BI/CH 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic

Exam-1 material

Fermentations
Pyruvate
pyruvate dehydrogenase (ox-decarbox; S-ester)
Krebs' Cycle
How did he figure it out?
Overview
8 Steps
Citrate Synthase (C-C)
Aconitase (=, -OH)
Isocitrate dehydrogenase (ox-decarbox; =O)
Ketoglutarate dehydrogenase (ox-decarbox; S-ester)
Succinyl-CoA synthetase (sub-level phos)
Succinate dehydrogenase (=)
Fumarase (-OH)
Malate dehydrogenase (=O)
Energetics
Regulation
Summary

Chemiosmotic theory: Phosphorylation — Exam-2 material

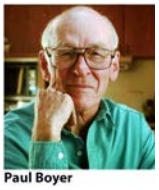
ATPase
Mitchell Hypothesis
Binding-Change Model
Connection to the proton motive force
Net ATP production
Regulation

See *Achieve*:
Ch19: Case Study: The Narrow Window

Oxidative Phosphorylation
Energetics (−0.16 V needed for making ATP)
Mitochondria
Transport (2.4 kcal/mol needed to transport H^+ out)
Electron transport
Discovery
Four Complexes
Complex I: NADH → $CoQH_2$
Complex II: Succinate → $CoQH_2$
Complex III: $CoQH_2$ → Cytochrome C (Fe^{2+})
Complex IV: Cytochrome C (Fe^{2+}) → H_2O

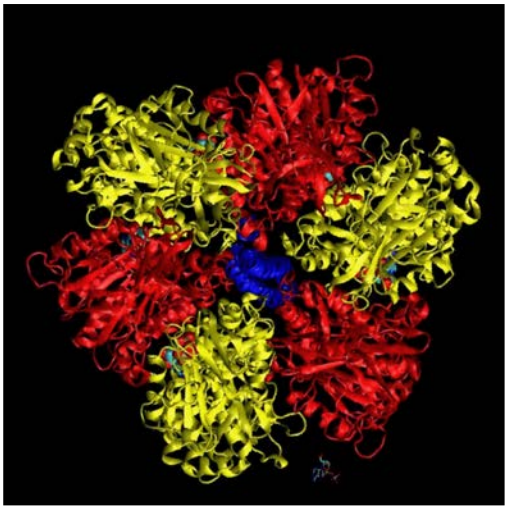
Phosphorylation

Mechanism
Binding-Change Model


Paul Boyer


John E. Walker

Nobel Prize in Chemistry 1997

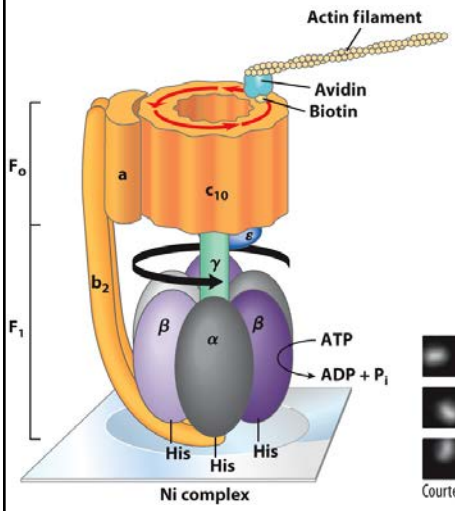


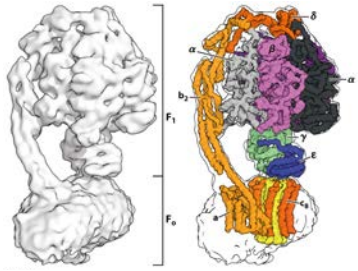
WHAT drives the γ-subunits motion?

ATP cannot be released from one site unless and until ADP and P_i are bound at the other·····without substrates IT ALL GRINDS TO A HALT!

