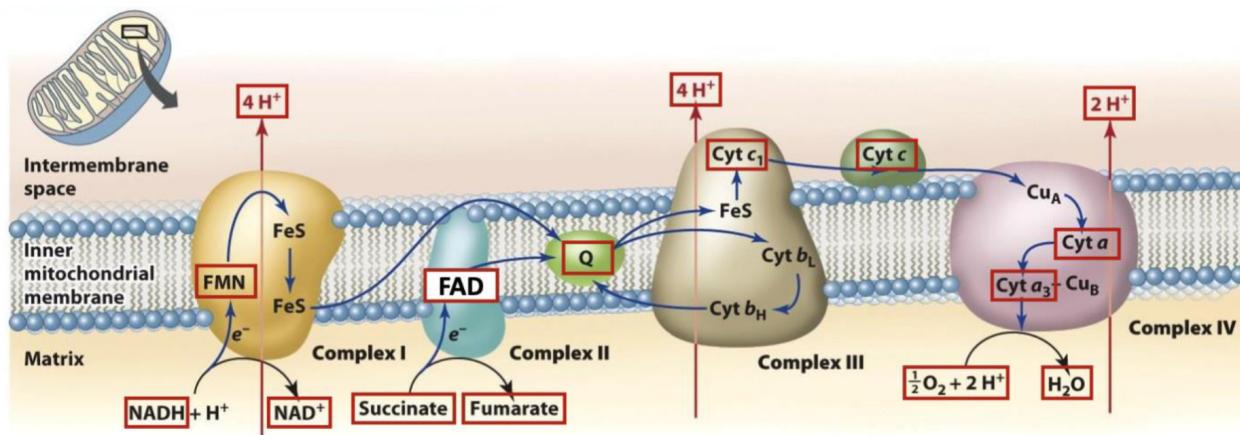


The ETC is a series of oxidation-reduction reactions

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- The flow of electrons in the ETC is "downhill" energetically, moving from molecules with lower E_o values (e.g., NADH at -0.315) to those with higher E_o values (e.g., O₂ at 0.815V)
- For example, Q at +0.045V to Cyt b at +0.077V

Electron Transport Chain: Complexes I to IV



Protein Components of the Mitochondrial Respiratory Chain

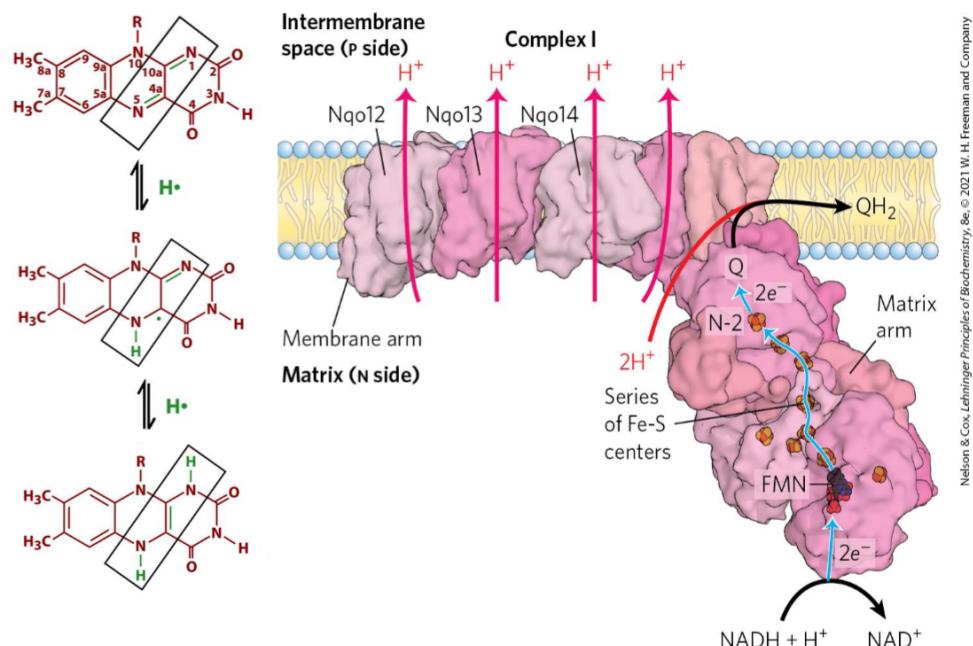
Table 19-3 The Protein Components of the Mitochondrial Respiratory Chain

Enzyme complex/protein	Mass (kDa)	Number of subunits	Prosthetic group(s)
I NADH dehydrogenase	850	45 (14 in Bacteria)*	FMN, Fe-S
II Succinate dehydrogenase	140	4*	FAD, Fe-S
III Ubiquinone: cytochrome <i>c</i> oxidoreductase	250	11*	Hemes, Fe-S
Cytochrome <i>c</i>	13	1*	Heme
IV Cytochrome oxidase	204	13 (3–4 in Bacteria)*	Hemes; Cu _A , Cu _B

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

Complex I: NADH Oxidoreductase

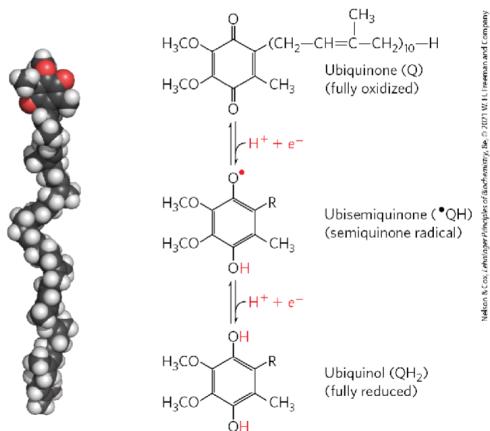
- Also known as NADH oxidoreductase or NADH dehydrogenase
- Large, large L-shaped enzyme with >40 polypeptide chains
- Accepts 2 electrons from NADH and passes them to FMN (Flavin Mononucleotide)
- Then passes electrons through 8+ Fe/S clusters to Ubiquinone **one at a time**
- This complex uses this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



(Review) Ubiquinone

- Ubiquinone, also known as coenzyme Q, is a lipid with a quinone ring structure at the top

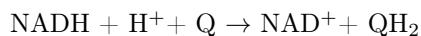
- Can be reduced with two electrons, then travels to Complex III: freely diffusible within the inner mitochondrial membrane
- plays a central role in coupling electron flow to proton movement



Complex I Catalyzes Two Simultaneous and Obligately Coupled Processes

Complex I catalyzes:

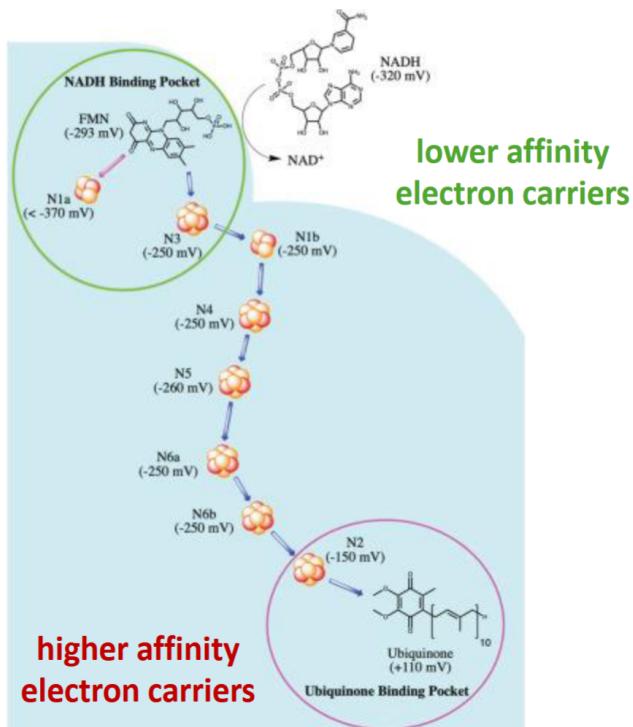
- the exergonic transfer of a hydride ion (hydrogen atom with two electrons) from NADH and a proton from the matrix to ubiquinone



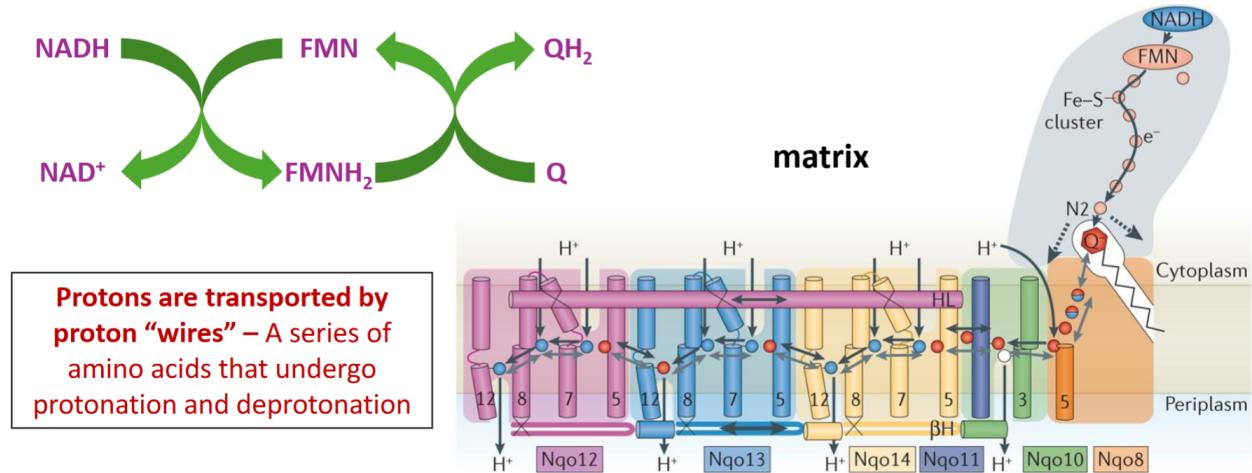
- the endergonic transfer of 4 protons from the matrix to the intermembrane space

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)

