BI/CH 422/622

OUTLINE:

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

Exam-1 material

Exam-2 material

Pyruvate
- pyruvate dehydrogenase (ox-decarbox; S-ester)

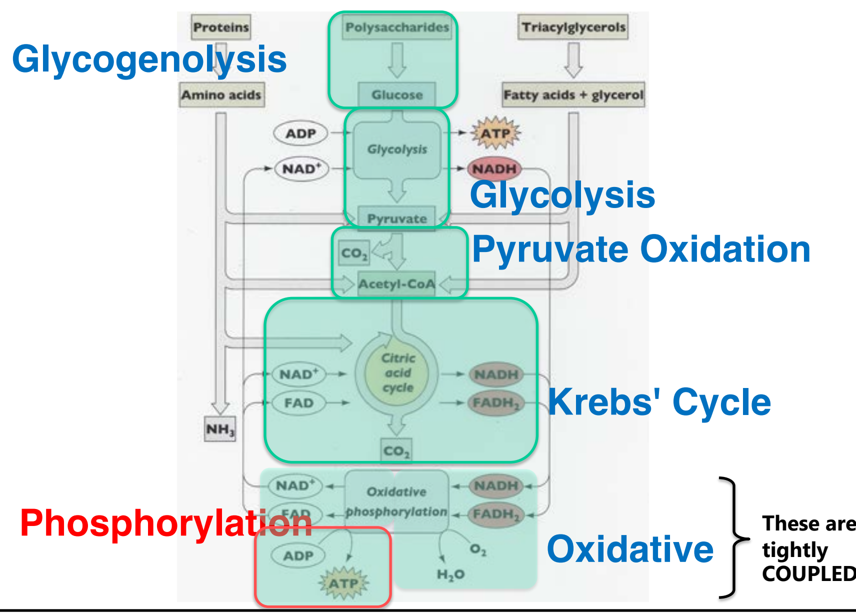
Krebs' Cycle
- How did he figure it out?
- Overview
- 8 Steps
  - Citrate Synthase (C-C)
  - Aconitase (=, -OH)
  - Isocitrate dehydrogenase (ox-decarbox; =O)
  - Ketoglutarate dehydrogenase (ox-decarbox; S-ester)
  - Succinyl-CoA synthetase (sub-level phos)
  - Succinate dehydrogenase (=)
  - Fumarase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

Oxidative Phosphorylation
- Energetics (-0.16 V needed for making ATP)
- Mitochondria
- Transport (2.4 kcal/mol needed to transport H+ out)
- Electron transport: Oxidative
  - 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

Chemiosmotic theory: Phosphorylation
- Ox/Phos is Coupled
- ATPase
- Mitchell Hypothesis
- Binding-Change Model
- Connection to the proton-motive force

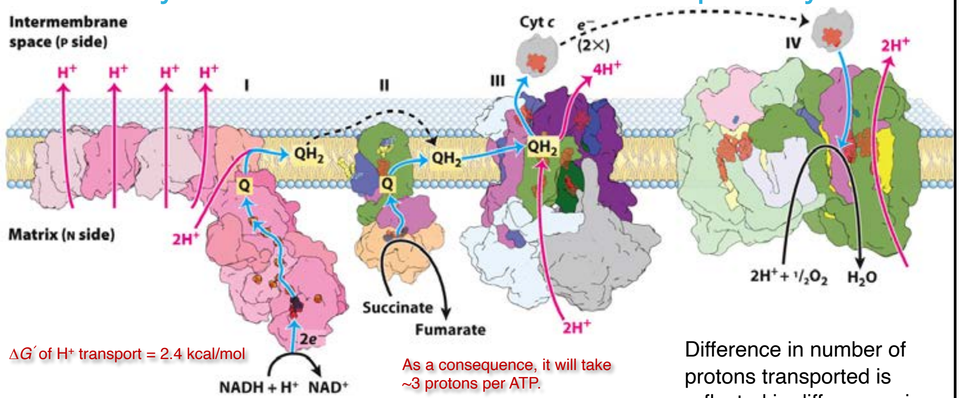
See *Achieve*:
Ch19: Case Study: The Narrow Window