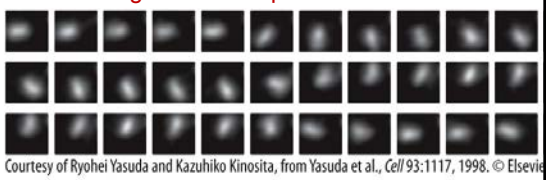
Phosphorylation

Experimental evidence for the rotary/binding-change mechanism





The γ-subunit is tightly bound to the C10 ring with the help of the ε-subunit



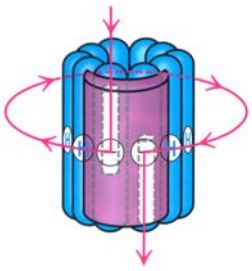
Courtesy of Ryohei Yasuda and Kazuhiko Kinosita, from Yasuda et al., *Cell* 93:1117, 1998. © Elsevie

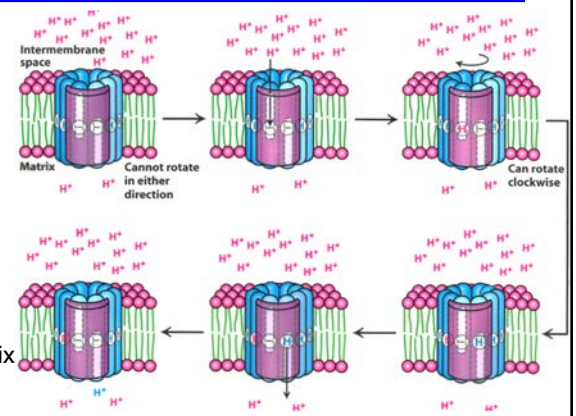
ATP Synthase moving a bead

(https://www.youtube.com/watch?v=oFgMTdVRi6I)

Phosphorylation

Mechanism

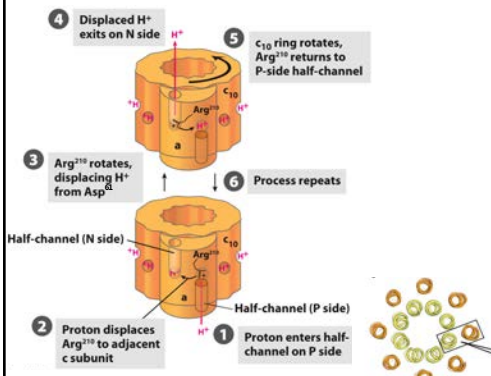




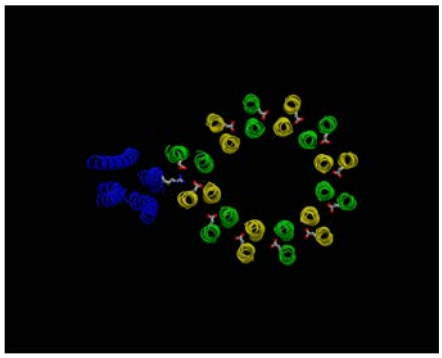
- Protons in excess outside the matrix forces them on a specific Asp residue in the C-subunit.
- But, it must enter through the **a-subunit**.
- Once bound, it causes a conformational change to rotate the c-ring and make available another proton binding site.
- Protons can't get access to the matrix until a complete revolution and contact with the **a-subunit** again, which has a tunnel to the matrix.
- Proton translocation causes a rotation of the F_0 subunit and the **central shaft γ**.

Phosphorylation

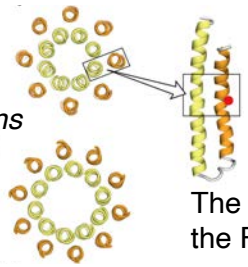
Mechanism



H. sapiens
[8]



E. coli
[12]



Bovine & S. cerevisiae
[10]

The number of c-subunits in the F_0 ring varies from 8-17.

Phosphorylation

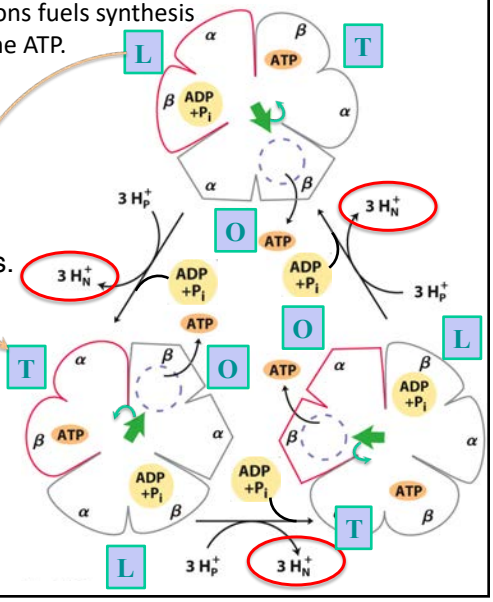
Mechanism

Coupling Proton Translocation to ATP Synthesis

(Lets assume there are 9 c-subunits in F_0)