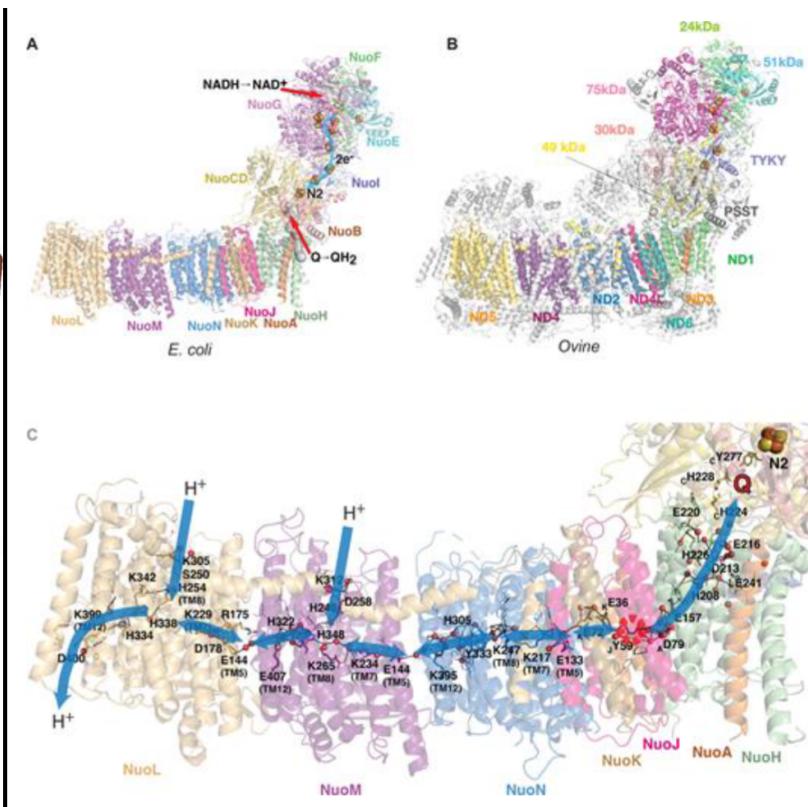
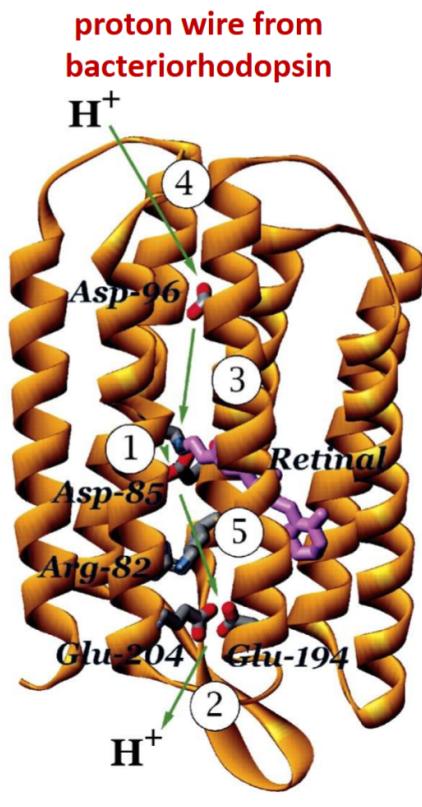


Complex I Overview



Proton Wires

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

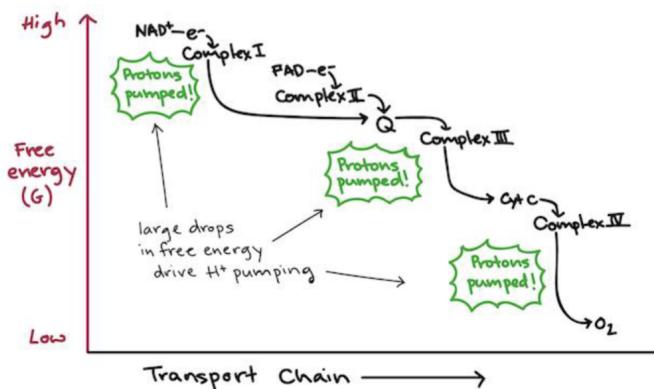


Proton transfer pathways, outlined by blue arrows. Membrane arm contains the central axis of charged residues, essential for the proton transfer and the coupling

Pumping Protons and Free Energy

Half-Reaction	E° (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O$	0.815
$NO_3^- + 2H^+ + 2e^- \rightleftharpoons NO_2^- + H_2O$	0.42
Cytochrome a_3 (F^{2+}) + $e^- \rightleftharpoons$ cytochrome a_3 (F^{2+})	0.385
$O_2(g) + 2H^+ + 2e^- \rightleftharpoons H_2O_2$	0.295
Cytochrome a (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome a (Fe^{2+})	0.29
Cytochrome c (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome c (Fe^{2+})	0.235
Cytochrome c_1 (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome b (Fe^{2+}) (mitochondrial)	0.077
Ubiquinone + $2H^+ + 2e^- \rightleftharpoons$ ubiquinol	0.045
Fumarate $^-$ + $2H^+ + 2e^- \rightleftharpoons$ succinate $^-$	0.031
FAD + $2H^+ + 2e^- \rightleftharpoons FADH_2$ (in flavoproteins)	~0.
Oxaloacetate $^-$ + $2H^+ + 2e^- \rightleftharpoons$ malate $^-$	-0.166
Pyruvate $^-$ + $2H^+ + 2e^- \rightleftharpoons$ lactate $^-$	-0.185
Acetaldehyde + $2H^+ + 2e^- \rightleftharpoons$ ethanol	-0.197
FAD + $2H^+ + 2e^- \rightleftharpoons FADH_2$ (free coenzyme)	-0.219
$S + 2H^+ + 2e^- \rightleftharpoons H_2S$	-0.23
Lipoic acid + $2H^+ + 2e^- \rightleftharpoons$ dihydrolipoic acid	-0.29
NAD $^+$ + $H^+ + 2e^- \rightleftharpoons$ NADH	-0.315
NAD $^+$ + $H^+ + 2e^- \rightleftharpoons$ NADPH	-0.320
Cysteine disulfide + $2H^+ + 2e^- \rightleftharpoons$ 2 cysteine	-0.340
Acetacetate $^-$ + $2H^+ + 2e^- \rightleftharpoons$ β -hydroxybutyrate $^-$	-0.346
$H^+ + e^- \rightleftharpoons \frac{1}{2}H_2$	-0.421
$SO_4^{2-} + 2H^+ + 2e^- \rightleftharpoons SO_3^{2-} + H_2O$	-0.515
Acetate $^-$ + $3H^+ + 2e^- \rightleftharpoons$ acetaldehyde + H_2O	-0.581

How much free energy is released through the redox reactions of Complex I?



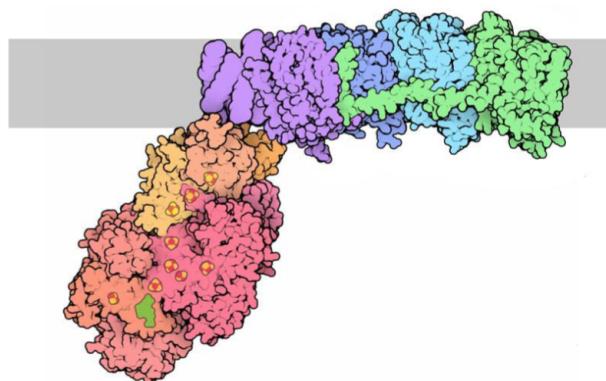
- Free energy is released on each step, with every reduction. Oxygen is the final electron acceptor, with the lowest free energy and the highest electronegativity.
 - To find how much free energy is released between two steps, subtract their values to find ΔE° , then plug into the equation $\Delta G^\circ = -nFE^\circ$.

- 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



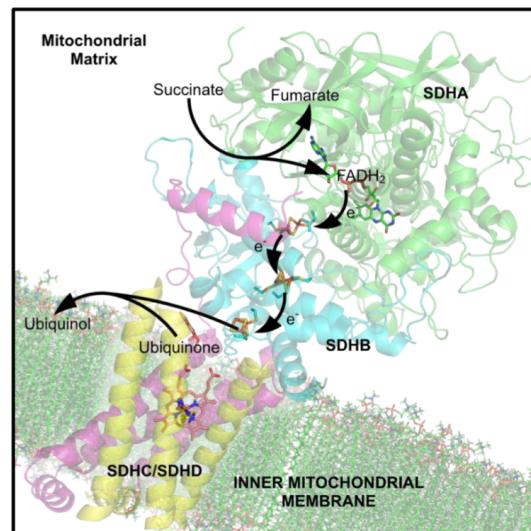
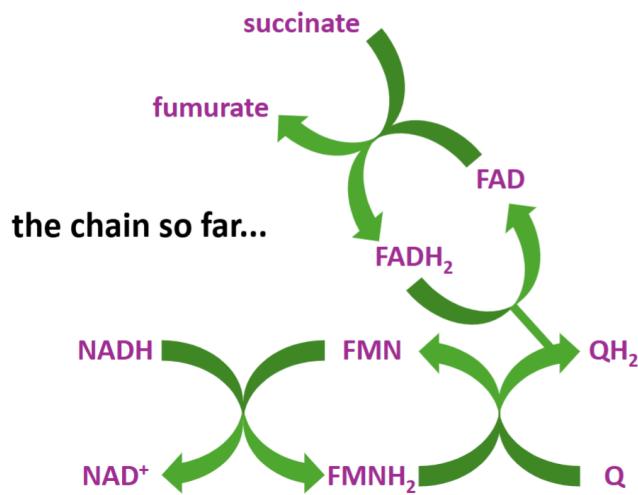
water flow powering grinding grain



electron flow powering proton pumping

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)

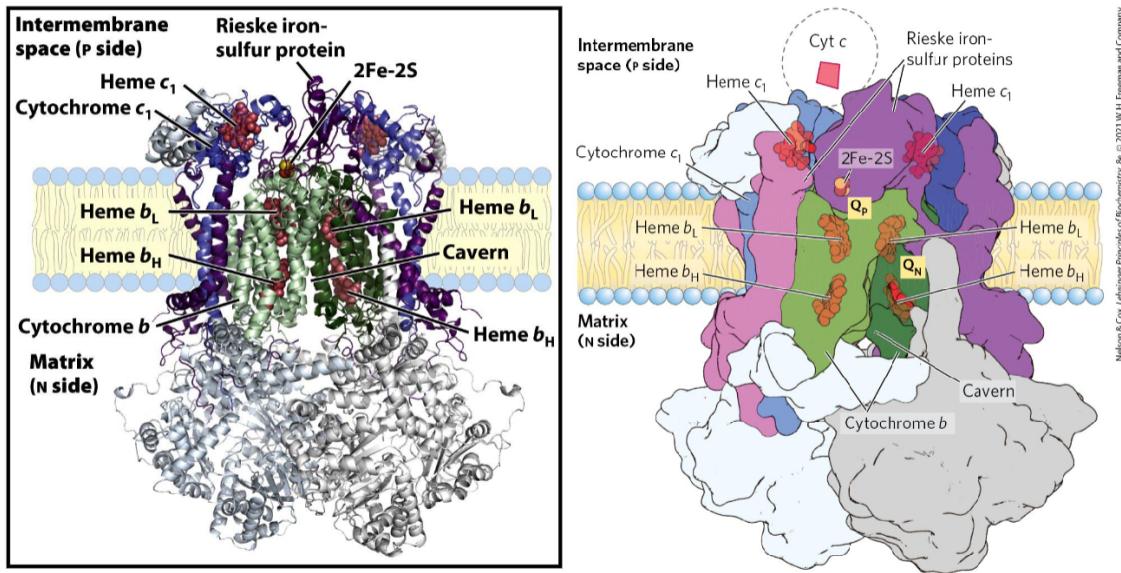


Why Doesn't Complex II Pump Protons?

