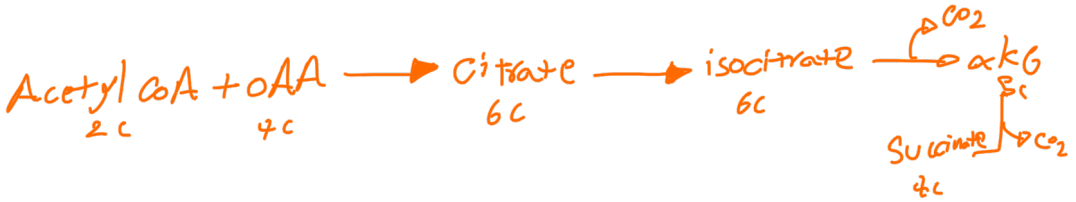
Krebs Cycle
TCA cycle
Citric acid cycle

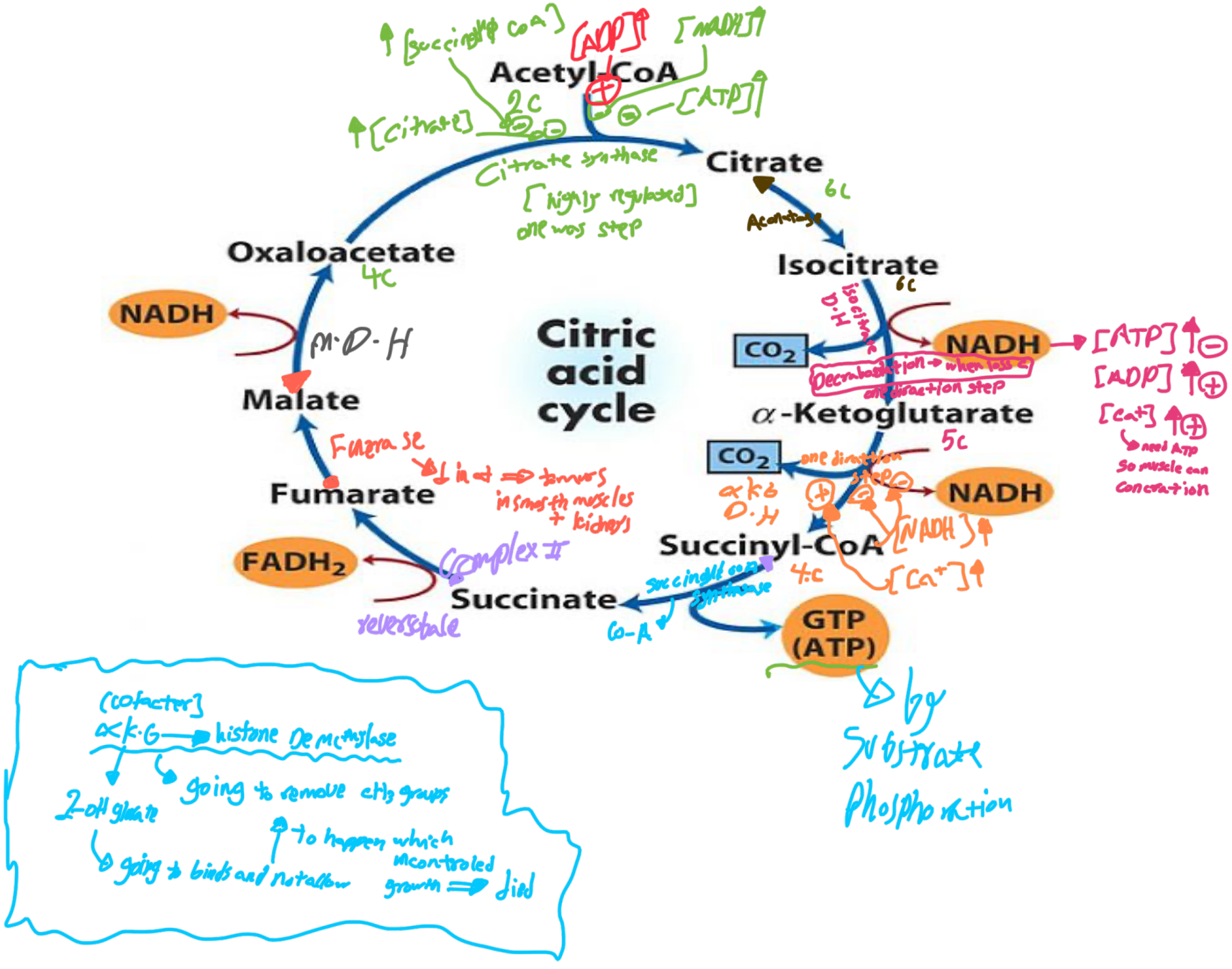
TCA Cycle

1. Acetyl-CoA donates its acetyl group to the four-carbon compound oxaloacetate to form the six-carbon citrate. Citrate is then transformed into isocitrate, also a six-carbon molecule
2. dehydrogenated with loss of CO2 to yield the five-carbon compound Alpha-ketoglutarate
3. alpha-Ketoglutarate undergoes loss of a second molecule of CO2 and ultimately yields the four-carbon compound succinate.
4. Succinate is then enzymatically converted in three steps into the four-carbon oxaloacetate—which is then ready to react with another molecule of acetyl-CoA.



The tricarboxylic acid (TCA) cycle

- Krebs cycle
- The citric acid cycle
- The enzymes of the TCA cycle are located in the mitochondrial matrix
- Fuel Molecules lose electrons, get oxidized and donate those electrons to energy carriers producing NADH and FADH2
- Main function of the TCA cycle is to release high-energy electrons that power the synthesis of ATP via oxidative phosphorylation



- Acetyl-CoA adds two carbons to oxaloacetate to start the cycle.
- Isomerization takes place by removing H2O and then adding it back.
- A CO2 is lost and a NADH is produced
- Another CO2 is lost and another NADH is produced.
- A substrate-level phosphorylation.
- FAD+ is reduced to form FADH2
- another NADH is produced

Each turn of the cycle forms

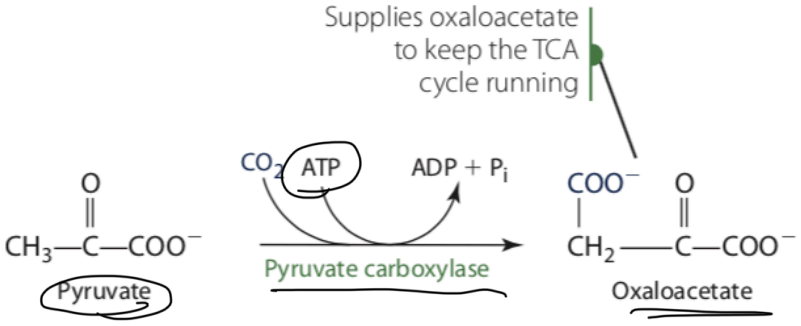
- 1 GTP
- 3 NADH molecules
- 1 FADH2 molecule
- Note: oxygen is not required until later
- Oxygen is required to recycle
  - NADH NAD+
  - FADH2 FAD
  - (Thus generating ATP)
- Cofactors must be recycled to be reused in the Krebs Cycle

- The role of the citric acid cycle not confined to the oxidation of acetate only
- The citric acid cycle is a "hub of intermediary metabolism"
- Four- and five-carbon end products of many catabolic processes feed into the cycle and serve as fuels.
- Oxaloacetate and alpha ketoglutarate are produced from aspartate and glutamate
- Under some metabolic circumstances, drawn out of the cycle to serve as precursors of the amino acids aspartate and glutamate by simple transamination reactions
- Oxaloacetate is converted to glucose in gluconeogenesis
- Succinyl- CoA is a central intermediate in the synthesis of the porphyrin ring of heme groups, which serve as oxygen carriers (in hemoglobin and myoglobin

- As intermediates of the citric acid cycle are removed to serve as biosynthetic precursors, they are replenished by anaplerotic **reactions**
- The most important anaplerotic reaction in mammalian liver and kidney is the reversible carboxylation of pyruvate by CO2 to form oxaloacetate
- Pyruvate carboxylase is a regulatory enzyme and is virtually inactive in the absence of acetyl-CoA.

- To keep the TCA cycle functioning, oxaloacetate—and other TCA cycle intermediates that can lead to oxaloacetate— must be replenished in the cycle.
- Oxaloacetate, fumarate, succinyl-CoA, and a-ketoglutarate can all be formed from certain amino acids
- We ne urbons) + CO

High levels of acetyl CoA activate pyruvate Carboxylase



Requires Biotin

Figure 3.22 Formation of oxaloacetate from pyruvate and CO_2.

