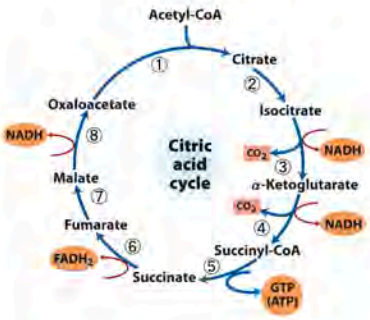
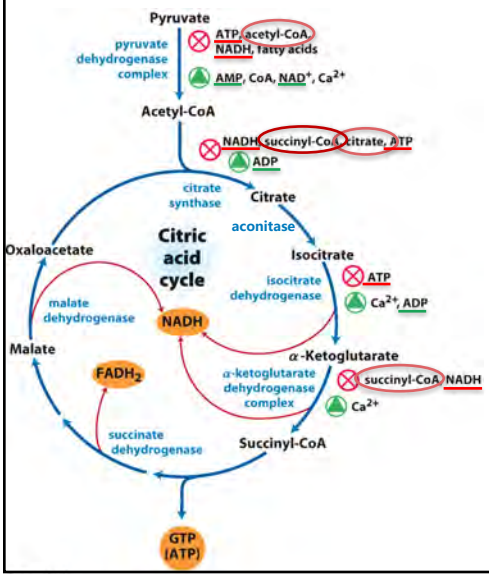
The Citric Acid Cycle

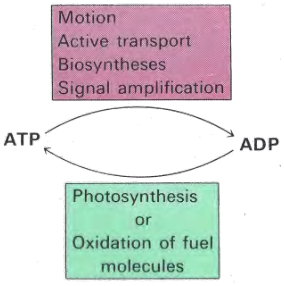
Citrate Synthase	✓	Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
Aconitase	✓	Step 2: Isomerization via dehydration/rehydration
ICDH & αKGDH	✓	Steps 3–4: Oxidative decarboxylations to give 2 NADH
Suc-CoA Synthetase	✓	Step 5: Substrate-level phosphorylation to give GTP
Succinate DH	✓	Step 6: Dehydrogenation to give $FADH_2$
Fumarase	✓	Step 7: Hydration
Malate DH	✓	Step 8: Dehydrogenation to give NADH

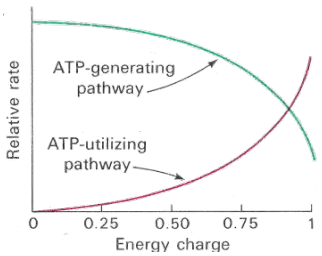


The Citric Acid Cycle

Regulation

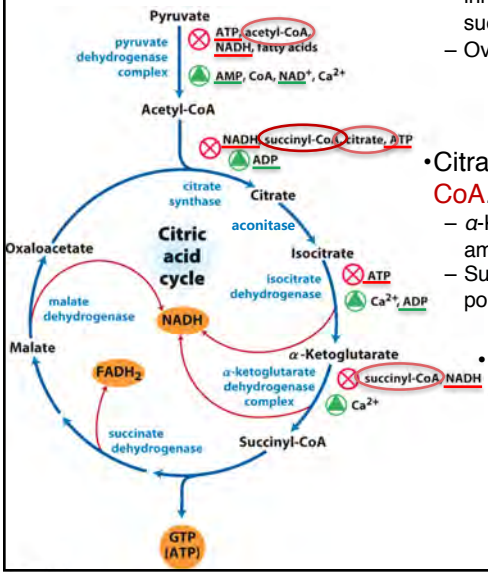






The Citric Acid Cycle

Regulation



- General regulatory mechanism
  - activated by substrate availability
  - inhibited by product accumulation (acetyl-CoA, succinyl-CoA, citrate)
  - Overall products of the pathway are NADH and ATP.
    - affects all regulated enzymes in the cycle
    - inhibitors: NADH and ATP
    - activators: NAD^+, ADP, and AMP
- Citrate synthase is also inhibited by succinyl-CoA.
  - α-Ketoglutarate is an important branch point for amino acid metabolism.
  - Succinyl-CoA communicates flow at this branch point to the start of the cycle.
- Regulation of isocitrate dehydrogenase controls citrate levels; important for fatty acid metabolism. Energy Charge.
  - Aconitase is reversible.
  - Inhibition of IDH leads to accumulation of isocitrate and reverses aconitase.
  - Accumulated citrate leaves mitochondria and inhibits phosphofructokinase in glycolysis.