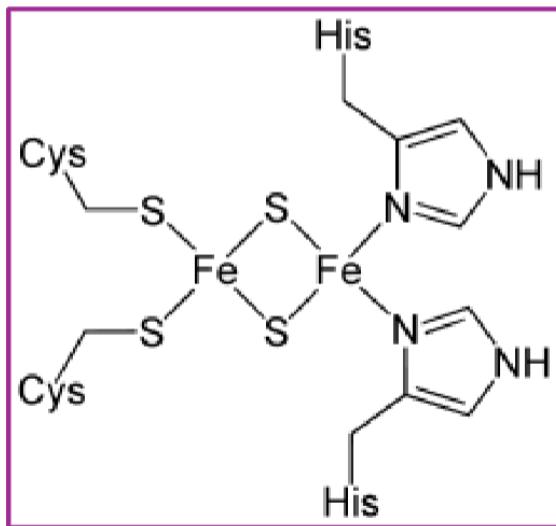
- Reduction of Fumarate and reduction of Ubiquinone only has a ΔE° of +0.014 V! There is not enough energy differential to pump protons.

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.

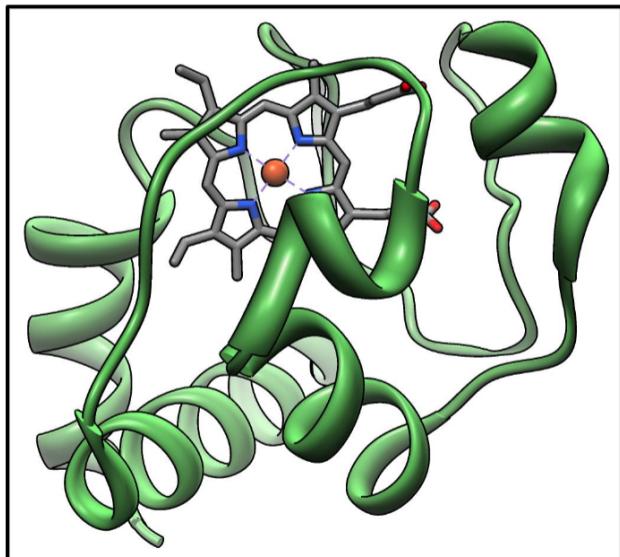


Rieske center



Cytochromes

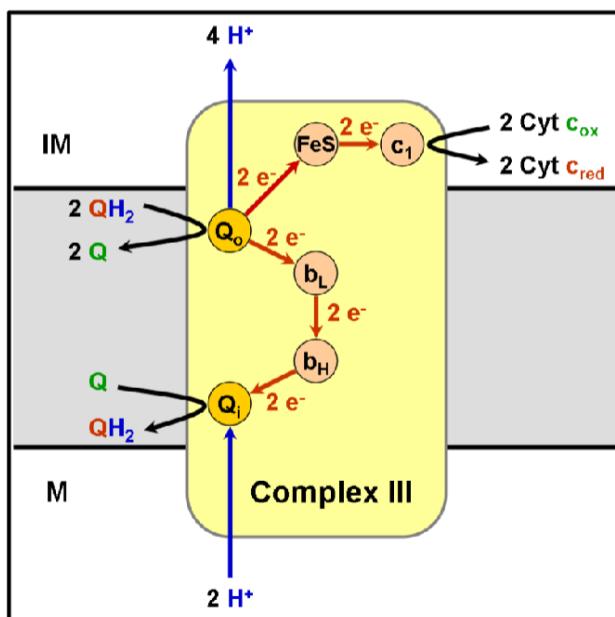
- Proteins with heme groups that are involved in redox reductions (as opposed to O₂ binding)
- **Oxidation state change in central iron allows for electron carrying**
- Can be embedded in complexes (e.g., cytochrome *b*, cytochrome *c*₁) or freely moving between them (cytochrome *c*)



Cytochrome c

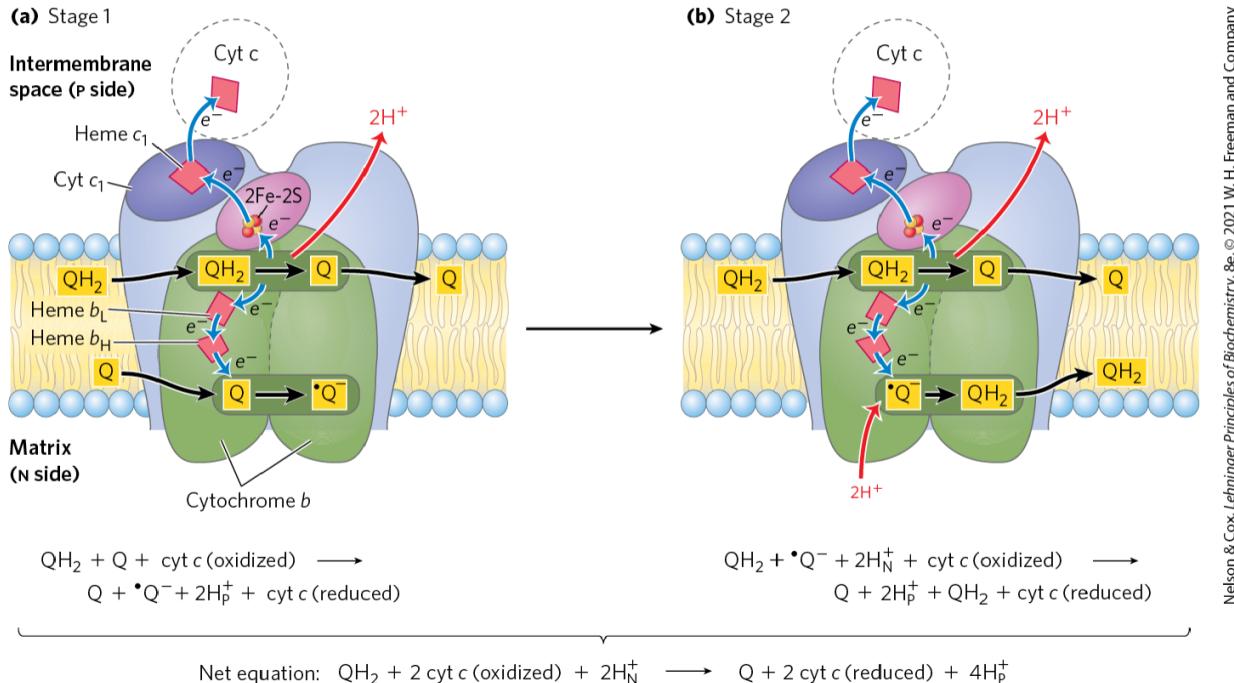
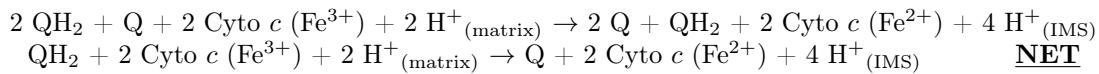
The Q Cycle

- The two cavities in Complex III can bind both reduced **ubiquinol (QH₂)** and **oxidized ubiquinone (Q)**
- When ubiquinol (QH₂) 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·⁻)**
- Same process occurs with a second ubiquinol, generating a second reduced cytochrome *c*, but **fully reduces one CoQ**
 - This ensures that while two QH₂ molecules are oxidized, one QH₂ is regenerated, maintaining the balance of the ubiquinone pool



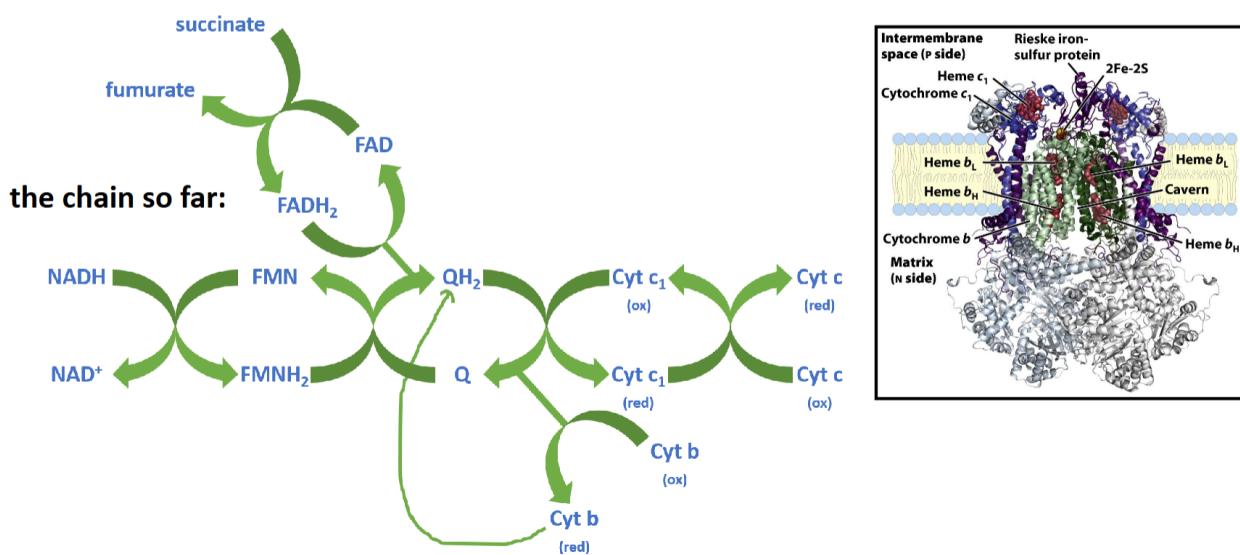
- One reason for this cycle (besides lack of FAD/FMN) is that H^+ ions can be donated directly to the intermembrane space from oxidized QH_2

– Four protons transported for every two electrons reaching cytochrome *c*



Nelson & Cox, Lehninger Principles of Biochemistry, 8e, © 2021 W.H. Freeman and Company