Anaplitic reaction

Regulation of the Citric Acid Cycle

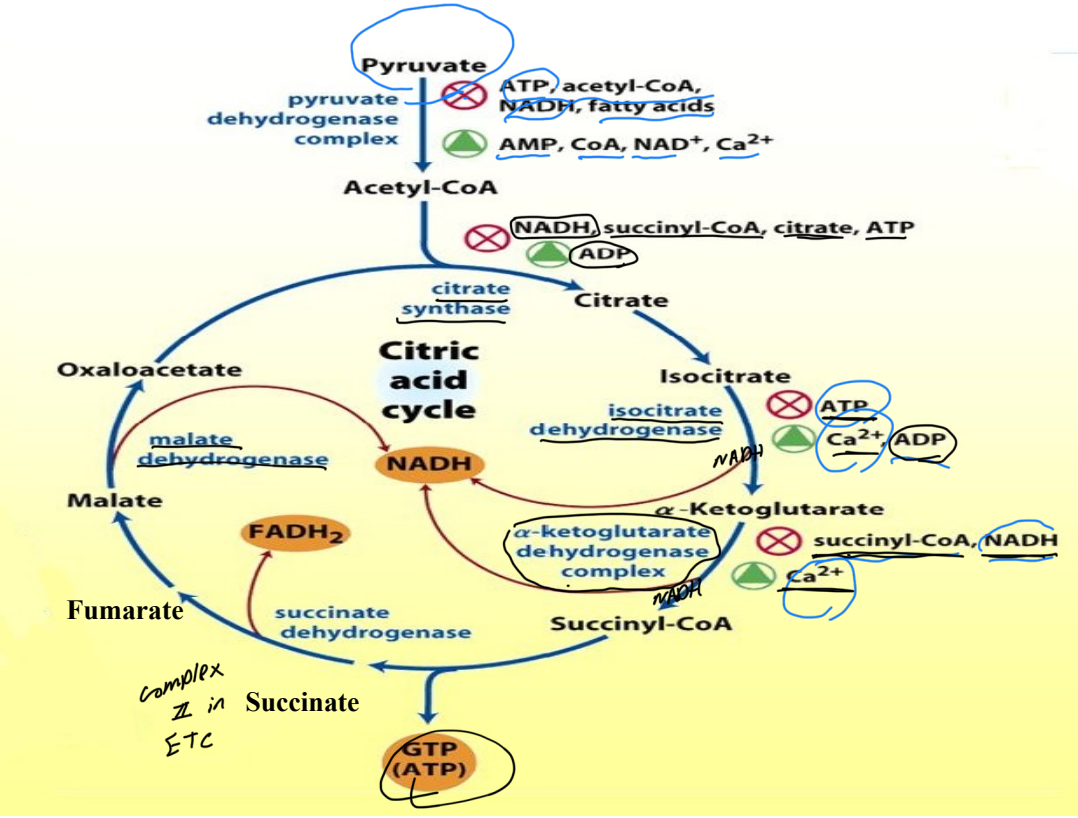
- No hormonal regulation
- Allosteric regulation only (molecules in cell bind to enzymes part of the TCA cycle, not on active site (on a different allosteric site , causing enzyme to undergo a conformational change, either making enzyme work better or be inhibited)
- Substrate availability

Example

- Low concentration of acetyl CoA will slow down citric acid cycle
- Citrate being shuttled out of citric acid cycle for FA synthesis Slow down cycle
- In starvation state, amino acids break down and enter critic acid cycle as alpha ketoglutarate or oxaloacetate more substrate speeding cycle

Allosteric regulation

- **The PDH complex (Pyruvate acetyl CoA)**
  - Inhibited by fatty acids and acetyl-CoA and when the cell's [ATP]/[ADP] and [NADH]/[NAD+] ratios are high
  - Regulatory protein enzymes- The PDH complex is inhibited by reversible phosphorylation of E1 complex (protein kinase and protein phosphatase)
- **The Citric Acid Cycle Is Regulated at Its Three Exergonic Steps**
  - Citrate synthase
  - Isocitrate dehydrogenase
  - alpha-ketoglutarate dehydrogenase



Formation of ATP

- Acts as the main energy currency and must be continually synthesized from the energy provided by macronutrients
  - Some ATP are synthesized by direct phosphorylation involving high-energy phosphate donors, referred to as substrate-level phosphorylation
  - Two reactions in glycolysis
  - one reaction in the TCA cycle produce GTP
- The production of ATP in mitochondria by oxidative phosphorylation

(Electron transport chain)

Oxidative phosphorylation is the process of making ATP by using the proton gradient generated by the ETC.

- The production of ATP in mitochondria by oxidative phosphorylation begins with the oxidation of fuel molecules by the TCA cycle and the release of electrons and protons.
- Electrons obtained from nutrients and metabolic intermediates are transferred to NAD+ and FAD
- AH2 + NAD+□ A+ NADH + H+
- BH2 + FAD□ B +FADH2

TCA CYCLE AND GLYCOLYSIS

- The electrons and protons are captured by NADH and FADH2 and delivered to the inner mitochondrial membrane.

NAD+ and FAD must be recycled

Recycling is accomplished by oxidation and transfer of electrons to oxygen.

$$NADH + H^+ + 1/2\ O_2 \xrightarrow{ADP + P_i \rightarrow ATP} NAD^+ + H_2O$$

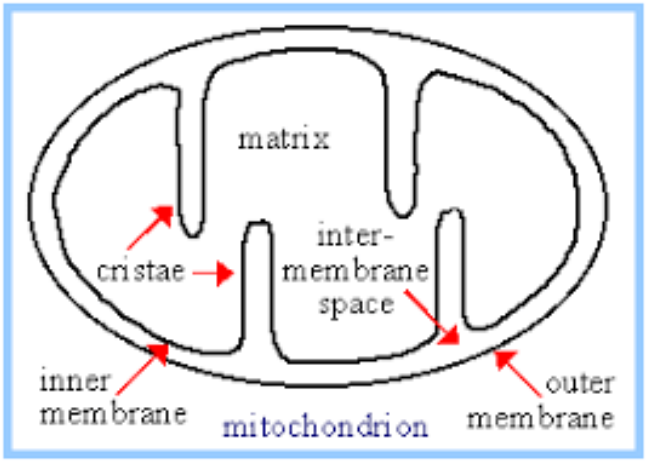
$$FADH_2 + 1/2\ O_2 \xrightarrow{ADP + P_i \rightarrow ATP} FAD + H_2O$$