Oxidative Phosphorylation



Electron Transport

Summary of the Electron Flow in the Respiratory Chain



This scheme is for 2e⁻ coming down from **1** NADH or **1** $FADH_2$

Complex I → Complex IV

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

Complex II → Complex IV

$FADH_2 + 6H^+_{(N)} + \frac{1}{2}O_2 \rightarrow FAD + 6H^+_{(P)} + H_2O$

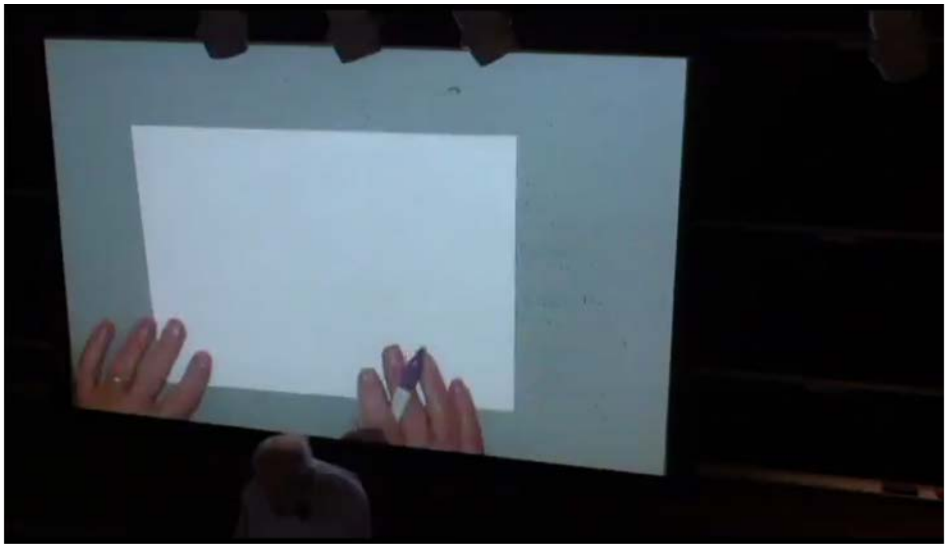
Difference in number of protons transported is reflected in differences in ATP synthesized=P/O ratios:

Substrate	P/O ratio
Pyruvate	3
Acetyl CoA	3
NADH	3
Succinate	2

Phosphorylation

(The Chemiosmotic Theory)

The Chemiosmotic Theory

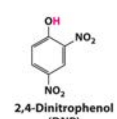


Dr. Kornberg: **Lecture 02.17.17 (45:00-48:25)- mitochondria coupling (3.5 min)**

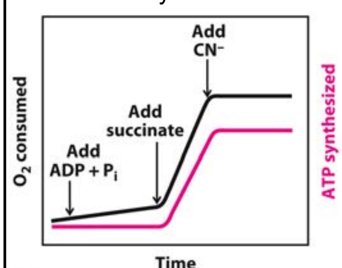
Oxidative Phosphorylation

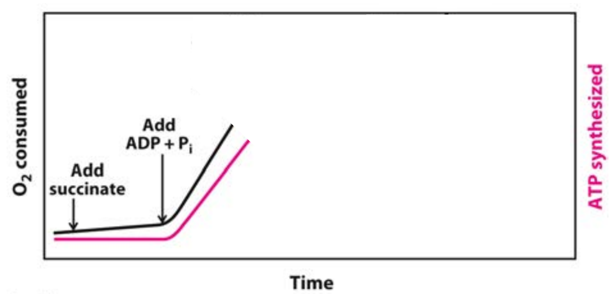
Relationship of ETC and ATP Synthesis

The Chance/Williams experiment showed that fuel oxidation is COUPLED to ATP synthesis



Here, same experiment done the other way:





- As described, ATP synthesis requires electron transport.
- But electron transport does not require ATP synthesis.

