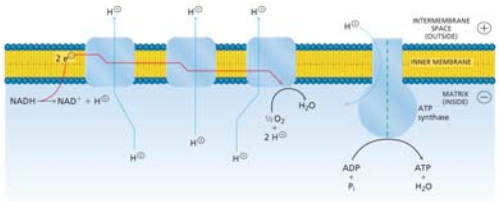
The Proton-Motive Force

Chapter 21 Stryer Short Course

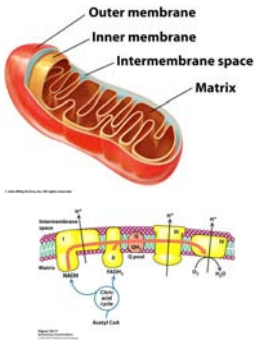
Overview

- Redox reactions
- Electron transport chain
- Proton gradient
- ATP synthesis
- Shuttles



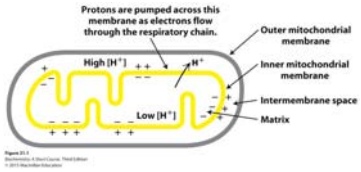
Analogy: How does burning coal put flour in the grocery store?

Compartmentalization



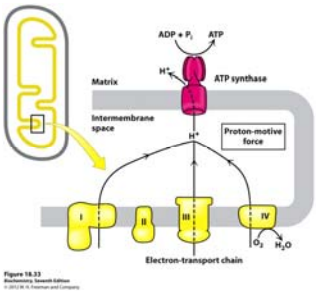
Chemiosmotic Hypothesis

- Electron transport is coupled to ATP synthesis through a proton gradient
- Generation of proton-motive force
  - pH gradient
  - Charge gradient



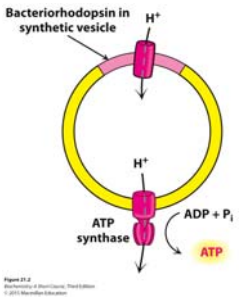
Protonmotive Force

- Flow of electrons is useless if not coupled to a useful process
  - Battery connected to wire
- Proton gradient across mitochondrial membrane



Experimental Evidence

- Artificial vesicle
- Exposure to light generates ATP only with intact membrane
- Respiratory chain and ATP Synthase are separate, and linked only by proton gradent

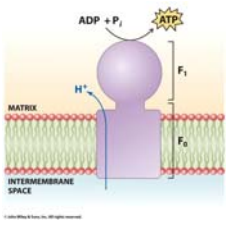


Problem

- How did these key experiments support the chemiosmotic theory of Peter Mitchell?
  - The pH of the intermembrane space is lower than the pH of the mitochondrial matrix.
  - Oxidative phosphorylation does not occur in mitochondrial preparations to which detergents have been added.
  - Lipid-soluble compounds inhibit oxidative phosphorylation while allowing electron transport to continue.

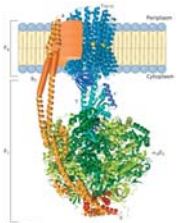
F_0F_1 ATP Synthase

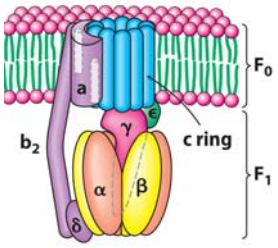
- Machine that couples to ATP synthesis to proton flow
- Uncouplers used to show link of oxygen uptake and ATP synthesis



ATP Synthase (Complex V)

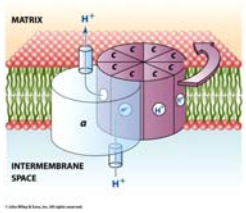
- Molecular motor
- Rotor: c, γ, ε
  - Proton channel