Complex III Overview



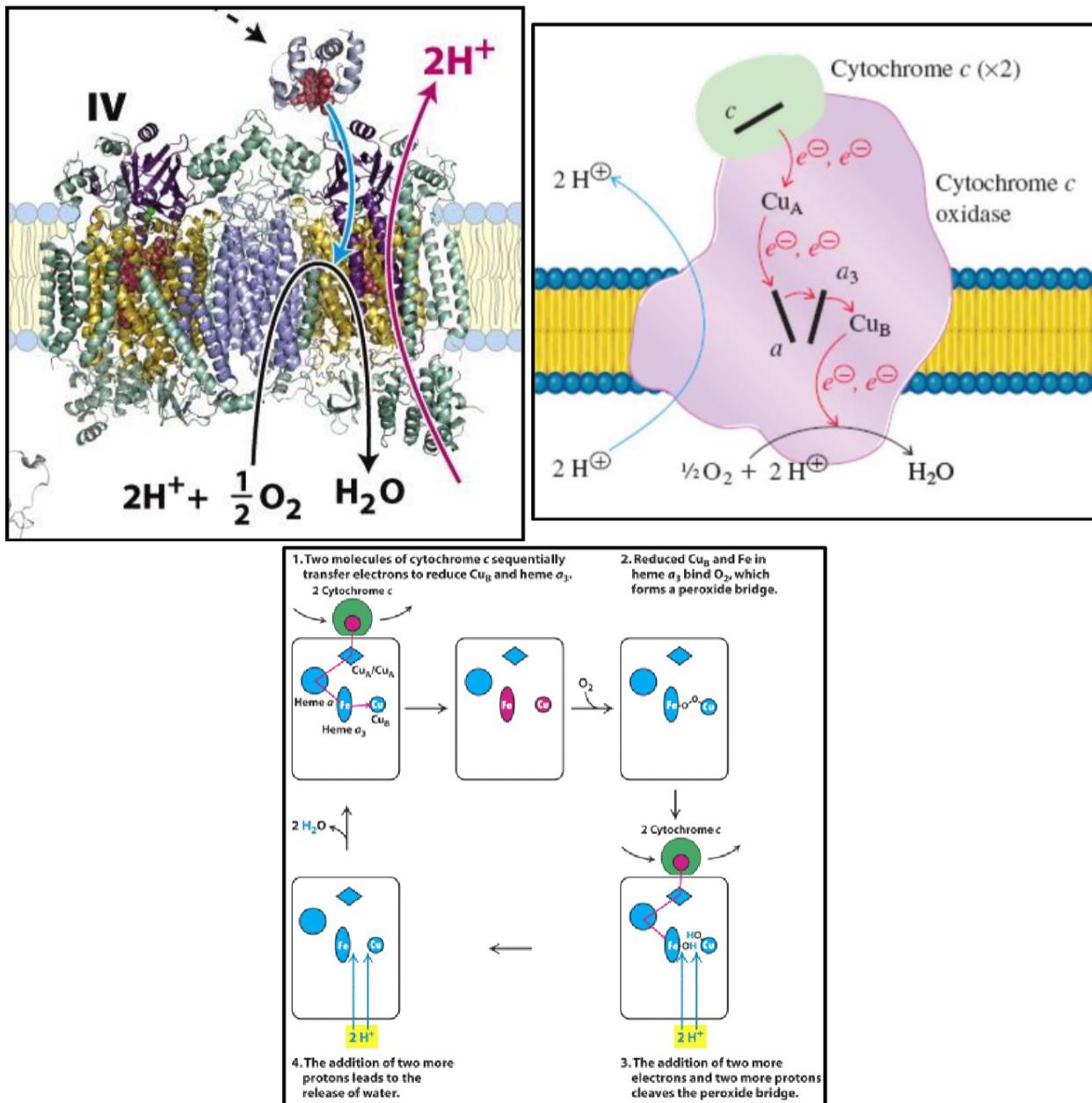
Complex IV: Cytochrome *c* Oxidase

- Two cytochrome *c* molecules each transfer one electron to Complex IV through two redox-active centers: two cytochrome groups (cyt *a* and cyt *a*₃) and two copper atom groups (Cu_A and Cu_B)

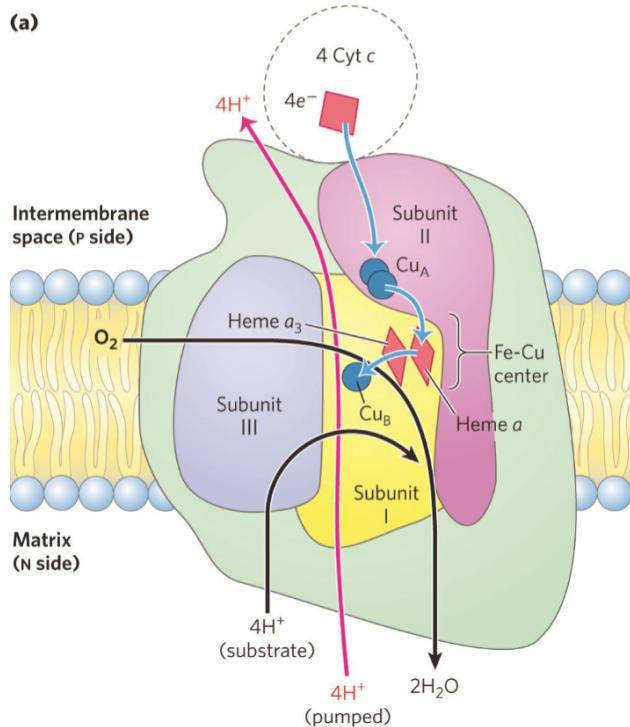
- One electron is held at the Cu_B center
- The other is held at cytochrome a3
- Once oxygen (O_2) binds to cytochrome a3 and Cu_B , it accepts the two electrons, forming a peroxide bridge

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 ($2 \text{ H}_2\text{O}$)

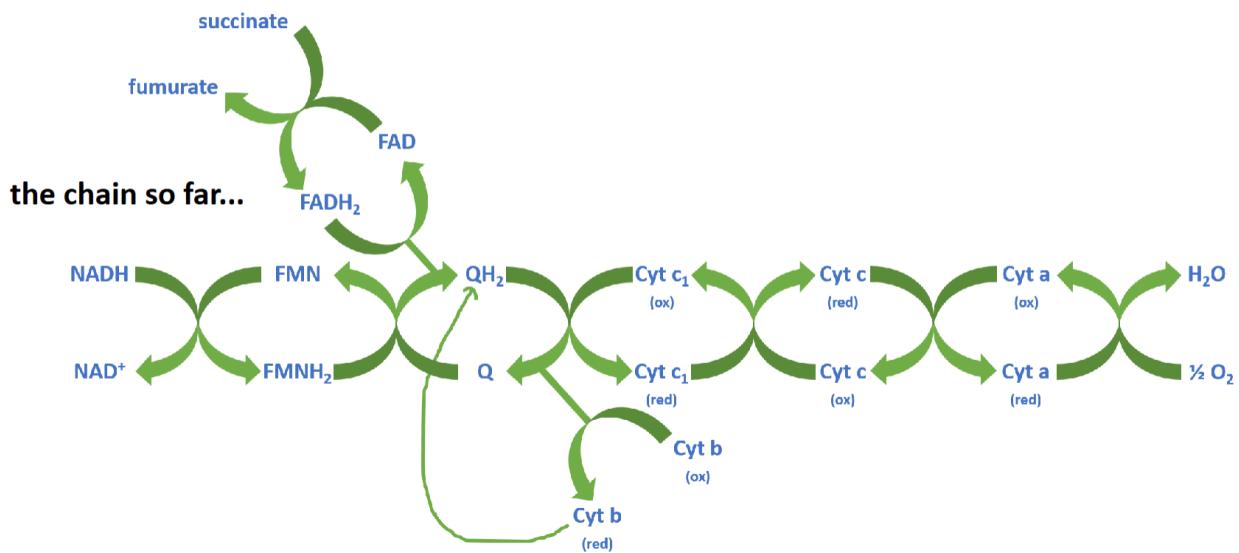
- The complete process involves the oxidation of **four cytochrome c molecules**, transferring four electrons to reduce one molecule of O_2 to two molecules of H_2O . This process requires the uptake of four protons from the matrix, contributing to the proton gradient necessary for ATP synthesis



Path of Electron Through Complex IV

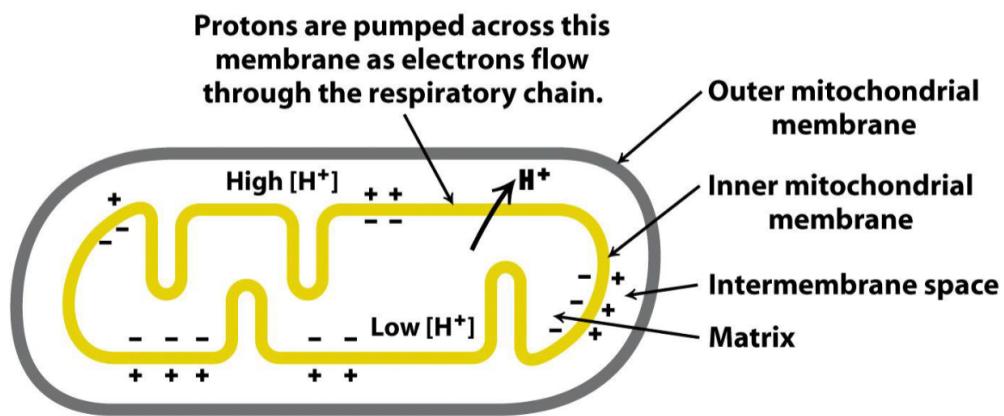


Complex IV Overview



Electrochemical Gradient Across the Inner Membrane

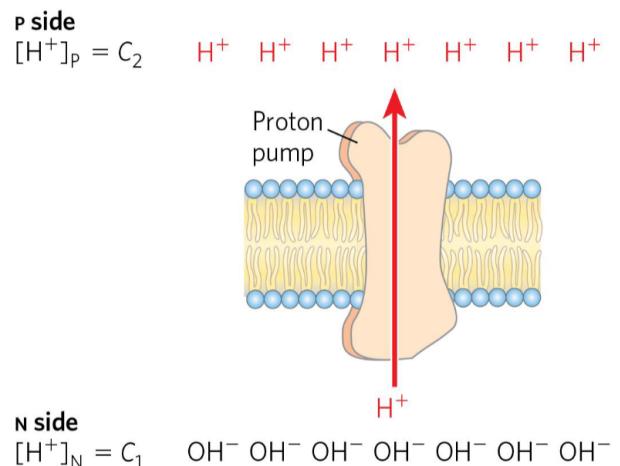
The free energy made available by "downhill" (exergonic) electron flow is coupled to the "uphill" transport of protons across a proton-impermeable membrane. The free energy of fuel oxidation is thus conserved as a transmembrane electrochemical potential



this generates proton motive force

Proton-Motive Force

- **proton-motive force** = the energy stored in an electrochemical proton gradient across the mitochondrial inner membrane
 - composed of chemical and electrical potential energy

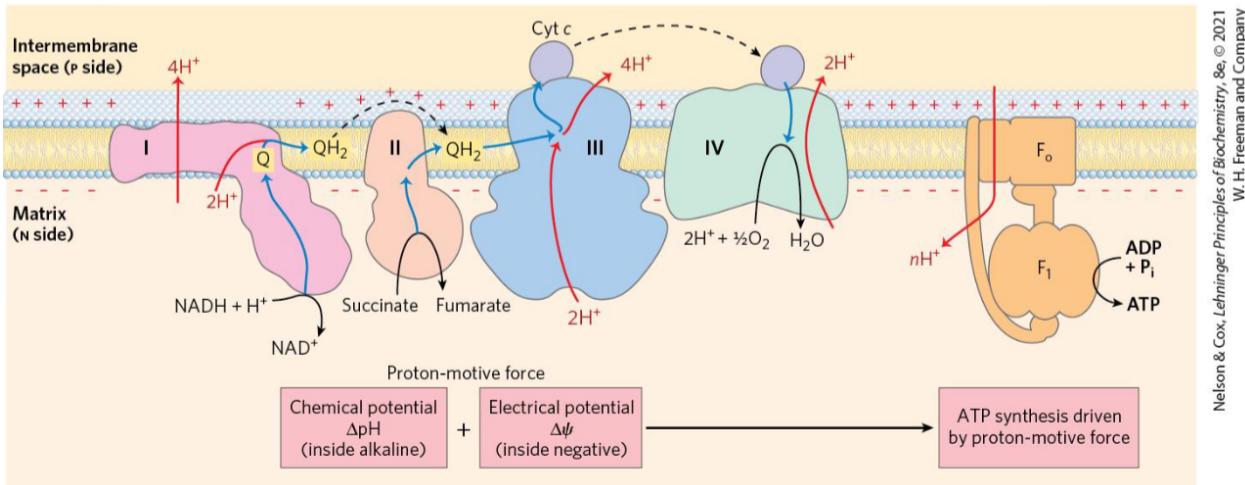


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$$\begin{aligned}\Delta G &= RT \ln(C_2/C_1) + ZF\Delta\psi \\ &= 2.3RT \Delta pH + F\Delta\psi\end{aligned}$$