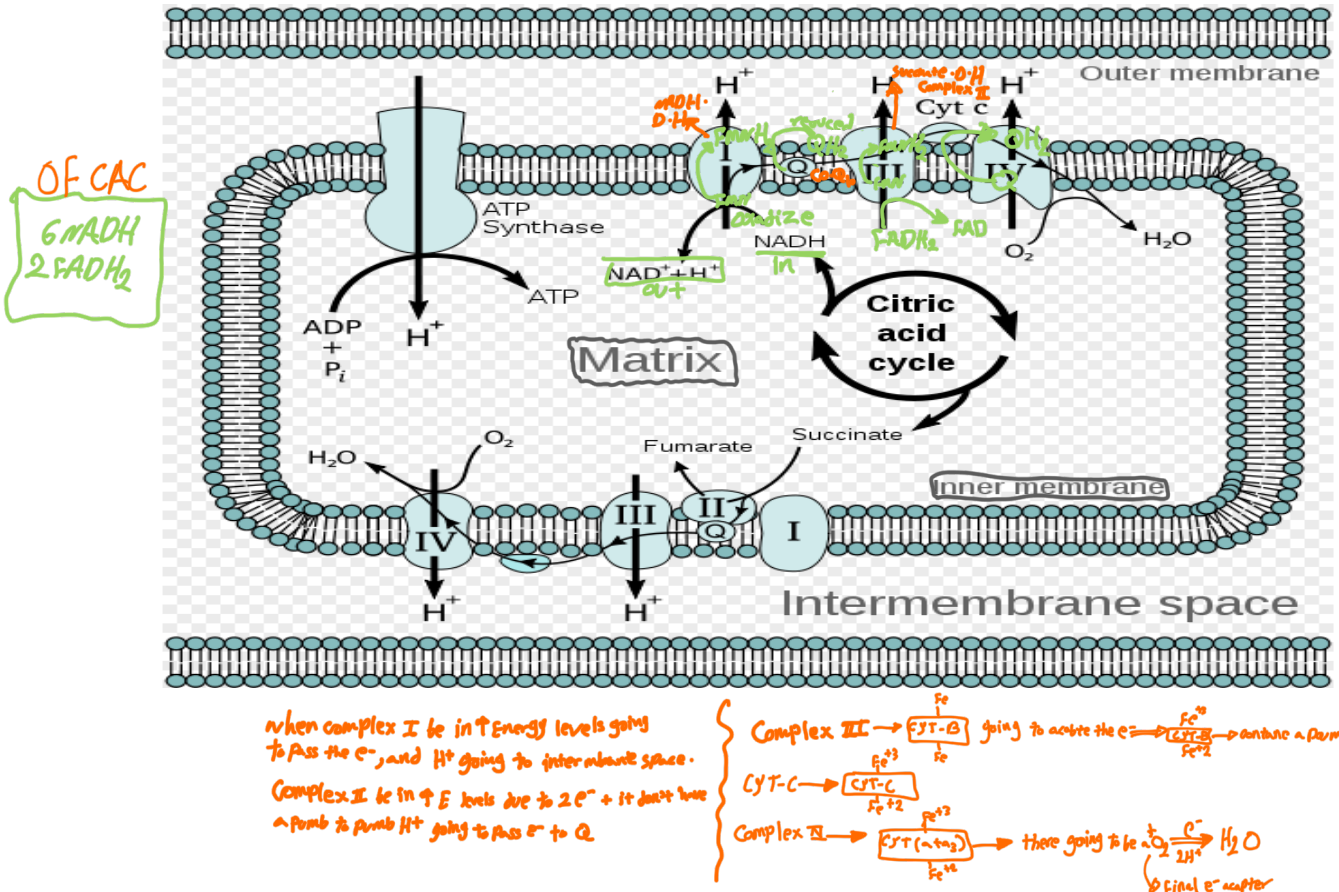
NAD^+ and FAD are then available for additional oxidative metabolism. The energy released during electron transport is coupled to ATP synthesis.

The electrons and protons are captured by NADH and FADH2 and delivered to the inner mitochondrial membrane.

Location of mitochondrial complexes

- Inner mitochondrial membrane:
  - **Electron transport chain:** oxidizes reduced coenzymes
  - **ATP synthase**: machinery to synthesize ATP

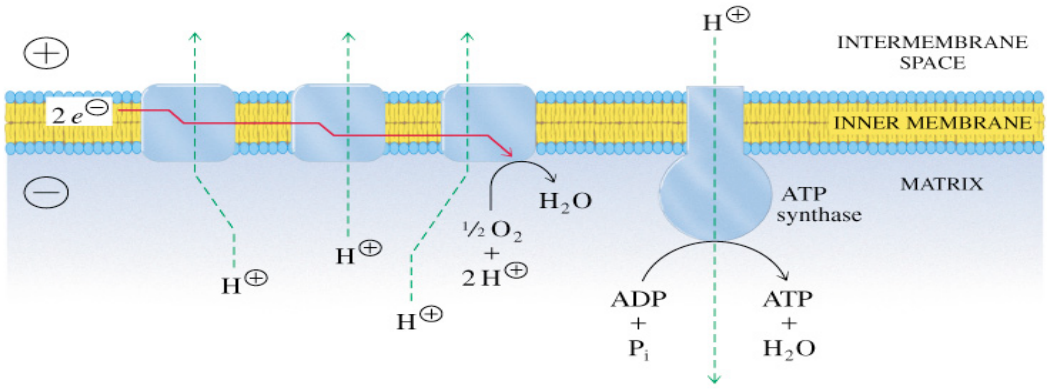




- The flow of electrons from NADH or FADH2 to O2 through protein complexes located in the mitochondrial inner membrane leads to the pumping of protons out of the mitochondrial matrix. The resulting uneven distribution of protons generates a pH gradient and a transmembrane electrical potential that creates a *proton-motive force*

- ATP is synthesized when protons flow back to the mitochondrial matrix through an enzyme complex (ATP synthase) . Thus, *the oxidation of fuels and the phosphorylation of ADP are coupled by a proton gradient across the inner mitochondrial membrane*

https://www.ncbi.nlm.nih.gov/books/NBK21208/



- The steps are coupled. Electrons do not flow to oxygen unless ATP is needed
- Each NADH produces 2.5 ATP
- Each FADH2 produces 1.5 ATP

- **Four protein complexes in the Inner membrane make up the ETC**
- Complexes I, II, III, IV
- Work together in succession to catalyze redox reactions
- Integral membrane proteins with prosthetic groups to move electrons
- Electrons move through the complexes in order
  - Electrons from **NADH** enter at **Complex I**
  - Electrons from **FADH2** enter at **Complex II**
- In each reaction, an electron **donor** is **oxidized** and an electron **acceptor** is **reduced**
- **Compounds differ from one another in how readily they will**

Compounds differ from one another in how readily they will be oxidized or reduced

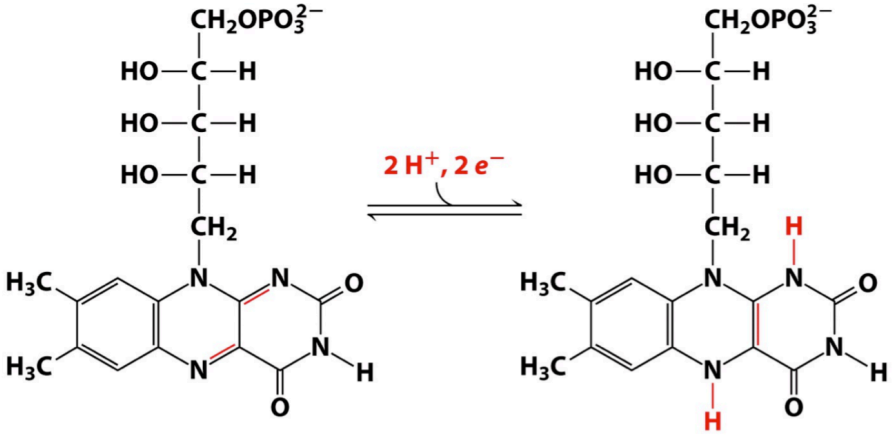
- Electrons flow downhill – spontaneously moving from molecules that are strong electron **DONORS** to strong electron **ACCEPTORS** = move from **high** energy state to **low** energy state
- Flow of electrons is spontaneous and thermodynamically favorable because the next carrier has greater affinity for electrons than the previous
- NADH is a strong electron donor: because its electrons are held in a high-energy linkage, the free-energy change for passing its electrons to many other molecules is favorable
- NADH is a good molecule for donating electrons to the respiratory chain, while O2 is a good electron Acceptor

Co-factors in Electron Transport

- Protein components use **metal- containing prosthetic groups** or **flavins** to carry electrons
- Metal-containing groups such **as iron-sulfur clusters, copper ions, hemes**

Co-factors in Electron Transport

1. **Flavins**:
   (Complex I) FMN – $FMNH_2$
   (Complex II) FAD - $FADH_2$



Flavin mononucleotide (FMN) $FMNH_2$

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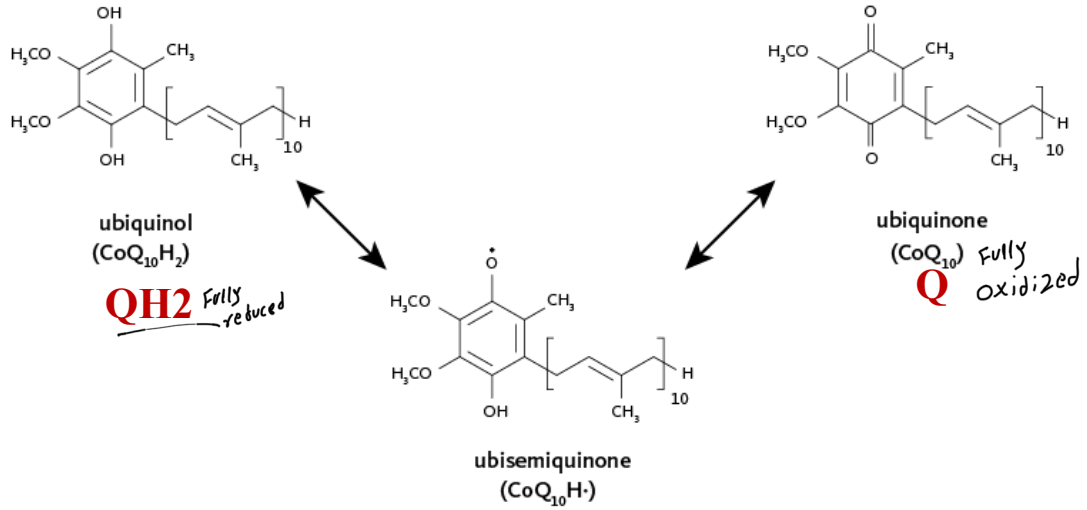
Co-factors in Electron Transport