Oxidative Phosphorylation

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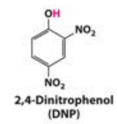

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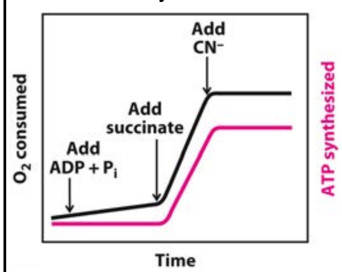

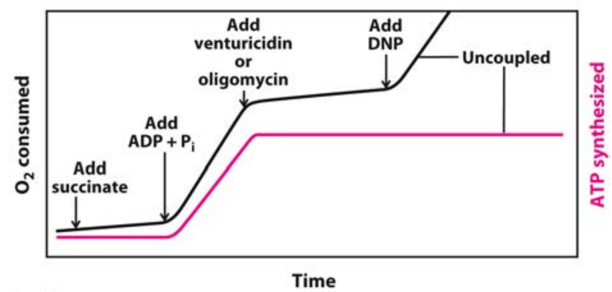

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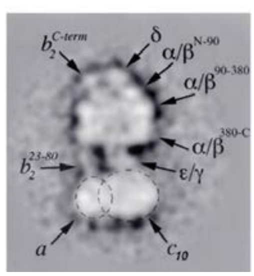
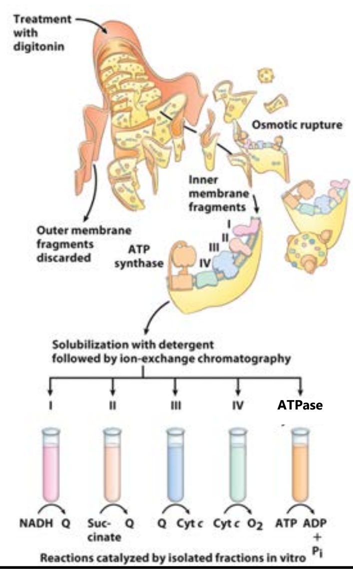
Phosphorylation

How Does Oxidative Phosphorylation Form ATP?



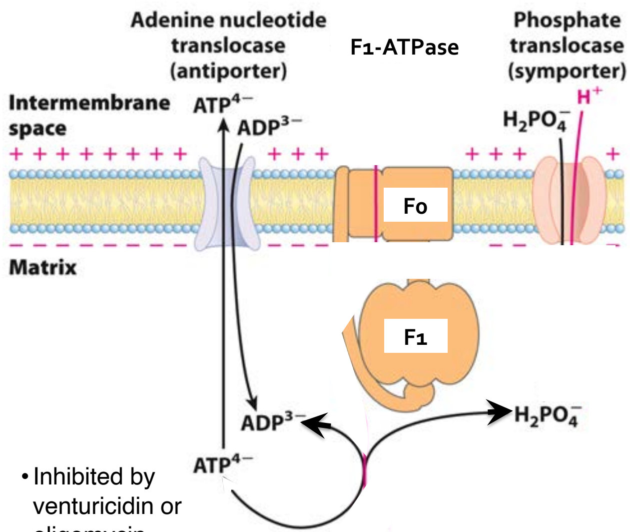


- Before the 1970's, it was thought that the ET chain used the energy of redox to make a "high-energy" intermediate for "substrate-level phosphorylation, as occurs in glycolysis and TCA cycle.
- It was clear that one of the most abundant proteins on the inner-mitochondrial membrane had a curious mushroom-shaped structure.
- It was purified, along with the other Complexes I-IV, initially called Complex V.

Phosphorylation

How Does Oxidative Phosphorylation Form ATP?

- Complex V, when purified separately from the membrane is an effective ATPase enzyme, hence it was initially called the F_1-ATPase.
- The F_1 part had 9 subunits ($\alpha_3\beta_3\gamma\delta\varepsilon$)
- When more careful purifications were performed, it was clear that its activity was closely coupled to an intact inner membrane with little activity.
- When membrane proteins were isolated, the F_0 part had 15 subunits of 3 different proteins (a, b_2, c_{10}).