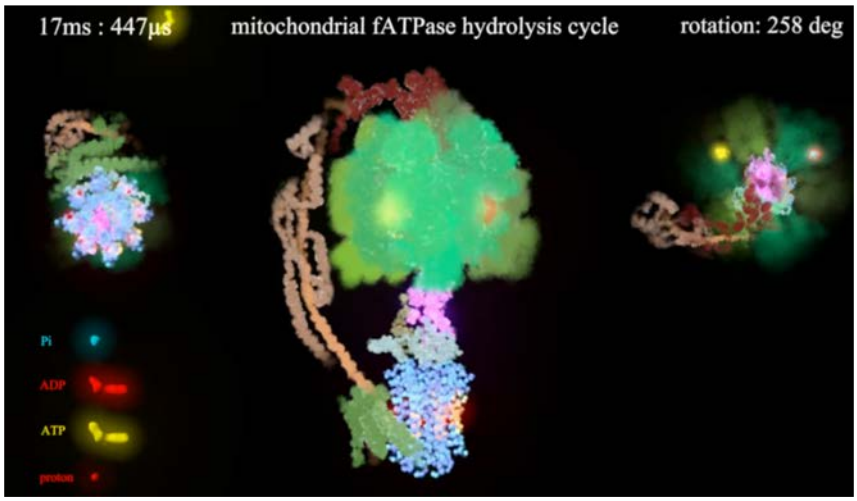
- Proton translocation causes a rotation of the F_0 subunit and the central shaft γ.
- This causes a conformational change within all the three $\alpha\beta$ pairs.
- The conformational change in one of the three pairs promotes condensation of ADP and P_i into ATP.
- The conformational change in another drives the release of ATP.
- This conformational change opens the binding site for ADP and P_i.

Translocation of three protons fuels synthesis of one ATP.



Phosphorylation

The Respiratory Chain and ATP Synthase Produce ATP by a Chemiosmotic Mechanism

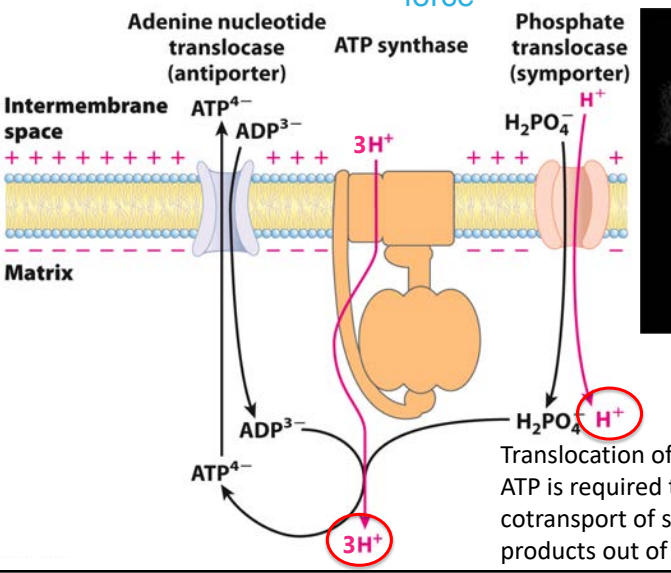


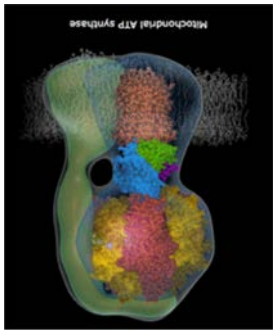
Animation of ATP Synthase

(https://www.youtube.com/watch?v=E1qTSwc1o3U)

Phosphorylation

Transport of ADP and P_i into the Matrix also uses proton-motive force





Translocation of a fourth proton per ATP is required to facilitate cotransport of substrates into and products out of the mitochondria.