2. **Ubiquinone (Q) (Mobile electron carrier)**
   - Also called coenzyme Q
   - A membrane-soluble compound
   - Long hydrophobic tail keeps Q anchored in the mitochondrial inner membrane
   - lipid soluble molecule that diffuses within the lipid bilayer, and shuttles electrons from Complexes I and II and pass them to III
   - **Not** a part of any complex

Figure 1. The Different Redox Forms of Coenzyme Q_{10}



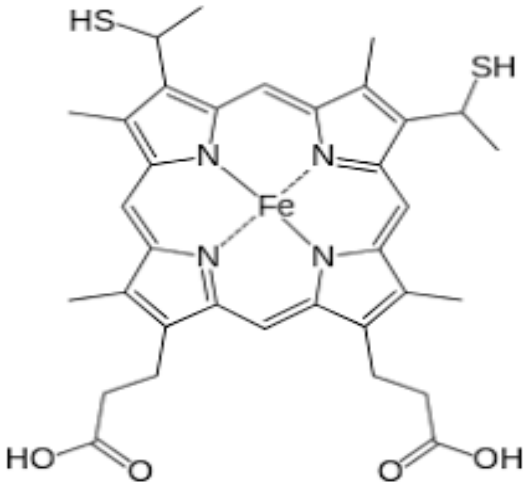
Coenzyme Q_{10} exists in three oxidation states: the fully reduced ubiquinol form ($CoQ_{10}H_2$), the radical semiquinone intermediate ($CoQ_{10}H\cdot$), and the fully oxidized ubiquinone form (CoQ_{10}).

https://lpi.oregonstate.edu/book/export/html/352

Co-factors in Electron Transport

3 Cytochrome c

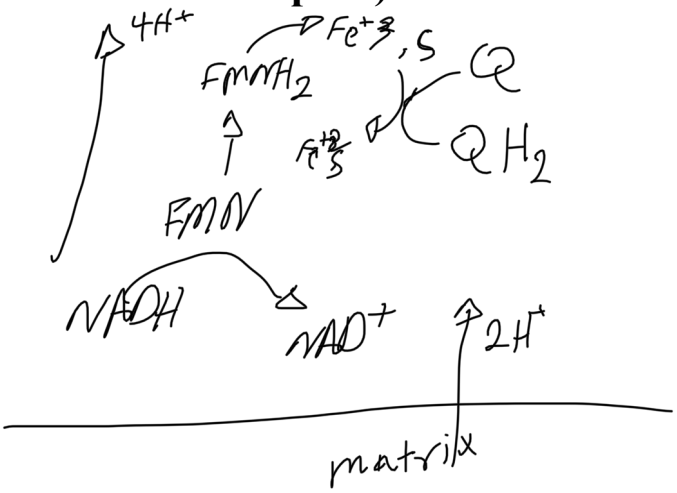
- A peripheral membrane protein associated with the outer face of the membrane, transports electrons from III to IV
- **Not** a part of any complex
- Shuttles electrons and protons from Complex III to Complex IV



MIDTERM
Thursday/ NOV 18

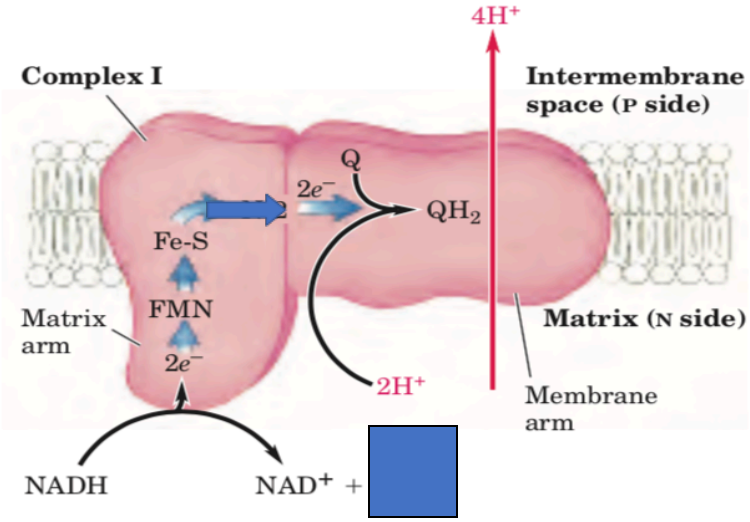
Complex I

- Complex I includes a **flavoprotein** (contains FMN – related to FAD) and proteins with **Fe-S centers (iron-sulfur clusters)**
- Electrons flow from NADH to Flavin mononucleotide (FMN)
- Electrons then flow to a prosthetic group on an iron sulfur cluster
- Iron cycles between 3+ and 2+ states
- Reduction of Q to QH2 requires 2 e-
- **About 4 H+ translocated per 2 e- transferred (pumped from matrix intermembrane space)**

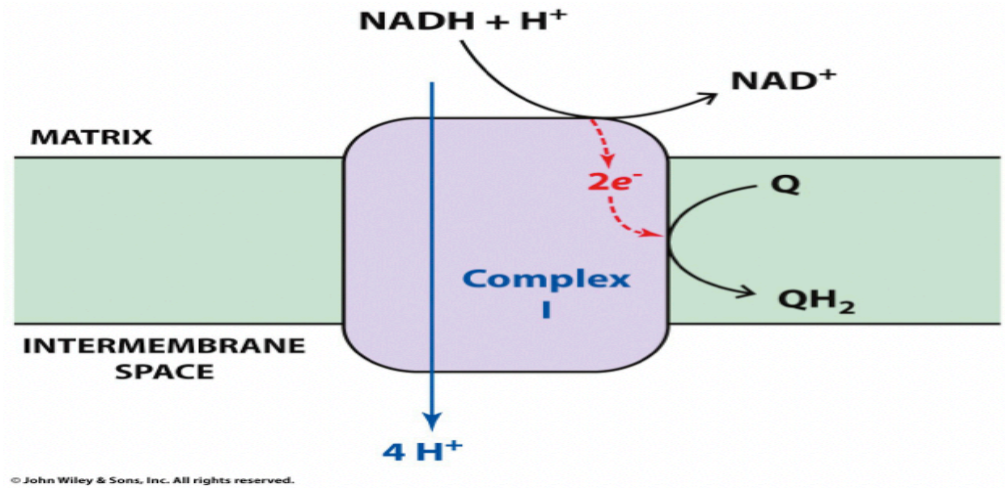


Complex 1

NADH dehydrogenase, is a very large, L-shaped structure that functions to accept high energy electrons from NADH molecules.



NADH binds to the vertical component of complex (within the matrix)
NADH gives off two electrons onto an acceptor molecule called flavin mononucleotide (FMN)
the FMN also uptakes two hydrogen ions (one from the matrix and one from the NADH molecule) to form the fully reduced FMNH2.

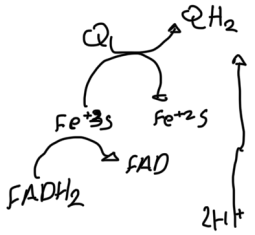


The electrons then move through a series of iron-sulfur clusters (Iron cycles between +2 and +3 end up being transferred onto ubiquinone.(Q)
Q uptakes two protons from the matrix to form ubiquinol (QH2)

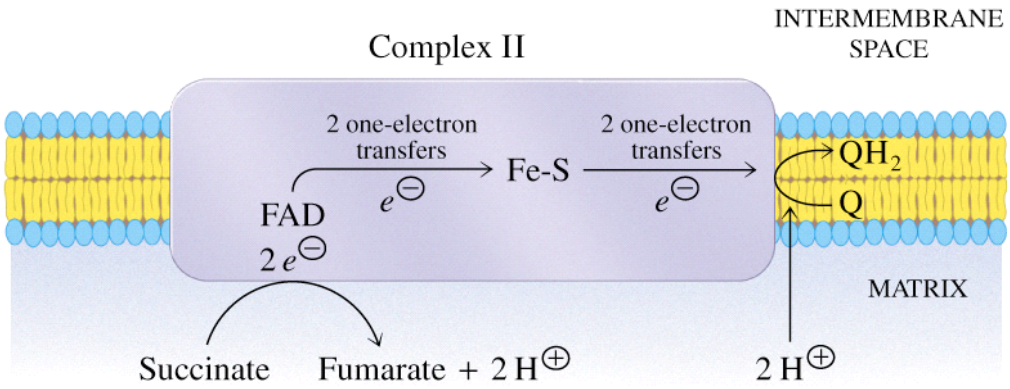
- The transfer of electrons to CoQ occurs one electron at a time.
- The Q molecule is lipid soluble and freely moves through the hydrophobic core of the membrane. Once it is reduced, (QH2), ubiquinone delivers its electrons to the next complex in the electron transport chain.