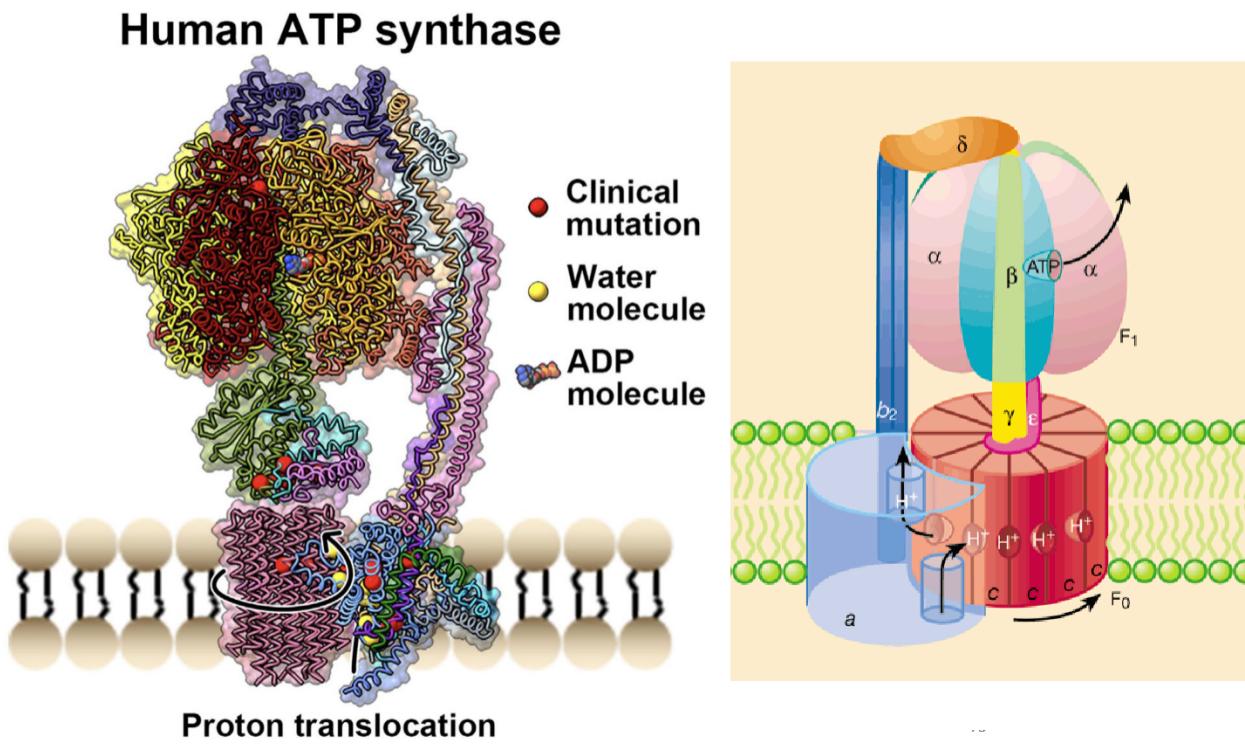
In the Chemiosmotic Model, Oxidation and Phosphorylation are Obligately Coupled

- **chemiosmotic model** = describes the coupling of ATP synthesis to an electrochemical proton gradient (the proton-motive force)



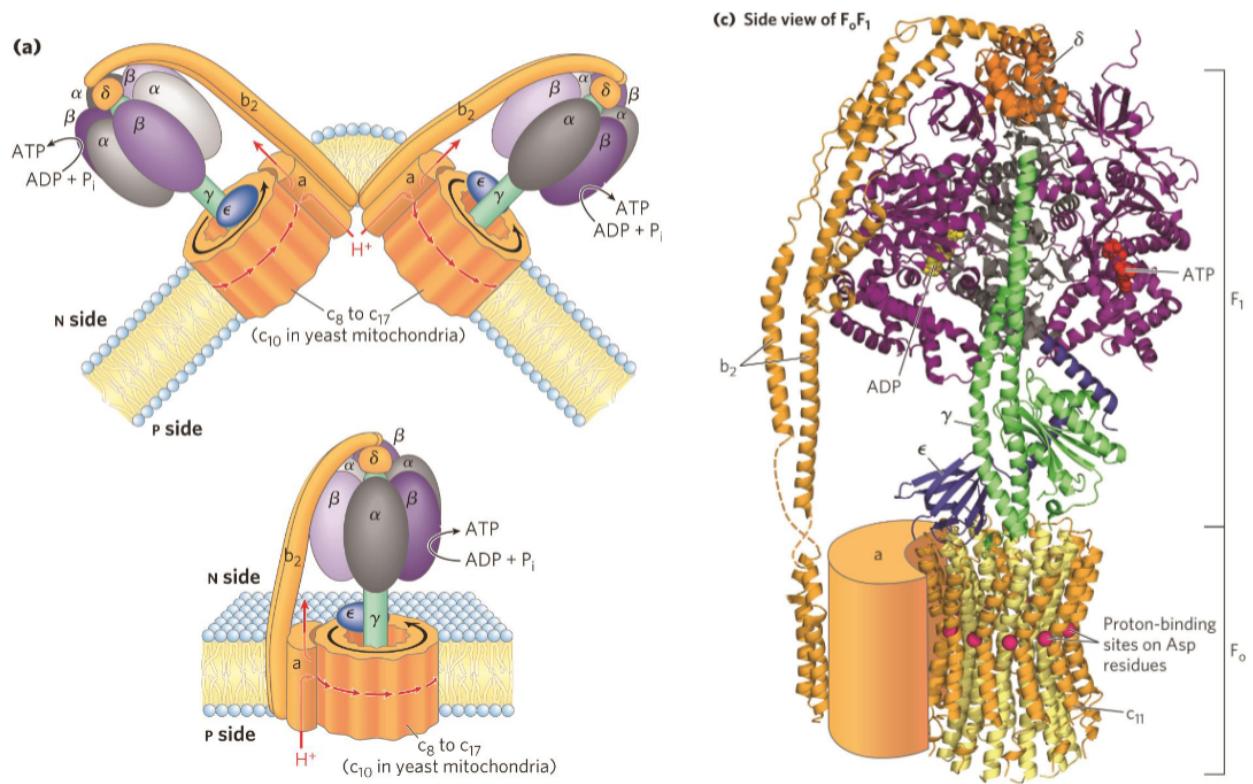
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Complex V: ATP Synthase



- Proton translocation**
- Molecular Cell 83, 2137–2147, June 15, 2023 © 2023 Elsevier Inc.
- ATP synthase uses the electrochemical gradient produced by the protons pumped into the IMS to produce ATP
 - Two major portions
 - F₀ stalk region** that translates the electrochemical energy into mechanical motion
 - Subunit **a** and **c ring**
 - F₁ catalytic region** that translates mechanical motion into ATP synthesis
 - α** and **β** subunits
 - γ (gamma) subunit connects them both - aka it translates the mechanical motion!

The Structure of the F₀F₁ Complex

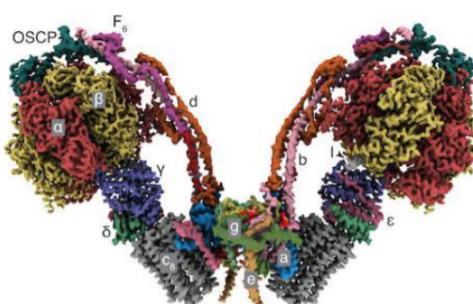
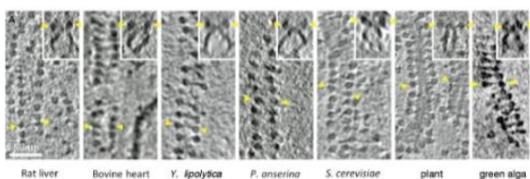


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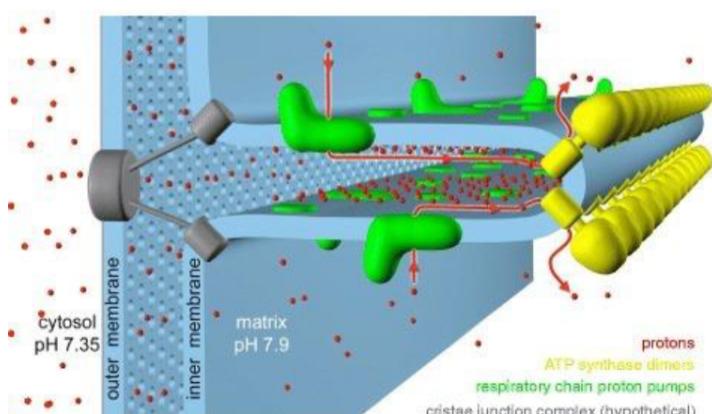
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Dimer rows of the mitochondrial ATP synthase in different organisms