The Citric Acid Cycle

Yield (TCA): Acetyl-CoA + $3NAD^+$ + FAD + GDP + P_i + $2\ H_2O$ →

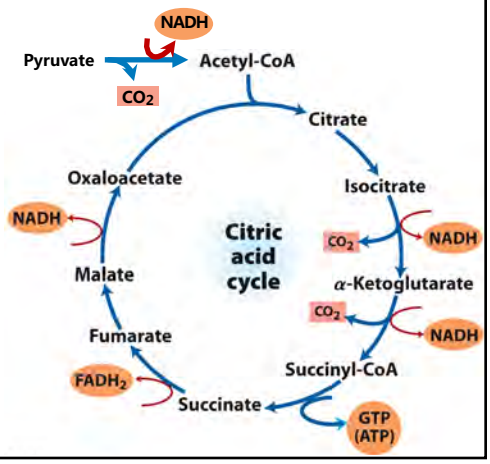
$2CO_2$ + 3NADH + $FADH_2$ + GTP + CoA + $3H^+$

Yield (from pyruvate):

Pyruvate + $4NAD^+$ + FAD + GDP + P_i + $2\ H_2O$ → $3CO_2$ + 4NADH + $FADH_2$ + GTP + CoA + $3H^+$

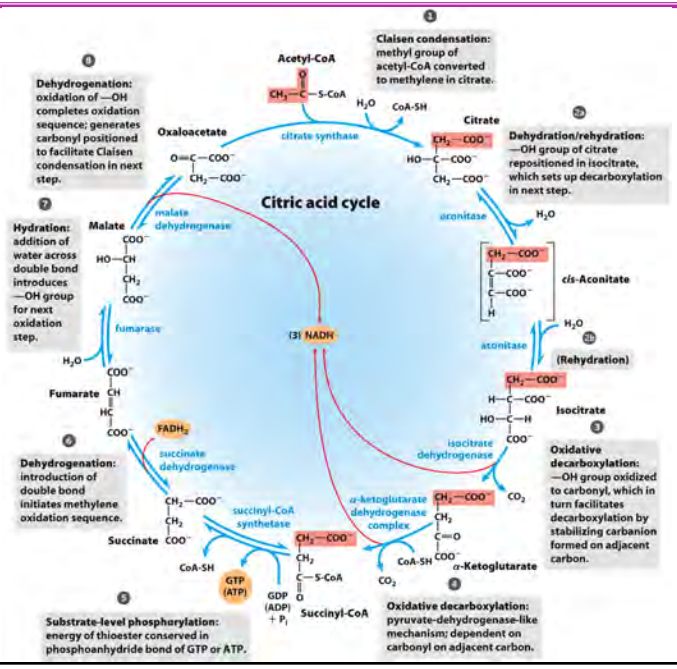
From acetyl-CoA:

- Net oxidation of two carbons to CO_2
  - equivalent to two carbons of acetyl-CoA
  - but NOT the exact same carbons
- Energy captured by electron transfer to NADH and $FADH_2$
- Generates 1 GTP, which can be converted to ATP
- Cycle acts as a unit; its basically a furnace for burning carbon



……..except we don't have the water yet!

The Citric Acid Cycle: Summary



Pyruvate Oxidation & Citric Acid Cycle

Summary

We learned that:

- a large multi-subunit enzyme, pyruvate dehydrogenase complex, converts pyruvate into acetyl-CoA
- several cofactors are involved in reactions that harness the energy from pyruvate
- the citric acid cycle is an important catabolic process: it makes reduced cofactors (NADH & $FADH_2$), plus GTP, that could yield ATP
- the rules of organic chemistry help to rationalize reactions in the citric acid cycle
- the citric acid cycle is largely regulated by availability of substrates and product inhibition (especially NADH and ATP)

Oxidative Phosphorylation

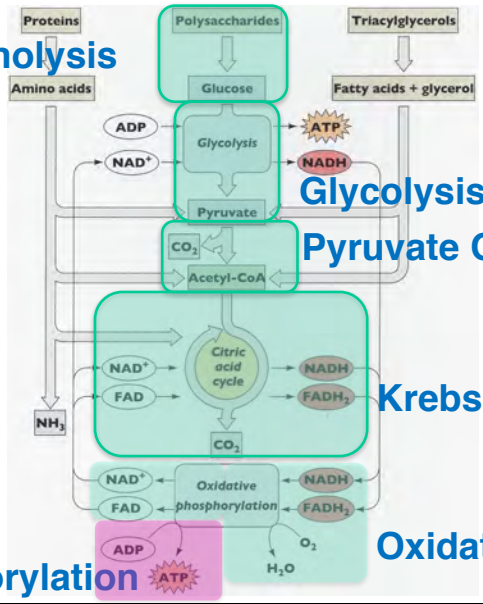
Oxidative Phosphorylation

Learning goals:

- Function of electron-transport chain in mitochondria…... make water
- Building up the proton-motive force
- Synthesis of ATP in mitochondria and chloroplasts

- Fuels for the cell, in the form of reduced carbon compounds (sugars), have been burned to carbon dioxide.
- Electrons from reduced fuels are transferred to reduced cofactors NADH or $FADH_2$.
- In oxidative phosphorylation, energy from NADH and $FADH_2$ is used to make ATP. …... But how?