Complex II

- **Contains succinate dehydrogenase**, a component of the **TCA cycle**
- Complex II contains a FAD protein and Fe-S centers
- succinate is converted to fumarate in the TCA cycle, FAD is reduced to FADH2.
- FADH2 (produced in kreb cycle) remains bound to complex
- Complex II can oxidize the FADH2 back into FAD and move the free electrons through a series of iron-sulfur clusters and onto ubiquinone, thereby forming ubiquinol (The FADH2 is oxidized by electron transfer through the Fe-S centers to reduce Q to QH2)



no H+ pumps.



Complex II does **NOT** contribute to **proton gradient**, but supplies electrons from succinate. Less ATP generated from FADH2 compared to NADH

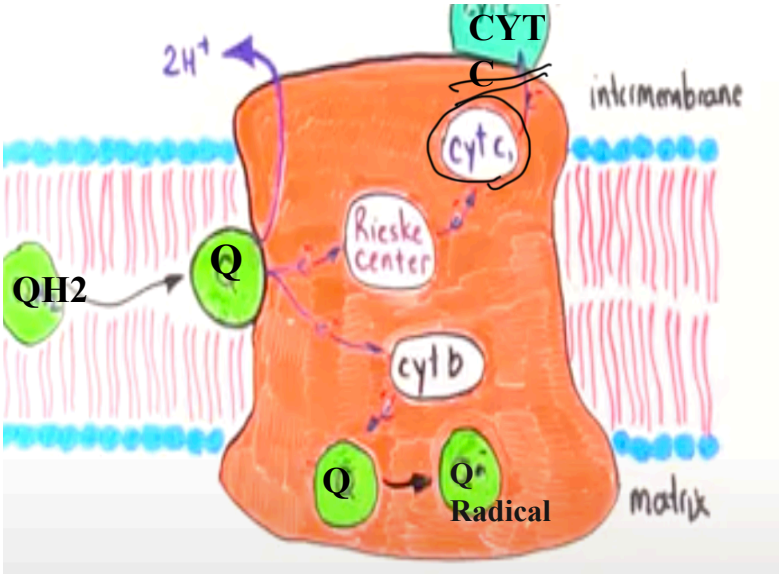
Electron transfer and proton flow in Complex III

- Electron transfer from Ubiquionol (QH2) to chytochrome c
- The electrons derived from both NADH and FADH2 are passed from QH2 to cytochrome *c* through the reactions of Complex III
- Complex III contains two different cytochromes and a Rieske center that contains the 2Fe-2S center group
  - **Cytochrome b** (two heme groups)
  - **Cytochrome c** (single heme group, carries one electron)
  - **Rieske center** that contains the 2Fe-2S center group

Complex III

* cyt c_1 and cyt c is different

- The process by which the electrons are transferred from the ubiquinol to cytochrome c is known as the Q cycle Two half cycles
- In the first half of the cycle, one electron flows to cytochrome *c*. A second electron flows to cytochrome *b* where it transfers to a Q molecule, creating an unstable semiquinone ion.



- One of these electrons moves onto the Rieske center, then onto cytochrome c1 and finally onto cytochrome c. Note that cytochrome c, unlike ubiquinone, can only carry a single electron at any given time.
- The other electron that comes from QH2 follows a different pathway and moves through the heme groups of cytochrome b and onto ubiquinone to form a partially reduced species called a semiquinone radical ion. The two protons that were originally attached to ubiquinol are transferred into the intermembrane space.
- In the second half-cycle of the Q cycle, another ubiquinol (QH2) attaches onto complex III. Upon binding, the two protons are moved into the intermembrane space and the two electrons follow the same pathways as before The fourth electron flows to cytochrome *b* where it joins the semiquinone(Q radical) to form QH2

Q cycle half one:

QH_2 attatch to complex 3 → Q

can be bind one e⁻ ← cyt c

cyt c_1

2 → 2 fe-2S

2 → cyt B

Q → Q^- Radical

Complex III

- In the second half-cycle of the Q cycle, another ubiquinol attaches onto complex III. Upon binding, the two protons are moved into the intermembrane space and the two electrons follow the same pathways as before

Summary

A single Q cycle

- **oxidation of 2 QH2**
- Reduces two cytochrome c molecules
- forms a single ubiquinol molecule
- pumps four protons into the intermembrane space

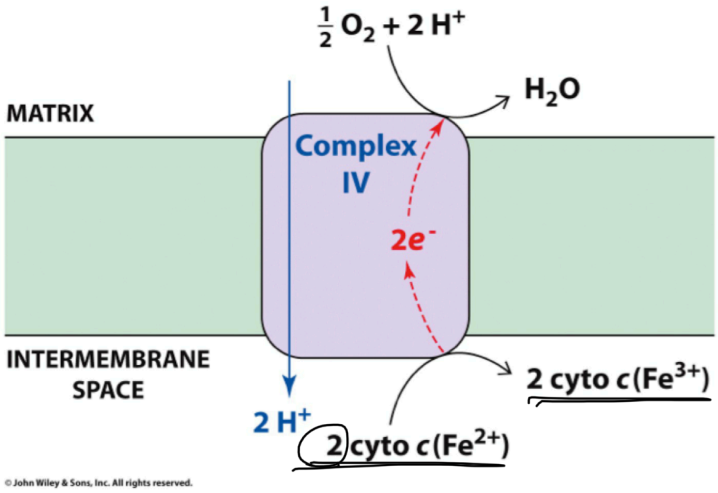
Complex IV

- Accepts electrons from cytochrome *c*
- Contains **2 cytochromes** (a and a3) and proteins with copper centers that provide multiple centers for **oxidation-reduction**
- Cytochromes a and a3 are the only species capable of direct transfer of electrons to oxygen
- two heme groups (one in each of the cytochromes a and a3

- oxygen molecule held very tightly between the iron and copper ions until the oxygen is completely reduced
- Catalyzes reduction of oxygen to form water.
- The metal ions cycle between their oxidized (Fe+3, Cu+2) and reduced (Fe+2, Cu+)
- Moves **H+** into the intermembrane space and contributes to the proton gradient

Complex IV

$$O_2 + 4\,e^- + 4H^+ \rightarrow 2\,H_2O$$



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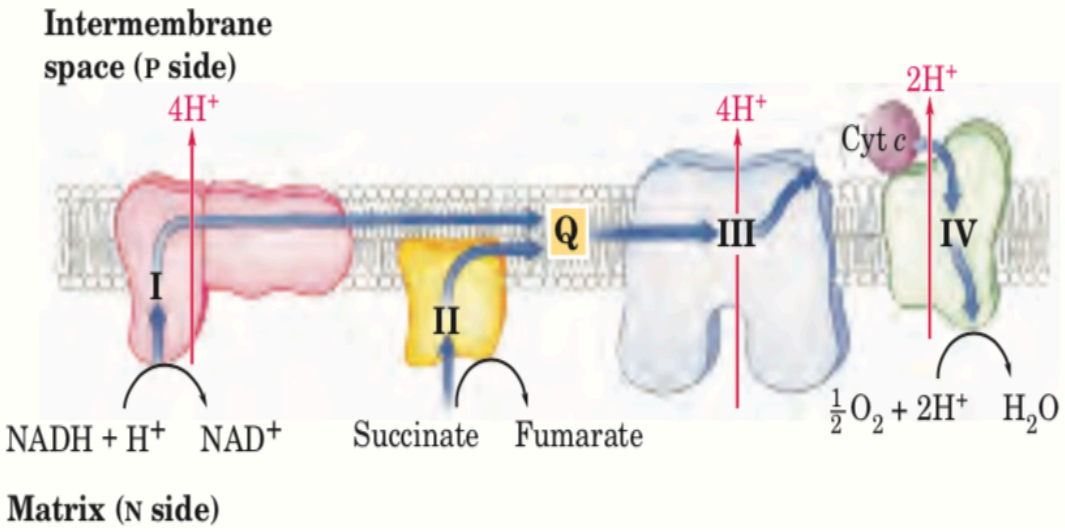


Oxidative phosphorylation. Electrons from NADH and FADH2 travel along the electron transport chain
a total of 10 protons are translocated from the matrix to the intermembrane space.