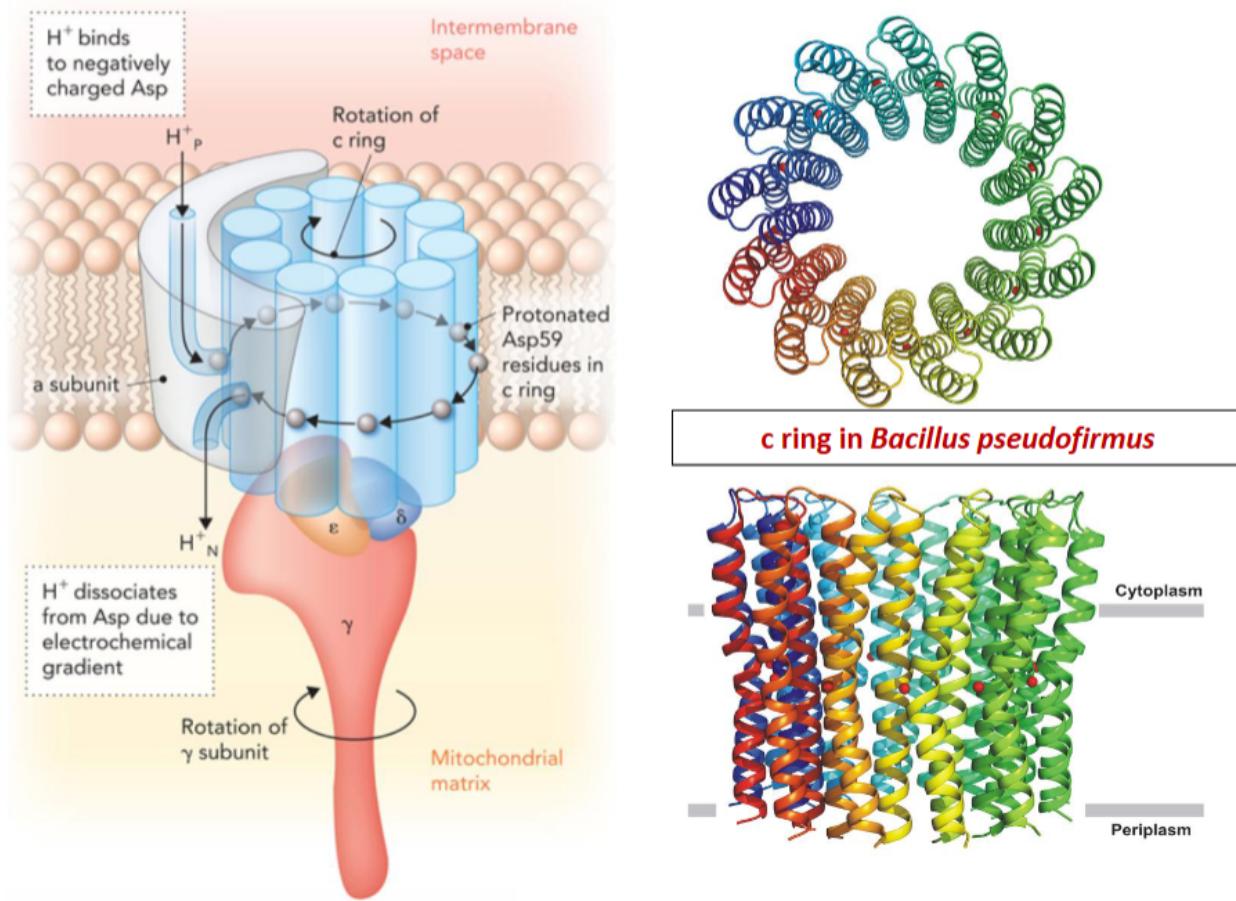


Organisation of mitochondrial cristae



F₀ portion: Proton motor / rotor

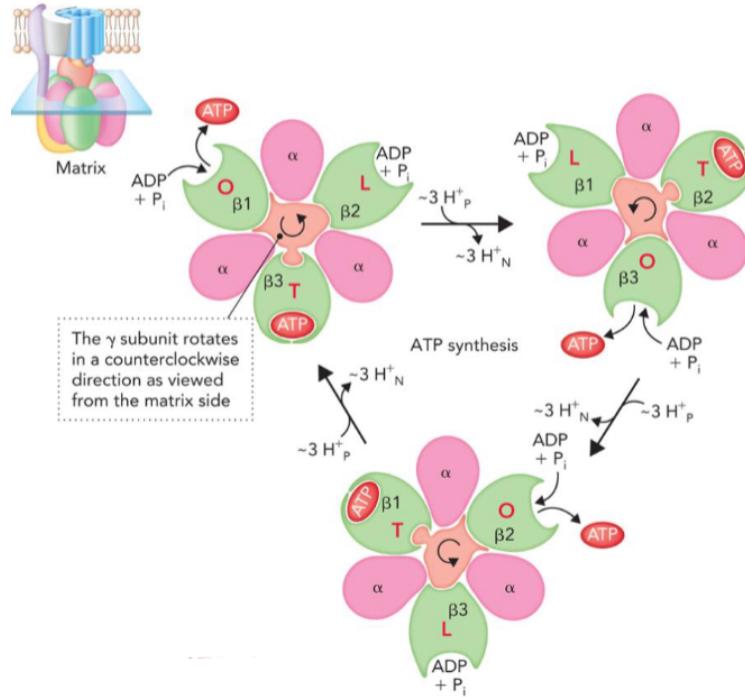
- The a subunit has two hydrophilic channels, one allowing for the translocation of protons from the intermembrane space and one allowing for translocation to the matrix (both connect to the c ring)
 - The electrochemical gradient pushes protons from the IMS into the a subunit which adds the proton to an un-protonated c subunit
- The c ring is (roughly) 10 alpha helix subunits attached in a circular loop
 - Each subunit has a key residue, Asp59 that can accept the proton from the a channel
 - After one full rotation, the second channel becomes available, allowing the proton to escape to the matrix
- This proton-powered rotation turns the gamma (γ) subunit!



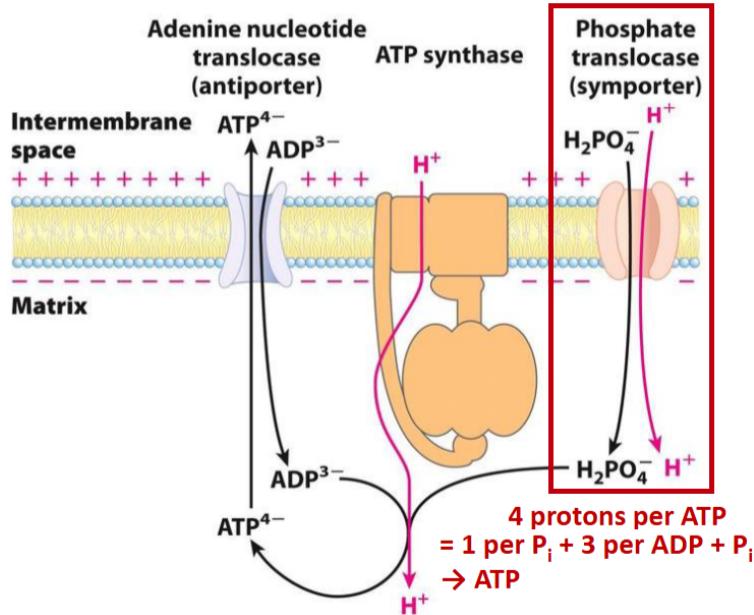
F₁ portion: ATP synthesis

- The α and β subunits form a hexamer (3 of each) with the γ subunit wedged in the middle
- As the γ subunit turns, it stimulates conformational change for the α and β subunits
 - $O \rightarrow L \rightarrow T$ (when counter-clockwise)
- The **open** conformation allows for ADP and P_i to bind (and allows previous ATP to leave)
- The **loose** conformation locks ADP and P_i in place

- The **tight** conformation promotes ATP formation
- One full turn produces 3 ATP!
- Around 10 protons makes 3 ATP. (3.33 protons/ATP)



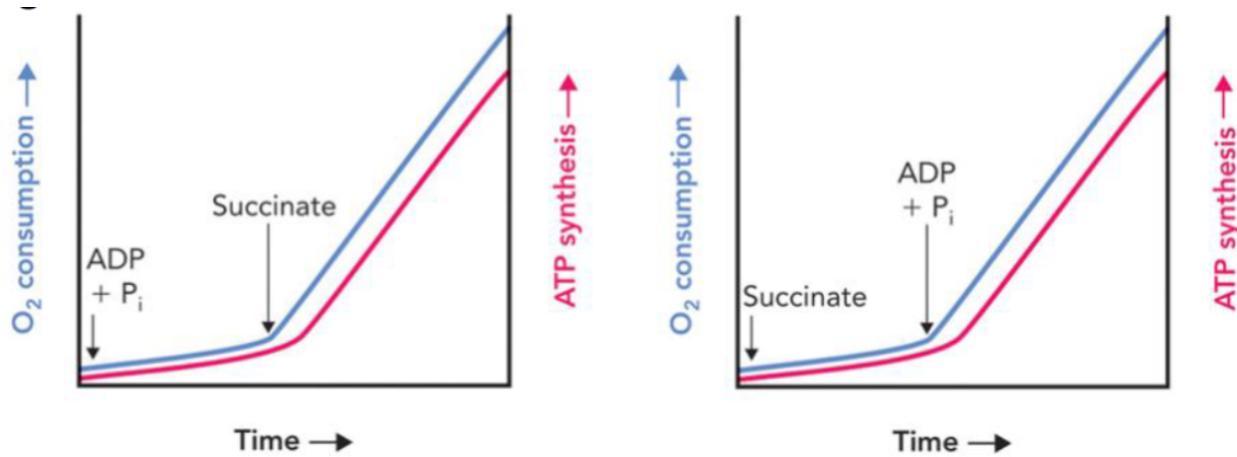
Extra Proton Used by Phosphate Translocase Symporter



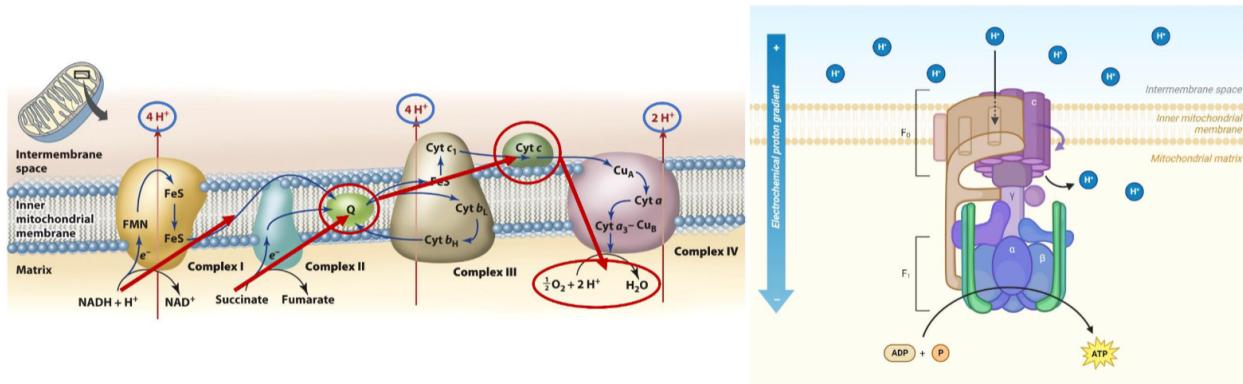
O_2 Consumption and ATP Synthesis are Tightly Linked

- Let's examine our model of oxidative phosphorylation

- Imagine an experiment with free floating mitochondria where we can control metabolites and measure environmental variables
- Need to provide ADP/ P_i as well as electron source for ATP synthesis and O_2 consumption
 - Everything stops if either is missing
- O_2 consumption and ATP synthesis are tightly linked
 - What links them? The electrochemical gradient



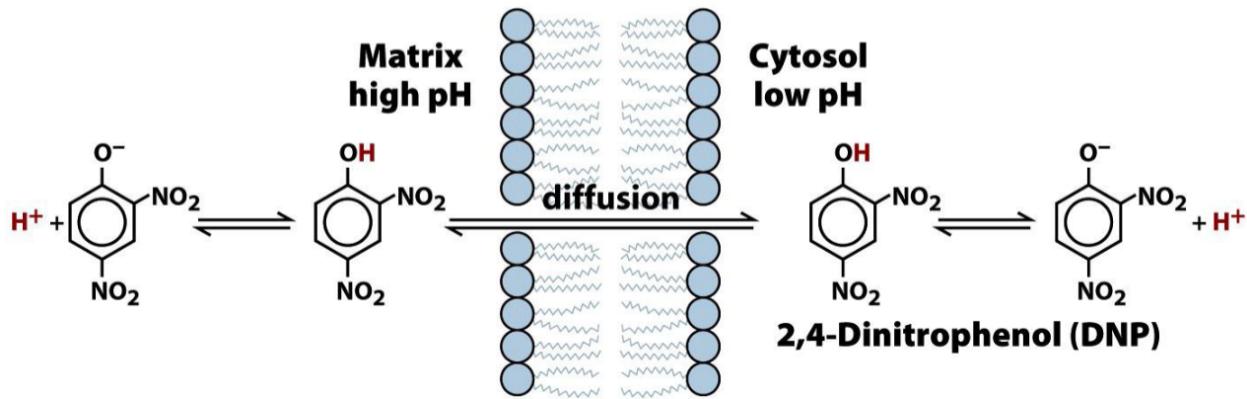
Respiratory Control and ATP Synthesis



- The electron transport chain pumps protons up their gradient
 - When this gradient becomes too high, electron transport becomes more difficult and ETC slows
 - This phenomenon is called **respiratory control**
- ATP synthase also relies on this gradient - when the gradient is too low, ATP synthesis slows
- This causes the correlation we saw earlier!