Oxidative Phosphorylation

Net Production of ATP by Oxidation of NADH

Complex I → Complex IV

$1NADH + 11H^+_{(N)} + \frac{1}{2}O_2 \rightarrow NAD^+ + 10H^+_{(P)} + H_2O$

- 10 protons pumped to P-side.
- For an F_0 with 9 c-subunits, 9 protons are allowed down their concentration gradient and one 360° rotation of the F_1 subunit produces 3 ATP molecules; or 3 protons per ATP.
- But, one proton is needed to get substrate P_i into matrix, so the total is 4 protons per ATP.
- So, for each $2e^-$ from NADH, the 10 protons will yield 2.5 ATP molecules
- For $2e^-$ from $FADH_2$, the 6 protons will yield 1.5 ATP molecules.
- Different organisms have different numbers of c-subunits, so this number varies species to species. Humans have 8.

Oxidative Phosphorylation

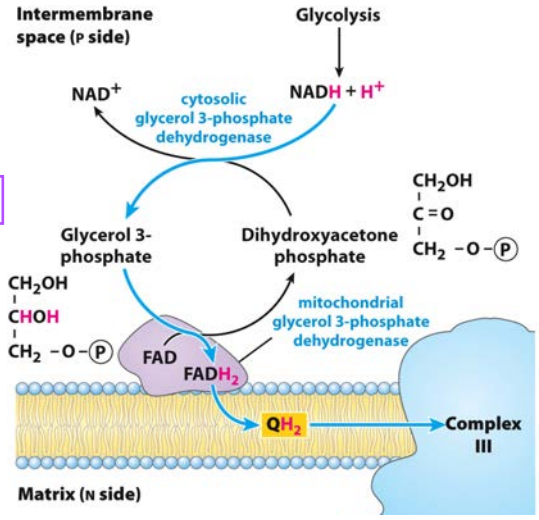
Net Production of ATP by Oxidation of Glucose

- In prokaryotic systems, organelles do not segregate machinery, so all electron carriers can easily feed directly into the electron-transport chain.
- In eukaryotic systems, organellar segregation prevents NADH from the cytosol from directly entering the electron-transport chain at Complex I.
  - NAD^+ pools are kept segregated and cannot directly cross the mitochondrial inner membrane.
  - Two methods are used to feed the electrons from NADH from the cytosol into the mitochondria:
    - glycerol-3-phosphate shuttle
    - malate-aspartate shuttle

Oxidative Phosphorylation

Converting Cytosolic Electron Carriers (NADH) to the Mitochondria

Glycerol-3-Phosphate Shuttle



Malate-Aspartate Shuttle

- This more complicated shuttle is mostly present in liver, heart, and kidney.
- Will be discussed when we do amino-acid degradation
- It moves NADH equivalents form cytosol to NADH equivalents to the mitochondria.

Oxidative Phosphorylation

Net Production of ATP via Catabolic Pathways

TABLE 19-5 ATP Yield from Complete Oxidation of Glucose *

Process	Direct product	Final ATP
Glycolysis	2 NADH (cytosolic) 2 ATP	3 or 5[a] 2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix) 2 $FADH_2$ 2 GTP	15 3 2
Total yield per glucose		30 or 32

[a]If the malate/aspartate shuttle is used to transfer reducing equivalents into the mitochondrion, yield is 5 ATP. If the glycerol 3-phosphate shuttle is used, the yield is 3 ATP.

- Every F_0 turn uses 8-17 H^+
- Every turn gets 3 ATP
- Additional 3 H^+ to transport 3 P_i

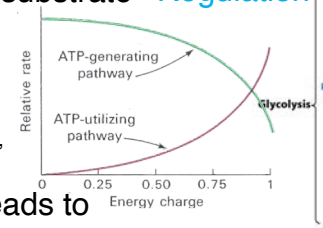
What is the yield for c_{17}?