ATP Synthesis

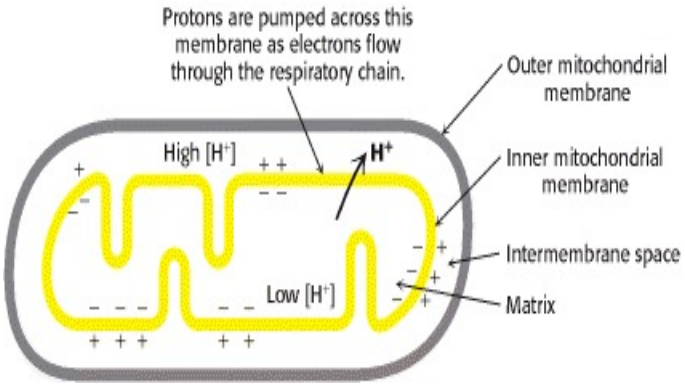
- How is a concentration gradient of protons transformed into ATP?
- **Complex I**, **Complex III** and **Complex IV** pump **protons** across the inner mitochondrial membrane
- Pumping uses the energy liberated from the oxidation of NADH and $FADH_2$
- Pumping generates a membrane potential because it generates an electrochemical gradient
  - Negative inside, positive outside
  - Alkaline inside, acidic outside

ATP Synthesis

The Chemiosmotic Hypothesis

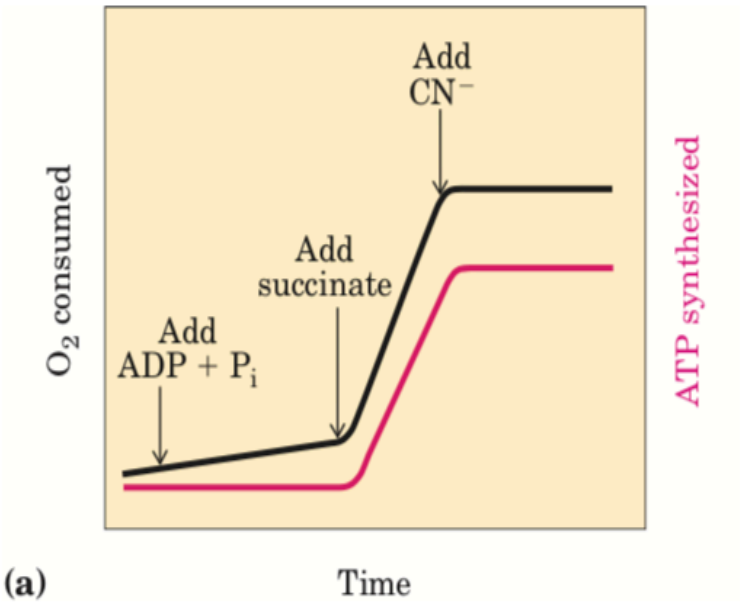
- **A proton concentration gradient serves as the energy reservoir for driving ATP formation**
- **Protonmotive force** (Δ**p**) is the energy of the proton concentration gradient
- Protons that are translocated into the intermembrane space by electron transport, flow back into the matrix via ATP synthase
- H+ flow forms a circuit (similar to an electrical circuit)
- The transmembrane protein, **ATP synthase**, catalyzes the phosphorylation of ADP in a reaction driven by movement of H+ across the inner membrane into the matrix

- In chemiosmotic theory transmembrane ATP synthases are very important. They convert energy of spontaneous flow of protons through them into chemical energy of ATP bonds.
- The proton gradient is COUPLED with ATP synthesis



Biochemistry. 5th edition.Berg JM, Tymoczko JL, Stryer L. New York: W H Freeman; 2002.

- In experiments to demonstrate coupling, mitochondria are suspended in a buffered medium and an O2 electrode monitors O2 consumption (Oxygen disappearance from closed vessel)
- At intervals, samples are removed and assayed for the presence of ATP.
- Addition of ADP and Pi alone results in little or no increase in either respiration (O2 consumption; black) or ATP synthesis (red). When succinate is added, respiration begins



When succinate is added, respiration begins immediately and ATP is synthesized. Addition of cyanide (CN-) , which blocks electron transfer between cytochrome oxidase and O2, inhibits both respiration and ATP synthesis.

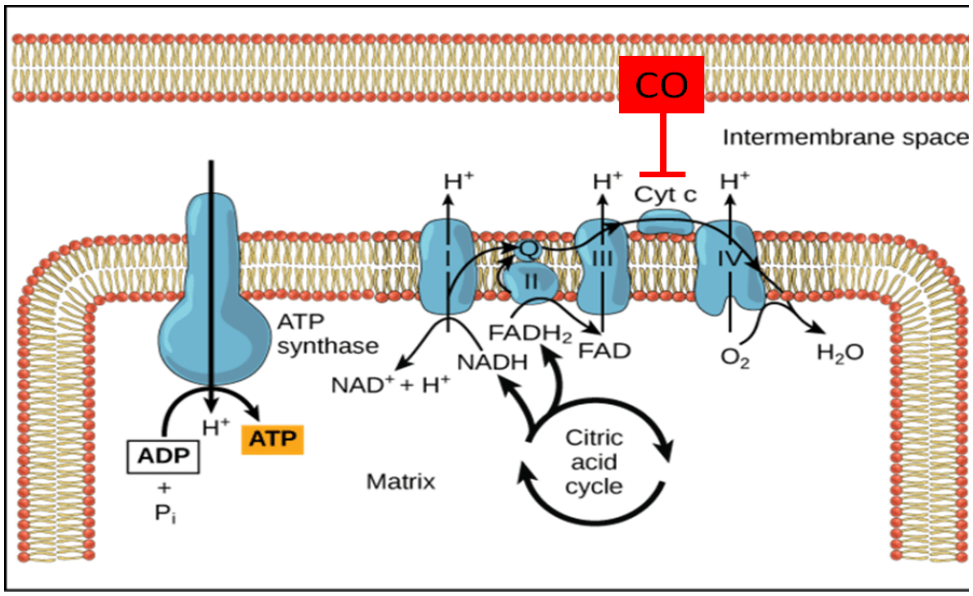
- Cyanide is a naturally occurring chemical, found in many plants, that has been used in conventional warfare

- Cyanide poisons the mitochondrial electron transport chain within cells and renders the body unable to derive energy (adenosine triphosphate—ATP) from oxygen.

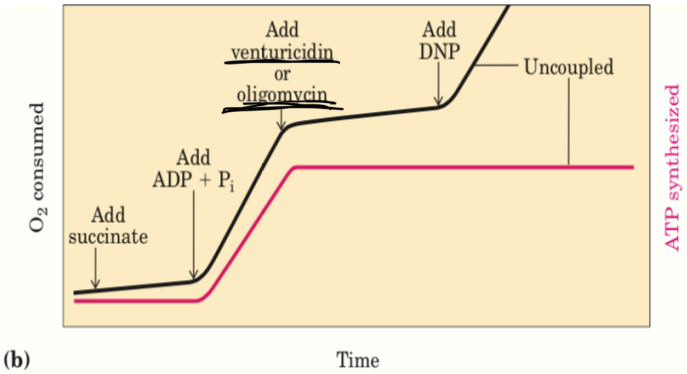
Cyanide poisons the mitochondrial electron transport chain within cells and renders the body unable to derive energy (adenosine triphosphate—ATP) from oxygen. Specifically, it binds to the a3 portion (complex IV) of cytochrome oxidase and prevents cells from using oxygen, causing rapid death

Carbon Monoxide works similarly to Cyanide

Carbon monoxide binds to and inhibits cytochrome c oxidase (complex IV)



https://blogs.brown.edu/emergency-medicine-residency/winter-is-coming-think-co-toxicity/



Experiments demonstrate that the reverse is true as well!
inhibition of ATP synthesis blocks electron transfer in intact mitochondria (providing O2 and oxidizable substrates, but no ADP) no ATP synthesis can occur and electron transfer to O2 does not proceed

venturicidin or oligomycin, inhibitors of ATP synthase, blocks both ATP synthesis and respiration

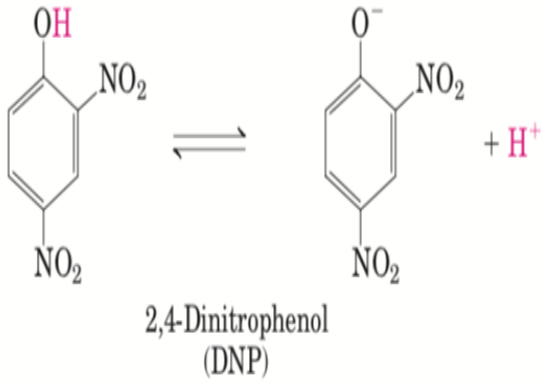
Dinitrophenol (DNP) is an uncoupler, allowing respiration to continue without ATP synthesis.