Oxidative Phosphorylation



Glycogenolysis

Glycolysis

Pyruvate Oxidation

Krebs' Cycle

Oxidative Phosphorylation

Oxidative Phosphorylation

Energy of the reduced cofactors

Is there enough energy in NADH & $FADH_2$ to drive the synthesis of ATP?

Each ATP synthesis is about +7.3 kcal/mol (opposite of hydrolysis)
We can do this calculation two ways:

1. Calculate the $\Delta E^{o'}$ needed to get 1 ATP made: compare to $\Delta E^{o'}$ of NADH→O_2
2. Calculate the $\Delta G^{o'}$ for the NADH→O_2 : compare to the $\Delta G^{o'}$ for ATP synthesis

#1

$\Delta E^{o'}$ of ATP

$$\Delta G^{o'} = -n\mathcal{F}\Delta E^{o'}$$

$$\frac{\Delta G^{o'}}{-n\mathcal{F}} = \Delta E^{o'}$$

$$\frac{+7.3\ \text{kcalmol}^{-1}}{-(2)(23.06\text{V}^{-1}\text{kcalmol}^{-1})} = -0.16\text{V}$$

$\Delta E^{o'}$ of NADH→ ½ O_2

$$\Delta E^{o'} = E^{o'}_{(reduction)} - E^{o'}_{(oxidation)}$$

$$= +0.82\ \text{V} - (-0.32\ \text{V})$$

$$= +1.14\ \text{V}$$

7.1 times more energy in $2e^-$ than needed to drive the synthesis of ATP

Dr. Kornberg: **Lecture 02.17.17 (12:51-15:31)-Faraday (2.7 min)**

Oxidative Phosphorylation

Energy of the reduced cofactors

Is there enough energy in NADH & $FADH_2$ to drive the synthesis of ATP?

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1. Calculate the $\Delta E^{o'}$ needed to get 1 ATP made: compare to $\Delta E^{o'}$ of NADH→O_2
2. Calculate the $\Delta G^{o'}$ for the NADH→O_2 : compare to the $\Delta G^{o'}$ for ATP synthesis

#2

$\Delta G^{o'}$ of NADH→ ½ O_2

$\Delta E^{o'} = E^{o'}_{(reduction)} - E^{o'}_{(oxidation)}$

$= +0.82\ V - (-0.32\ V)$

$= +1.14\ V$

$\Delta G^{o'} = -n\mathcal{F}\Delta E^{o'}$

$= -(2)(23.06 V^{-1} kcal mol^{-1})(+1.14 V)$

$\Delta G^{o'} = -52.6$ kcal/mol

$\Delta G^{o'}$ of ATP

7.2 times more energy in $2e^-$ going from NADH to oxygen than needed to drive the synthesis of ATP

6.5 times more energy in $2e^-$ going from $FADH_2$ to oxygen than needed to drive the synthesis of ATP