- Inhibited by venturicidin or oligomycin

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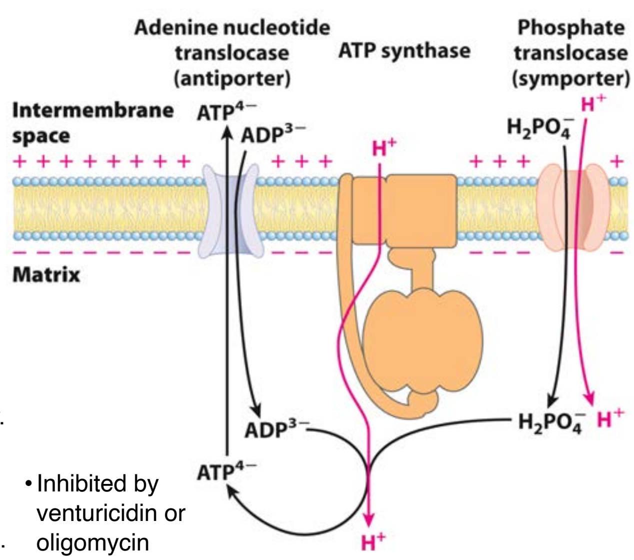

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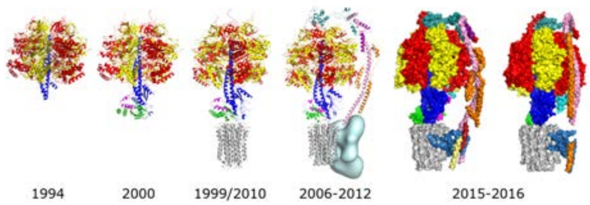
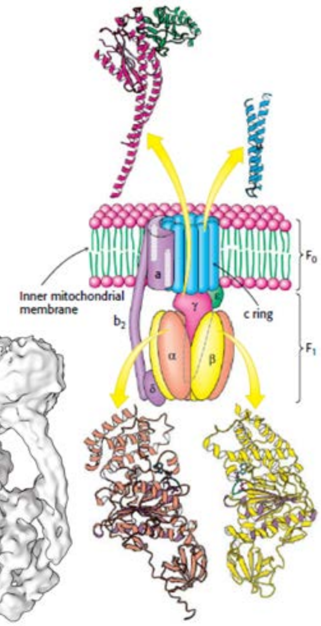
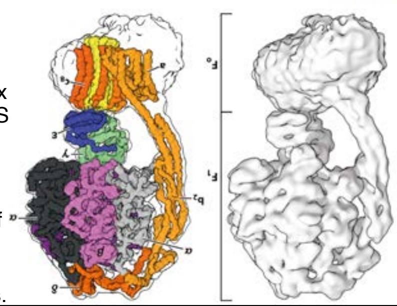
Phosphorylation



Contains two functional units:

- F_1
  - soluble complex in the matrix
  - individually catalyzes the hydrolysis of ATP
- F_0
  - integral membrane complex
  - transports protons from IMS to matrix, dissipating the proton gradient
  - energy transferred to F_1 to catalyze phosphorylation of ADP.
  - This mechanism was only fully accepted in late 1970s.





Phosphorylation

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

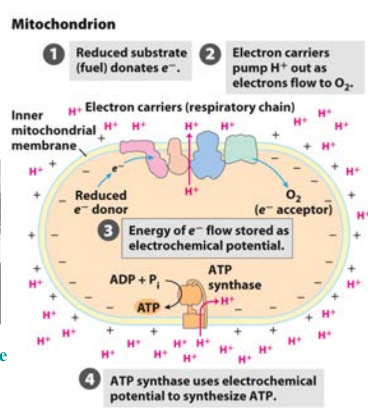
VS.