- What would happen to the energy stored in the proton gradient if it weren't used to synthesize ATP or do other cellular work?

- The proton-motive force builds up

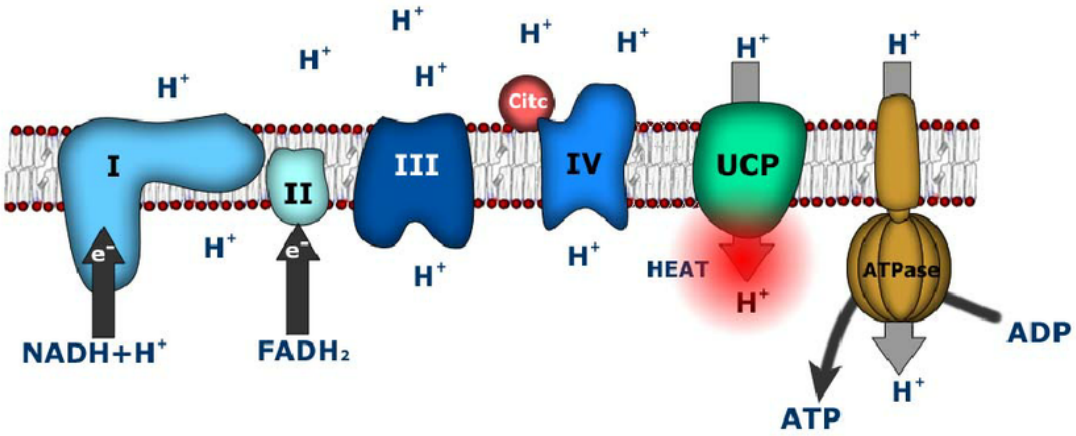
- **Uncoupling agents**: molecules that disrupts oxidative phosphorylation in by dissociating the reactions of ATP synthesis from the electron transport chain

- Mitochondria still capable of catalyzing electron transfer from succinate or NADH to O2
- O2 consumed **but no ATP generated**



2,4-Dinitrophenol
(DNP)

DNP has dissociable proton/ hydrophobic molecule. Diffuse readily across mitochondrial membranes. After entering the matrix in the protonated form, they can release a proton



Mitochondrial transporters present in the inner membrane of mitochondria

Allow proton-leak : they act as proton channels to redirect protons back into the matrix and away from ATP synthase

dissipation of oxidation energy as heat

Implicated in thermogenesis

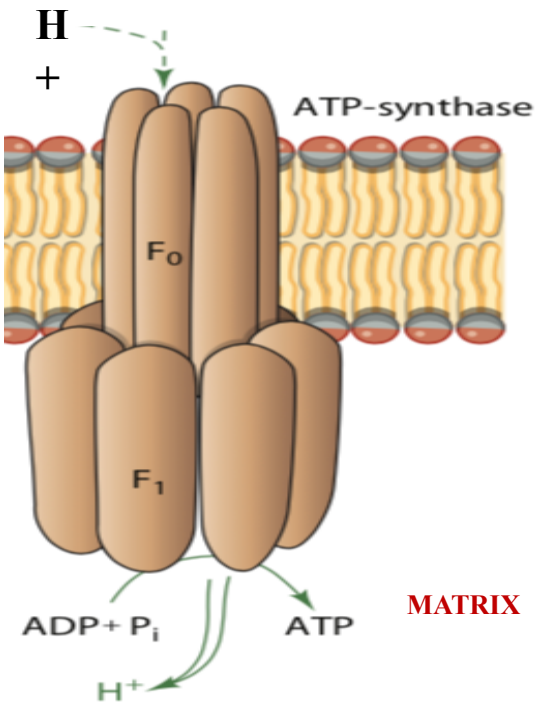
Valle, Adamo & Oliver, Jordi & Roca, Pilar. (2010). Role of Uncoupling Proteins in Cancer. C 567-591. 10.3390/cancers2020567.

Uncoupling Protein-1 (UCP1)

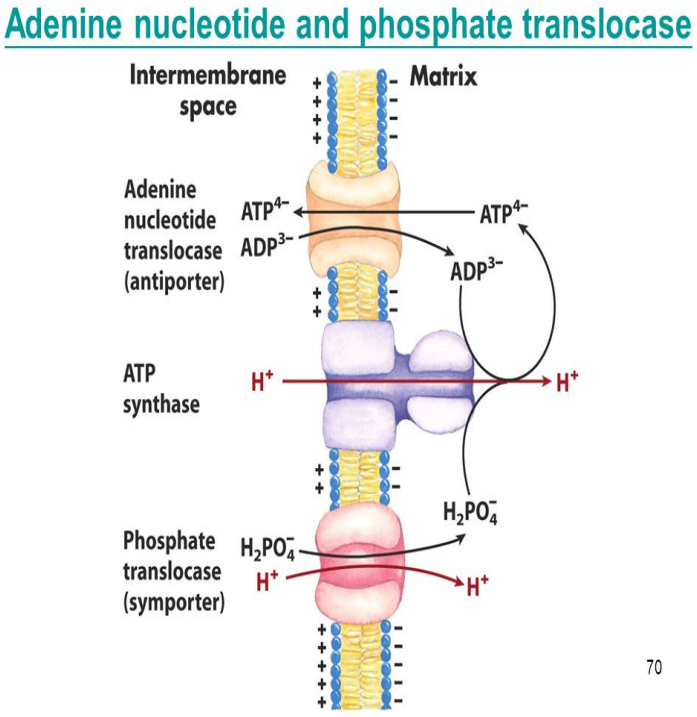
- Uncoupling proteins found in mammalian brown adipose tissue (BAT)
- **"Brown fat" dark color due to high levels of mitochondria which contain thermogenin (uncoupling protein)- produce heat- non shivering thermogenesis**
- In humans and other large mammals, BAT disappears after infancy

ATP Synthase

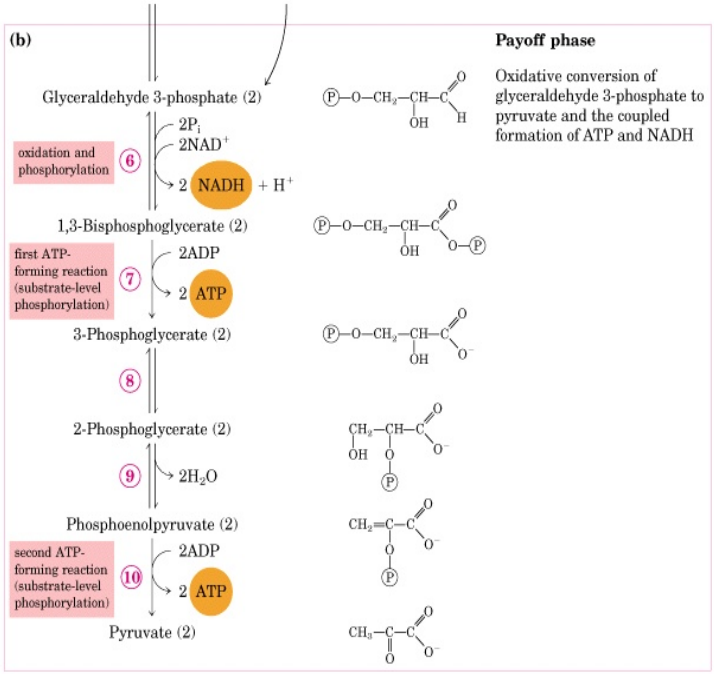
- Knob like Projections into the matrix side
- Two units
- F1 Contains the catalytic site for ATP synthesis
- F0 transmembrane channel for H+
- Proton flow back into the matrix through (F0) component of the ATP synthase while F1 catalyzes the synthesis of ATP