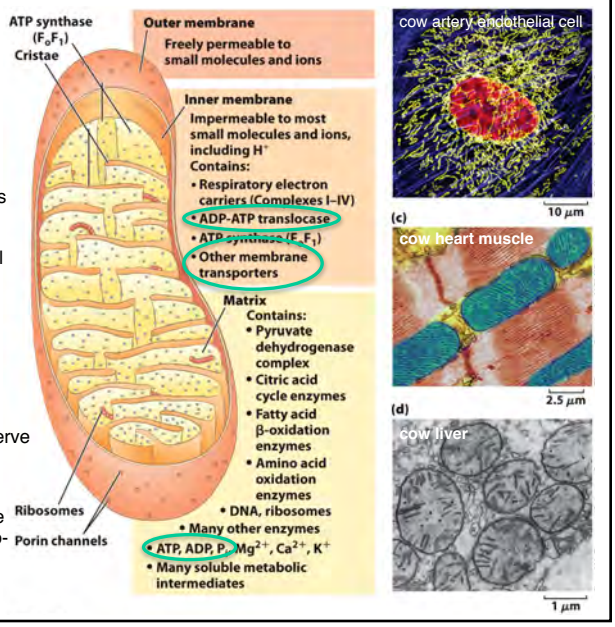
$\Delta G^{o'} = +7.3$ kcal/mol

Oxidative Phosphorylation

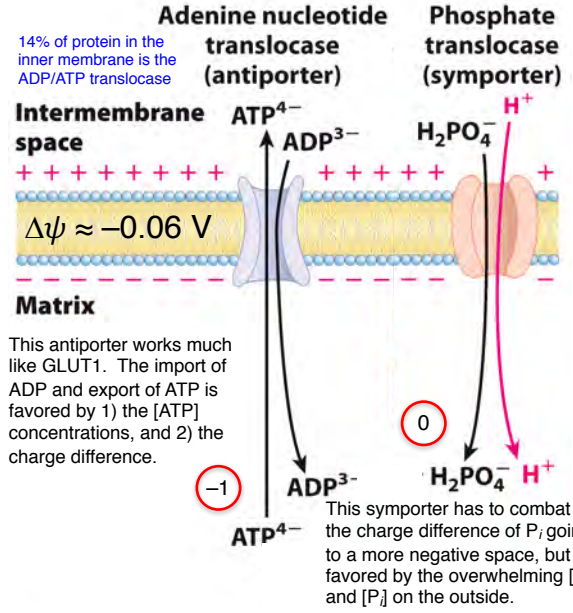
Structure of Mitochondria

Double membrane leads to four distinct compartments:

1. Outer membrane:
   - relatively porous membrane; allows passage of metabolites
2. Intermembrane space (IMS):
   - similar environment to cytosol
   - higher proton concentration (lower pH) +++++
3. Inner membrane
   - relatively impermeable, with proton gradient across it
   - location of electron transport chain complexes
   - Convolutions called cristae serve to increase the surface area.
4. Matrix
   - location of the citric acid cycle and parts of lipid and amino-acid metabolism
   - lower proton concentration (higher pH) -----



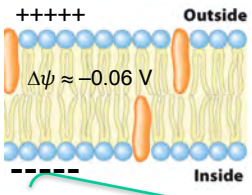
Oxidative Phosphorylation



- These translocases cost the membrane potential, which must be restored; costs 25% of Electron Transport.
- These translocases require energy from both the electrochemical gradient across inner mitochondrial membrane plus the proton gradient. Together they are called the Proton Motive Force.
- What generates this gradient?
- How much energy does this take to pump H^+ out?

Oxidative Phosphorylation

Energy required to pump a single proton *against* a pH gradient



$H^+_{in} \rightleftharpoons H^+_{out}$

$$\Delta G' = RT\ln\frac{[H^+]_{out}}{[H^+]_{in}} + z\mathcal{F}\Delta\psi$$

Switch the sign here because reaction is opposite that of transport

Top

$\ln[H^+]_{out} = 2.3\log[H^+]_{out}$

$= -2.3\ pH_{out}$

Bottom

$\ln(1/[H^+]_{in}) = 2.3\text{-}\log[H^+]_{in}$

$= +2.3\ pH_{in}$

$pH_{out} \approx 6.75$

$pH_{in} \approx 7.5$

$= RT2.3(pH_{in} - pH_{out})$

$= 5.9(7.5 - 6.75)$

$= 5.9(0.75)$

$= 4.4$ kJ/mol

$= 1.0$ kcal/mol

$= z\mathcal{F}\Delta\psi$

$= (+1)(96480)\ \Delta\psi$

$= (+1)(96480)(+0.06)$

$= 5.8$ kJ/mol

$= 1.4$ kcal/mol

$\Delta G' = 1.0 + 1.4 = 2.4$ kcal/mol

As a consequence, it will take ~3 protons per ATP.

Oxidative Phosphorylation

P/O ratios

Using tissues rich in mitochondria like pigeon muscle, and eventually using isolated mitochondria, biochemists would add different carbon compounds and measure two things:

1) Oxygen consumption
2) Amount of ATP made

The ratio of ATP synthesized to the oxygen ($\frac{1}{2}O_2$) consumed was termed the P/O ratio

The $\frac{1}{2}O_2$ represents $2e^-$ going through electron transport:

NADH was ~3
Pyruvate was ~3
Succinate was ~2
Ascorbate was 1

$\Delta G^{o'}$ of NADH→ $\frac{1}{2}$ O_2

$\Delta G^{o'}$ = –52.6 kcal/mol

$\Delta G^{o'}$ = +7.3 kcal/mol

22/53 = 42% efficient

What makes this proton motive force?

Electron Transport