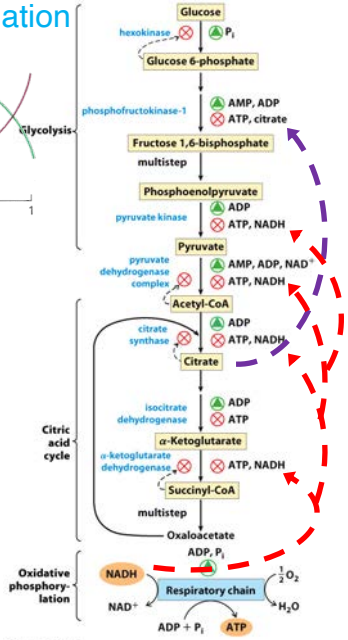
*
- This Table assumes F_0 is c_9 and uses 9 H^+ per turn
- Additional 3 H^+ to transport P_i needs 12 H^+ per 3 ATP
- This is 4 H^+ per ATP
- NADH pumps10 H^+, so 10/4 = 2.5 ATP/NADH oxidized

Oxidative Phosphorylation

- Primarily regulated by substrate availability
  - NAD^+ and ADP/P_i
- Local feedback inhib.
  - Succ-CoA, Citrate, Ac-CoA, and Glc6P.
- Inhibition of OxPhos leads to accumulation of NADH.
  - causes feedback inhibition cascade up to Pyruvate Kinase in Glycolysis
  - lack of NAD^+ in TCA causes accumulation of citrate, which feeds back to inhibit PFK-1
- Inhibitor of F_1 (IF_1)
  - prevents hydrolysis of ATP during low oxygen
  - IF_1 only active at lower pH in matrix, encountered when electron transport is stalled (i.e., low oxygen)

Regulation



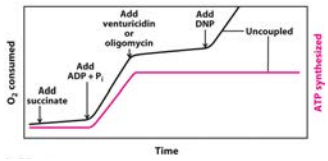


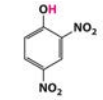
Oxidative Phosphorylation

UNCOUPLING

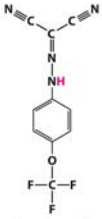
Chemically uncoupling ET and ATP biosynthesis:

Recall:



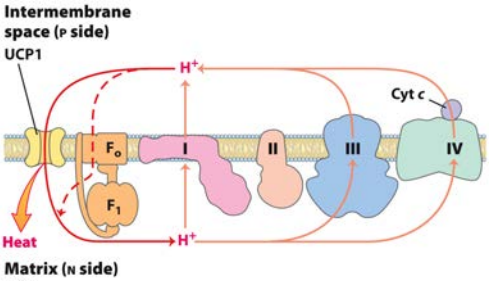


2,4-Dinitrophenol (DNP)



Carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP)

- In addition to chemical uncouplers (DNP & FCCP), there are times when uncoupling is needed physiologically
- Uncoupling protein 1 (UCP-1) in babies
- Hibernating animals



Reactive Oxygen Species Can Damage Biological Macromolecules

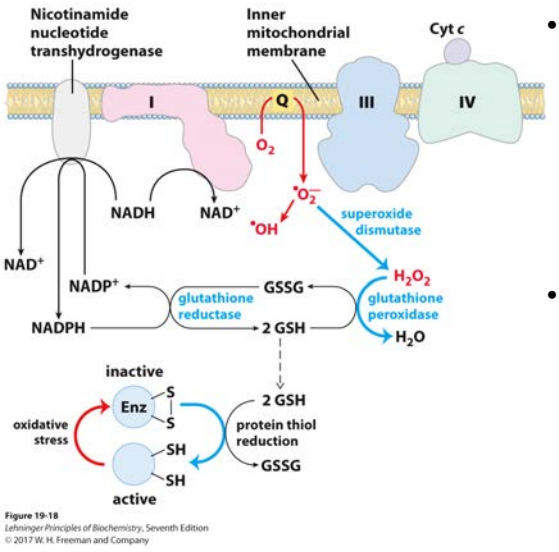


Figure 19-18
Lehninger Principles of Biochemistry, Seventh Edition
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- Ubiquinone is naturally "leaky" and facilitates partial reduction of non-Complex III targets.
  - Single electron tranfers result in free radicals.
- One method by which the cell can correct free-radical production of reduced glutathione, which fuels the glutathione shuttle

Summary:Oxidative Phosphorylation

We learned that:

- the reduced cofactors pass electrons into the electron- transport chain in mitochondria
- stepwise electron transport is accompanied by the directional transport of protons across the membrane against their concentration gradient
- the energy in the electrochemical proton gradient drives synthesis of ATP by coupling the flow of protons via ATP synthase to conformational changes that favor formation of ATP in the active site
- Summary video:
  https://www.youtube.com/watch?v=LQmTKxI4Wn4

End of material for Exam 2