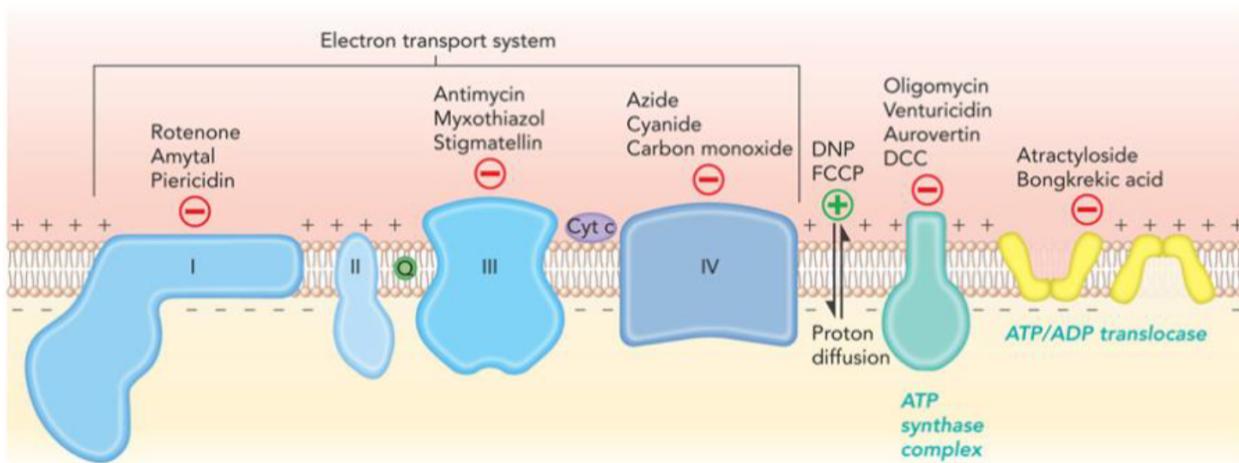
Uncoupling O₂ Consumption and ATP Synthesis

- Certain molecules can allow protons to cross the inner membrane of mitochondria
 - This would immediately destroy our proton motive force
- These molecules are called **uncouplers** because they uncouple ATP synthesis and O₂ consumption



Some idiots tried to use a drug like this in the late 20th century to lose weight. They died.

Inhibitors of ETC



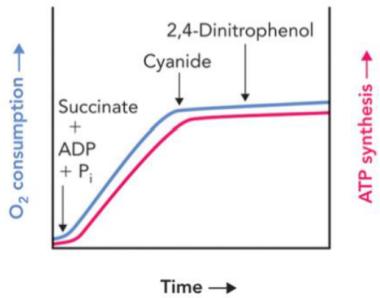
No need to memorize these.

The Impact of Inhibitors/Uncouplers

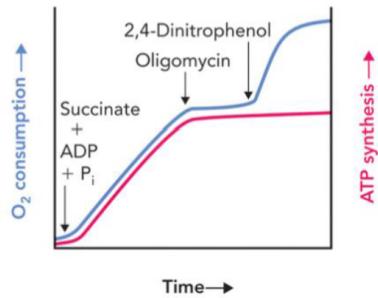
- Cyanide inhibits Complex IV (stops ETC → O₂ consumption and ATP synthesis stop)
- Oligomycin inhibits ATP synthase (ETC slows down due to backed-up proton gradient)
- DNP (2,4-Dinitrophenol) is an uncoupler, destroys the proton gradient (ETC runs without ATP production)

Why do these graphs make sense?

ETC is going to keep working (futilely) to re-establish the gradient
The first four complexes of ETC are tied to O₂ consumption



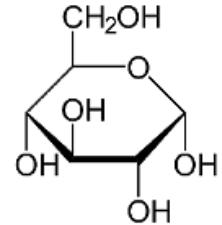
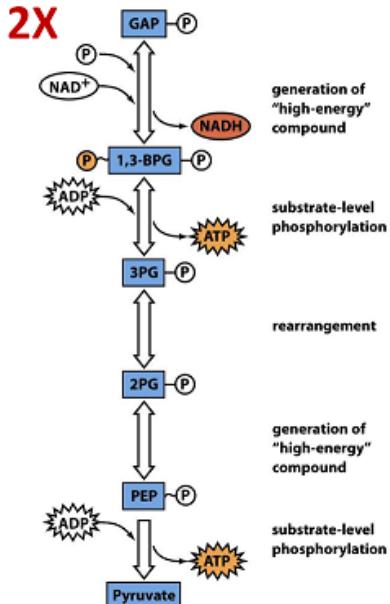
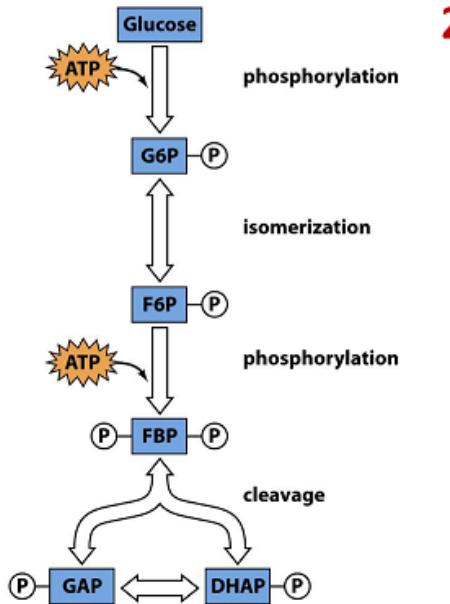
Cyanide stops ETC → O₂ consumption stops



Electrochemical gradient regulates ETC action

Shuttles

Glycolysis Products

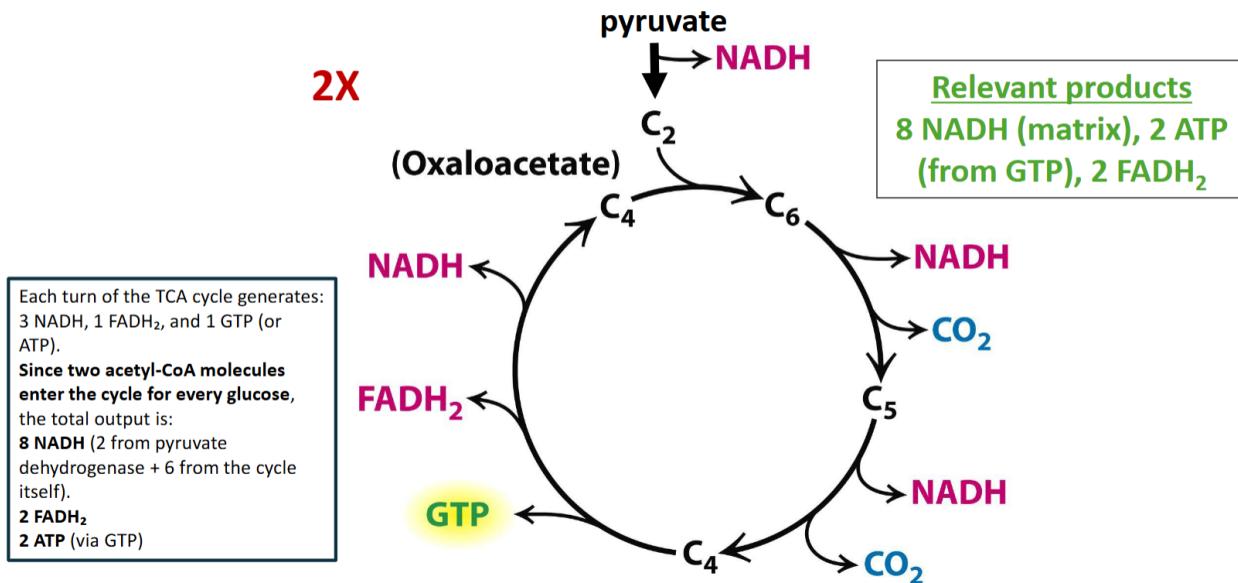


What ATP yield would we get if we fully oxidized one glucose?

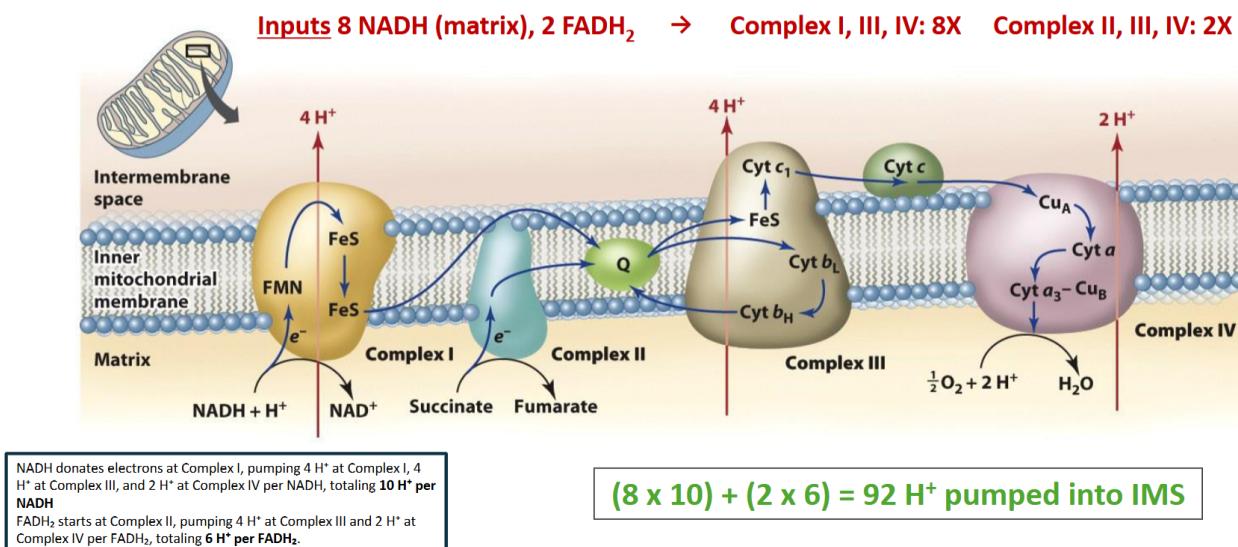
Relevant products
2 NADH (cytosol), 2 ATP

these 2 NADH are going to find their way to the matrix through a shuttle mechanism