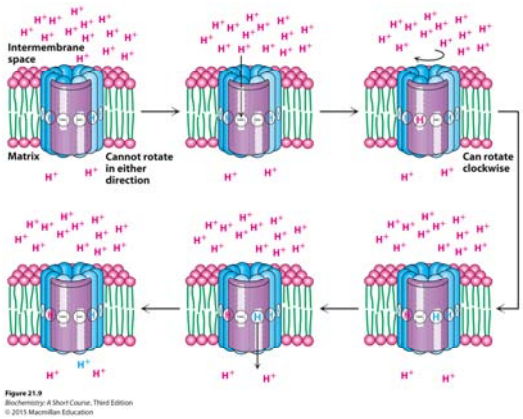




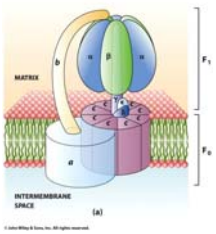
Proton Channel

- Protons enters channel between rotor and stator
- Rotor rotates to release strain by allowing proton to enter matrix
- 8- 15 protons = full rotation
  - Species dependent

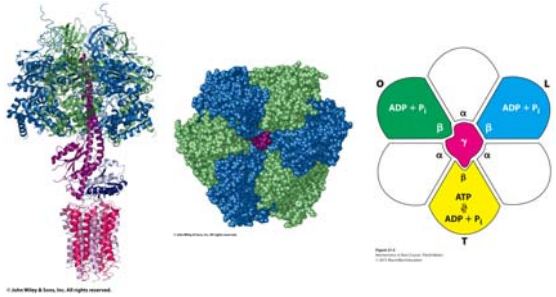




- "Stalk" (γ) moves inside the"knob"—hexameric ATP synthase
- Knob held stationary by "b"

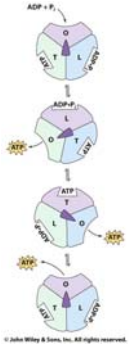


Hexameric Knob



Binding-Change Mechanism

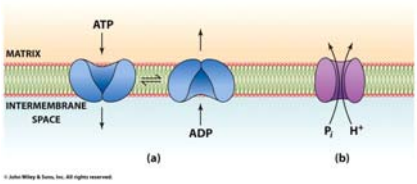
- Stalk causes ATP synthase to have three different conformations: open, loose, tight
- In "tight" conformation, energy has been used to cause an energy conformation that favors ATP formation



Remember Analogy

- Fuel→electricity→water pumped uphill→flows down to grind flour
- But we don't have bread until flour is transported to where it needs to go!
- Compartmentalization: Shuttling
  - ATP/ADP
  - NADH
  - Organic/inorganic ions

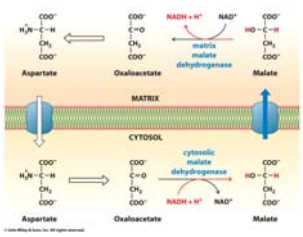
Active Transport of ATP



- ATP must go out, ADP and P_i must go in
- Together, use significant protonmotive force

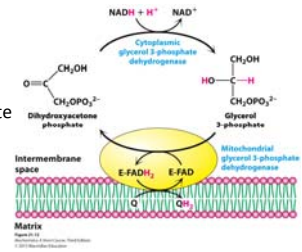
NADH into Matrix

- NADH of glycolysis must get "into" matrix
- Not direct
- Needs either
  - malate-aspartate shuttle (liver)
  - Glycerol-3-phosphate shuttle (muscle)
    - Costs 1 ATP worth of proton gradients, but allows for transport against NADH gradient

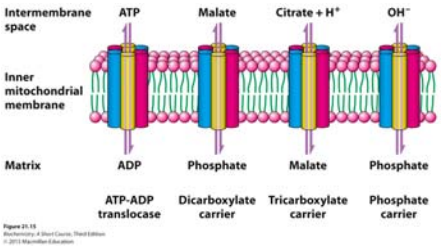


Glycerol-3-phosphate Shuttle

- Glycerol phosphate shuttle (1.5 ATP/NADH)
- Produces QH_2
- Operational in
  - Human muscle
  - Insects (replaces lactate dehydrogenase)