Hypothesis: The Respiratory Chain Produce ATP by a substrate-level-phosphorylation mechanism

Peter Mitchell
1920-1992

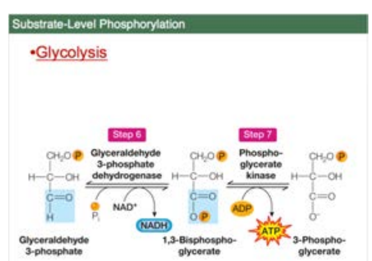
Peter Mitchell, 1920-1992

1978 **Nobel Prize for** Chemistry



Efraim Racker, 1913–1991

The search was for a compound, like 1,3 bisphosphoglycerate, that would be used by the ATP Synthase to make ATP.



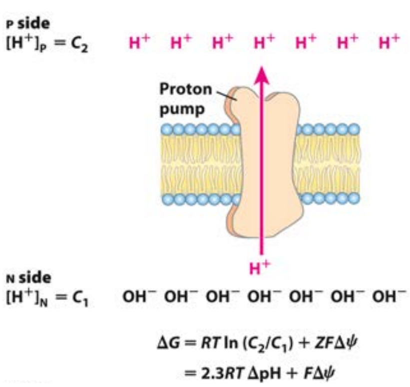
Phosphorylation

Chemiosmotic Theory

- ADP + P_i → ATP is highly thermodynamically unfavorable.
- How do we make it possible?
- Phosphorylation of ADP is not a result of a direct reaction between ADP and some high-energy phosphate carrier.
- The energy released by the exergonic flow of electrons to oxygen in electron transport is used to transport protons against the electrochemical gradient. Secondary active transport principles are at work.
- Energy needed to phosphorylate ADP is provided by the flow of protons down this electrochemical gradient. This can be calculated.
- If all that was needed was a proton gradient, could one be established without the ET chain and still drive ATP biosynthesis?

Phosphorylation

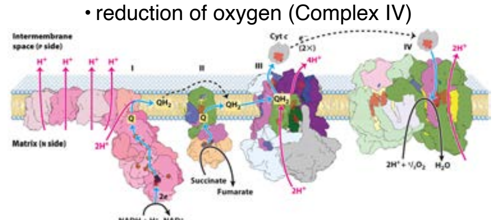
Chemiosmotic Theory



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

As a consequence, it will take ~3 protons per ATP. But, how many precisely?

- The proteins in the electron-transport chain created the electrochemical proton gradient (proton-motive force (PMF); a chemical AND electrical gradient) by one of three means:
  - actively transporting (pumping) protons across the membrane
    - Complex I and Complex IV
  - releasing protons into the intermembrane space
    - oxidation of QH_2 at Complex III
  - chemically removing protons from the matrix
    - reduction of CoQ in Q-cycle (Complex I, II, & III)
    - reduction of oxygen (Complex IV)

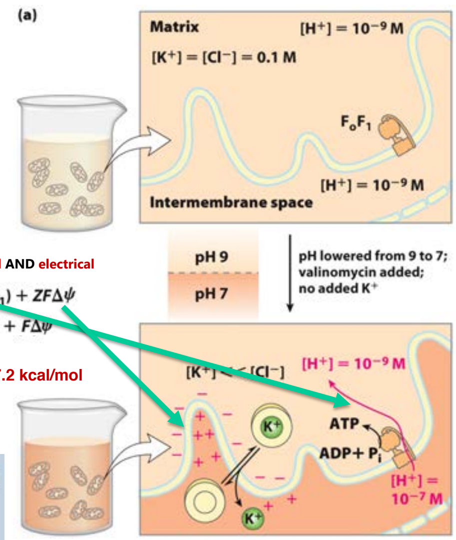


Phosphorylation

Chemiosmotic Theory

Nobel Prize in Chemistry
1978

Mitchell's experiment:



chemical AND electrical

$$\Delta G = RT \ln (C_2/C_1) + ZF\Delta\psi$$
$$= 2.3RT\,\Delta pH + F\Delta\psi$$