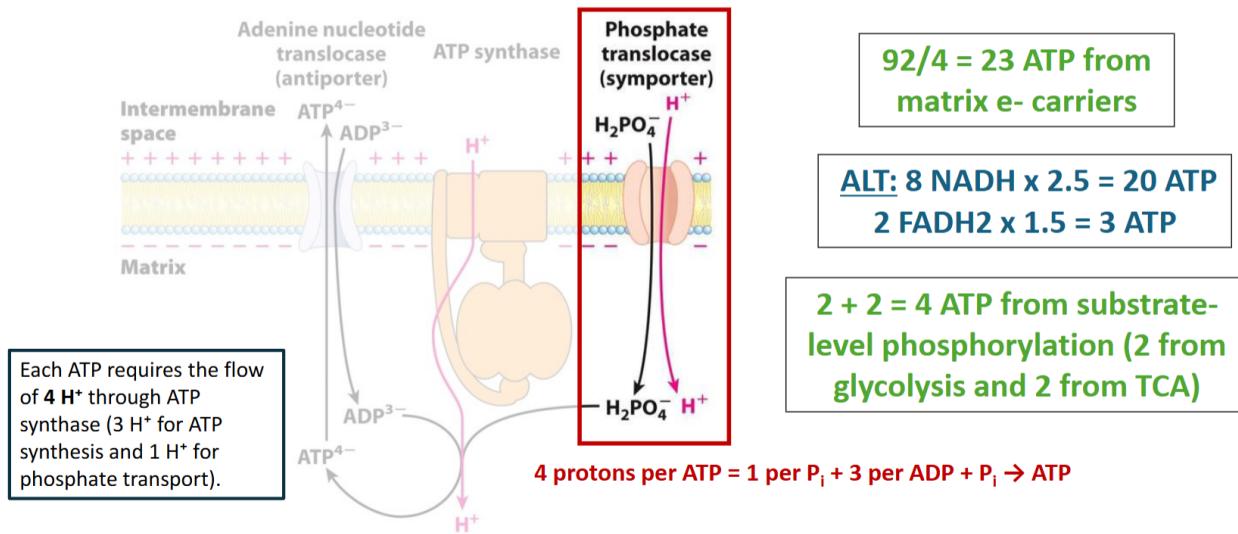
TCA Products



ETC Overview

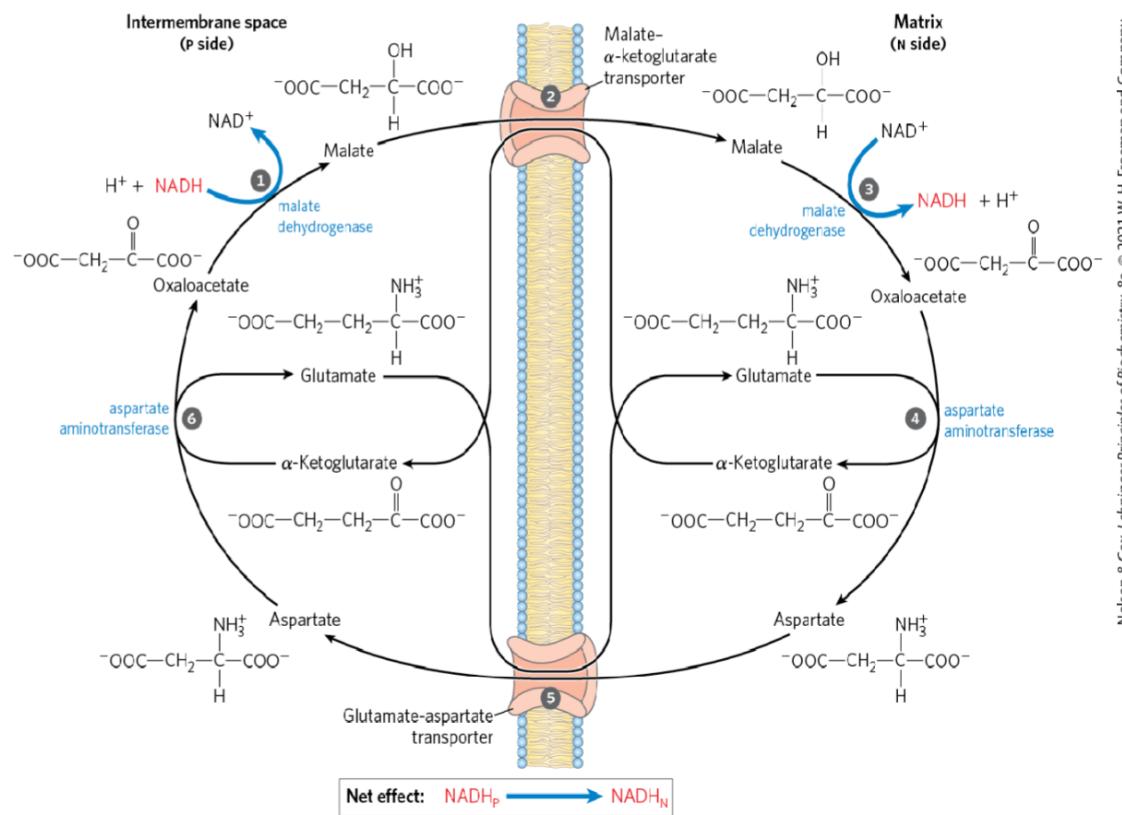


Protons to ATP and Yields



The Malate-Aspartate Shuttle

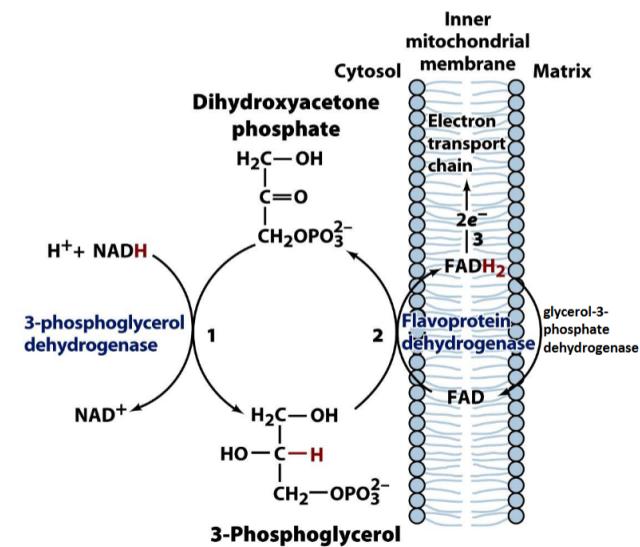
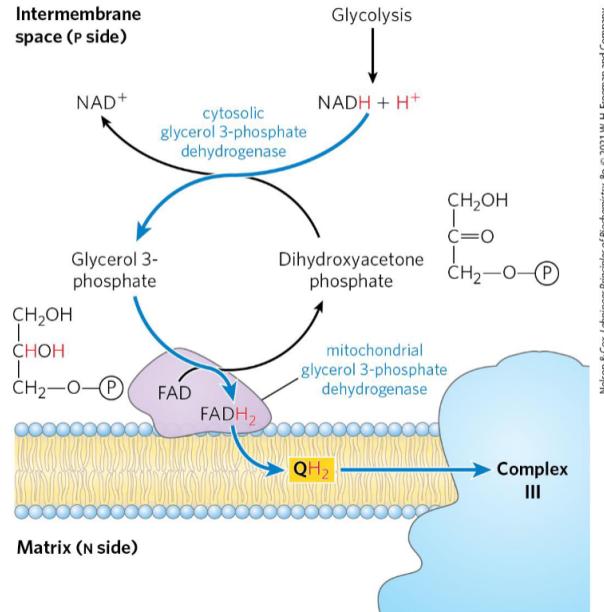
- NADH equivalents moved in by the **malate-aspartate shuttle** enter the respiratory chain at Complex I and yield a P/O ratio of 2.5
- These shuttles are going to pass the electrons from cytosolic NADH to an electron carrier in the matrix
 - Malate-aspartate passes these electrons to an NAD^+ , reforming NADH
 - The malate-aspartate shuttle is used by our **heart** and **liver** cells



$$\begin{aligned}
 2 \text{ NADH} (\text{cytosol}) &\rightarrow 2 \text{ NADH} (\text{matrix}) \\
 2 \text{ NADH} \times 2.5 &= 5 \text{ ATP} \\
 23 \text{ (e}^-\text{ carriers)} + 4 \text{ (SLP)} + 5 \text{ (shuttle)} &= 32 \text{ ATP}
 \end{aligned}$$

The Glycerol 3-Phosphate Shuttle

- NADH equivalents moved in by the **glycerol 3-phosphate shuttle** enter the respiratory chain at Complex III and yield a P/O ratio of 1.5.



- The glycerol-3-phosphate shuttle passes these electrons to a protein in the inner mitochondrial membrane
 - Cytosolic NADH reduces DHAP to glycerol-3-phosphate via cytosolic glycerol-3-phosphate dehydrogenase (which oxidizes NADH to NAD⁺)
 - Glycerol-3-phosphate enters the mitochondria and donates its electrons to **FAD** via **mitochondrial glycerol-3-phosphate dehydrogenase**
 - Electrons are passed to **Complex III** using **Coenzyme Q**
 - The G3P shuttle is used by **brain** and **muscle** cells

Stoichiometry of Coenzyme Reduction and ATP Formation in Aerobic Oxidation of Glucose

Table 16-1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed
Glucose → glucose 6-phosphate	-1 ATP	-1
Fructose 6-phosphate → fructose 1,6-bisphosphate	-1 ATP	-1
2 Glyceraldehyde 3-phosphate → 2 1,3-biphosphoglycerate	2 NADH	3 or 5
2 1,3-Biphosphoglycerate → 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate → 2 pyruvate	2 ATP	2
2 Pyruvate → 2 acetyl-CoA	2 NADH	5
2 Isocitrate → 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate → 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA → 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate → 2 fumarate	2 FADH ₂	3
2 Malate → 2 oxaloacetate	2 NADH	5
Total		30-32

Summary of Shuttles

The **Malate-Aspartate Shuttle** generates more ATP because it fully conserves the energy of cytosolic NADH by transferring it to NADH in the matrix, allowing the electrons to enter the ETC at Complex I. In contrast, the **Glycerol-3-Phosphate Shuttle** sacrifices some energy by transferring electrons to FADH₂, which enters at Complex III, resulting in lower ATP production. This efficiency tradeoff often depends on the cell type and its metabolic needs.