Many other metabolites…

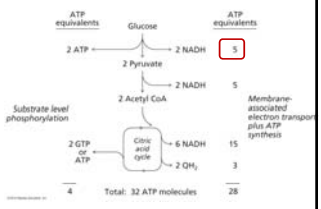


Energy Accounting

- ATP costs 2.7 protons
  - 8 protons produces 3 ATP
- NADH pumps 10 protons when 2 e^- reduce ½ O_2
  - 4 protons in Complex I, 4 protons in Complex III, and 2 protons in Complex IV
- P/O ratio--# of phosphorylation per oxygen atom
  - $10H^+$/NADH (1 ATP/2.7 H^+) = 3.7 ATP/NADH
  - $6H^+/QH_2$ (1 ATP/2.7 H^+) = 2.3 ATP/QH_2
- In vivo, P/O ratio closer to 2.5 and 1.5 due to other proton "leaking"
  - i.e. importing phosphate

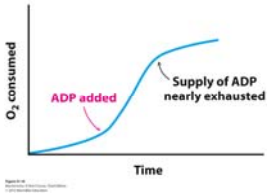
Net ATP Harvest from Glucose

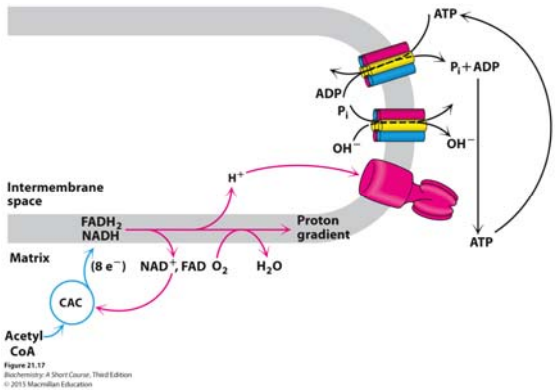
- Glycolysis = 2 ATP
  - Plus 3 or 5 ATP from NADH
  - What leads to difference in this case?
- Pyruvate DH = 5 ATP
- Citric Acid Cycle = 20 ATP
- Total: about 30 ATP/glucose



Regulation of Oxidative Phosphorylation

- Electron transport is tightly coupled to ATP production
  - Oxygen is not used unless ATP is being made
  - Avoid waste of fuels
  - Adding ADP causes oxygen utilization
  - Respiratory control



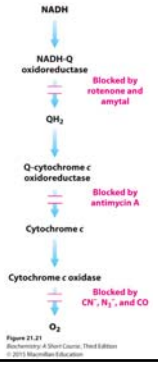


Problem

- A culture of yeast grown under anaerobic conditions is exposed to oxygen, resulting in dramatic decrease in glucose consumption. This is called the Pasteur effect. Explain.
- The [NADH]/[NAD^+] and [ATP]/A[ADP] ratios also change when an anaerobic culture is exposed to oxygen. Explain how the ratios change and what effect this has on glycolysis and the citric acid cycle in yeast.

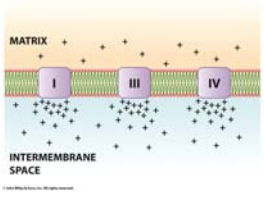
Respiratory Poisons

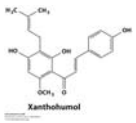
- Block electron transport chain
- Inhibit ATP synthase
- Block ATP export
- Decoupling of electron transport chain and ATP synthesis
  - Uncouplers

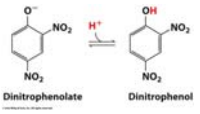


Uncouplers

- "Uncouple" protonmotive force from ATP synthase
  - DNP pKa / solubility perfectly suitable
- Act as respiratory poisons/obesity treatment







Regulated Uncoupling

- Heat generation through decoupling
- Brown fat
- Uncoupling protein-1 dissipates proton gradient, releasing energy as heat rather than storing as ATP