- ATP is synthesized in mitochondria but must be moved to the cytosol to supply energy for the cell. The enzyme *ATP-ADP translocase* shuttles ATP out of the mitochondria and ADP in.
- ATP must be transported to the cytosol, and ADP and Pi must enter the matrix
- ADP/ATP carrier exchanges mitochondrial ATP4- for cytosolic ADP3-
- The exchange causes a net loss of -1 in the matrix (draws some energy from the H+ gradient)



- Recall that the glycolytic pathway generates NADH in the cytosol in the oxidation of glyceraldehyde 3-phosphate
- NAD+ must be regenerated for glycolysis to continue.
- Under anaerobic conditions, NADH in the cytosol is used in the lactate dehydrogenase reduction of pyruvate to lactate, thereby becoming reoxidized to NAD+ without involving oxygen



- NADH cannot simply pass into mitochondria for oxidation by the respiratory chain, because the inner mitochondrial membrane is impermeable to NADH and NAD+
- **How is cytosolic NADH reoxidized under aerobic conditions?**

- ***Electrons from NADH,*****rather than NADH itself,** are carried across the mitochondrial membrane
- ***Glycerol 3-phosphate shuttle***
- specific to certain tissues. The glycerol-3-phosphate shuttle functions in the brain and skeletal muscle
- NADH in the cytosol transfers its electrons to dihydroxyacetone phosphate, forming glycerol-3-phosphate that freely diffuses across the outer mitochondrial membrane.
- Electrons of glycerol-3-phosphate are then transferred to FAD that is associated with a membrane-bound isoform of glycerol- 3-phosphate dehydrogenase located in the inner mitochondrial membrane

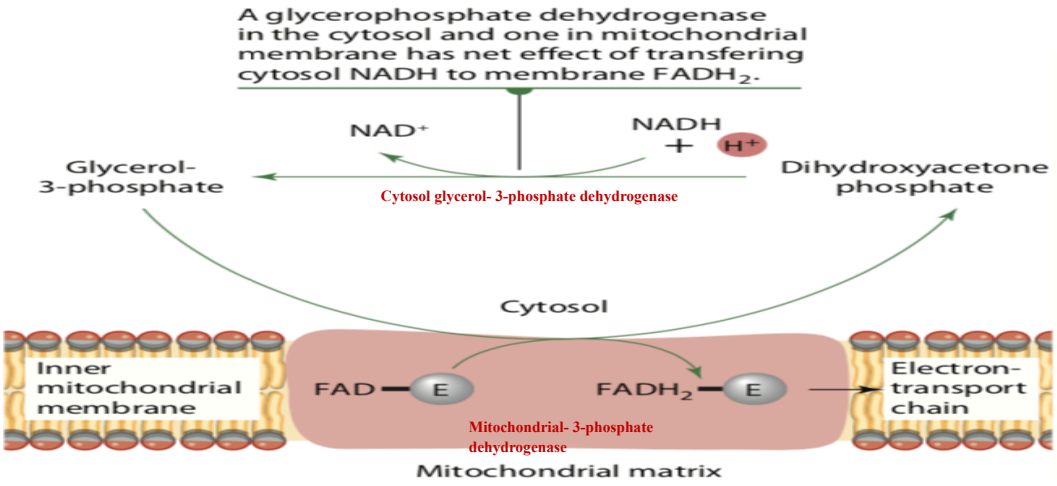
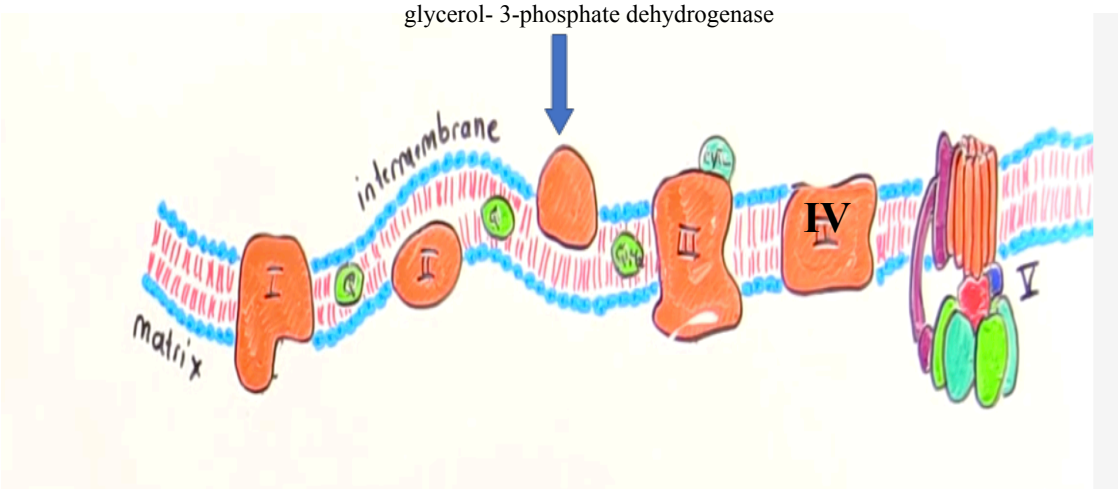


Figure 3.23 Glycerol-3-phosphate shuttle.

The first step in this shuttle is the transfer of a pair of electrons from NADH to dihydroxyacetone phosphate, a glycolytic intermediate, to form glycerol 3-phosphate This process occurs in the cytoplasm

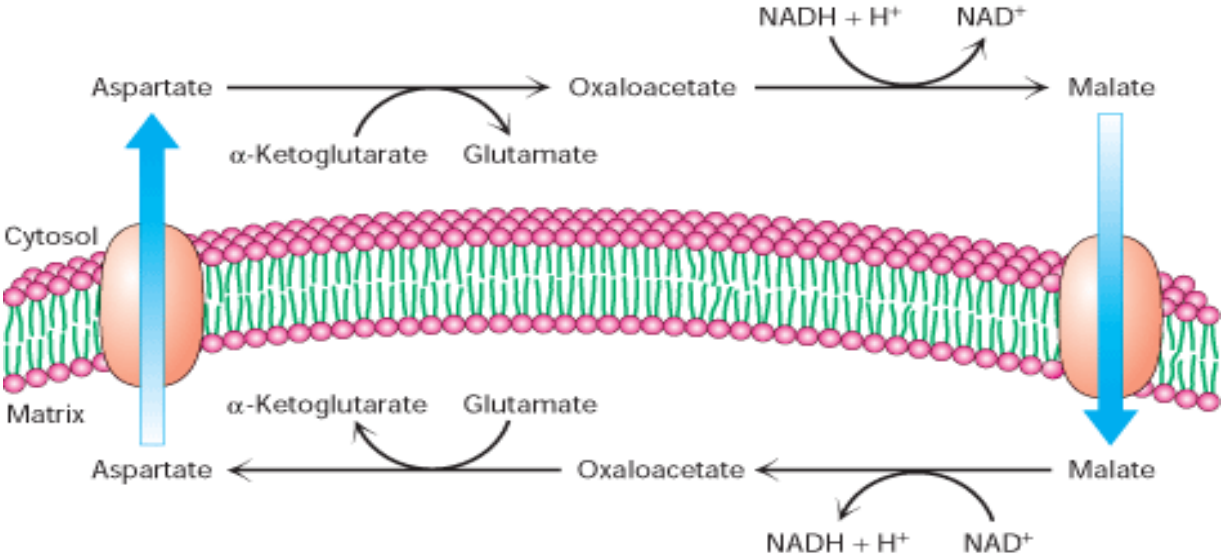
- Once G3P moves into the intermembrane space, it is then oxidized back into DHAP by the action of a membrane-bound enzyme called the mitochondrial glycerol 3-phosphate dehydrogenase
- An FAD molecule bound to this enzyme accepts the two high energy electrons and the two H+ ions to form FADH2. The FADH2 then gives up the two protons and electrons to a ubiquinone molecule to form ubiquinol. Ubiquinol then moves along the inner mitochondrial membrane to transfer the electrons onto complex III. Since this shuttle bypasses complex I, NADH molecules transported onto the ETC in this manner only produce a net result of 1.5 ATP molecules per NADH.



NADH molecules transported onto the ETC through the glycerol 3 phosphate shuttle produce 1.5 ATP molecules per NADH

Because bypassing complex 1

Malate–Aspartate Shuttle System



- The most active shuttle compound, malate, is freely permeable to the inner mitochondrial membrane. Oxaloacetate from the cytosol is reduced by the NADH to form malate and NAD1. The malate is oxidized by the enzyme malate dehydrogenase to oxaloacetate in the matrix of mitochondria, producing NADH that enters the electron transport chain and generates 2.5 moles of ATP per mole of NADH