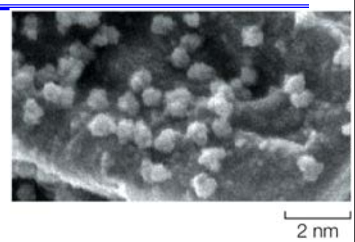
For 3 protons:
$\Delta G' = 3.0 + 4.2 = 7.2$ kcal/mol

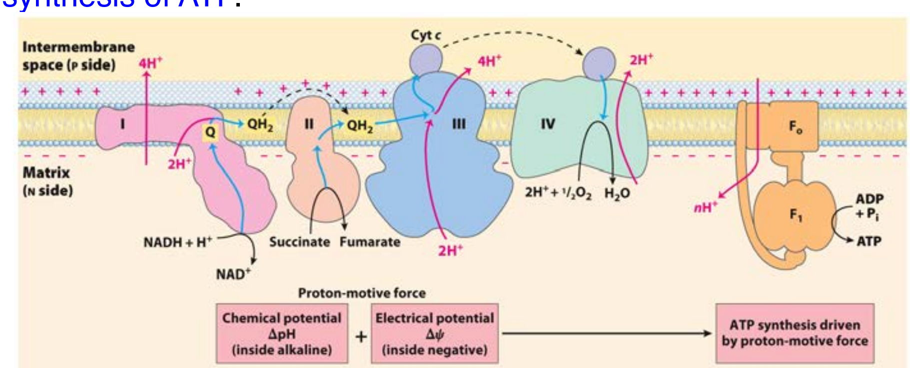
In the absence of electron transport, an artificial H^+ gradient is sufficient for ATP synthesis by mitochondria. (if there is also a sufficient membrane potential)

Phosphorylation

Chemiosmotic Theory for ATP Synthesis:

- Electron transport chain sets up a proton-motive force.
- Energy of proton-motive force drives synthesis of ATP.





Phosphorylation

Chemiosmotic Theory

Chemiosmotic Energy Coupling
Requires Membranes

- The proton gradient needed for ATP synthesis can be stably established across a membrane that is impermeable to ions.
  - plasma membrane in bacteria
  - inner membrane in mitochondria
  - thylakoid membrane in chloroplasts
- The membrane must contain proteins that couple the "downhill" flow of electrons in the **electron-transfer chain** with the "uphill" pumping of protons across the membrane.
- The membrane must contain a protein that couples the "downhill" flow of protons to the phosphorylation of ADP.

Phosphorylation

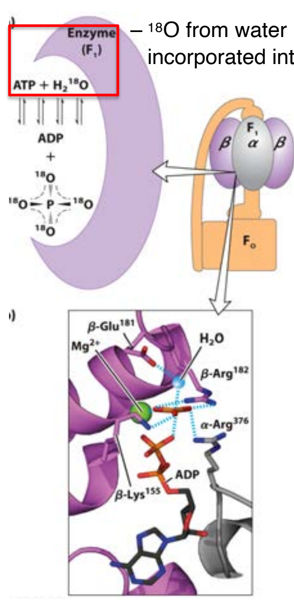
Mechanism: BINDING

The F_1 catalyzes ADP + P_i ⇄ ATP + H_2O

–Isotope studies

Both ATP hydrolysis and ATP synthesis have occurred several times during the incubation

– ^{18}O from water is quickly incorporated into P_i



–Kinetic studies

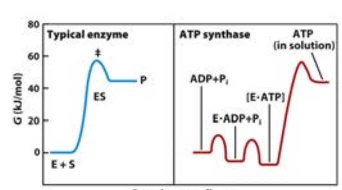
$$\text{Enz–(ADP + P}_i) \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Enz–(ATP + H}_2\text{O)}$$

$$K'_{eq} = \frac{k_1}{k_{-1}} = \frac{24\ s^{-1}}{10\ s^{-1}} = 2.4$$

($\Delta G'^\circ$ = **–0.5 kcal/mol**)

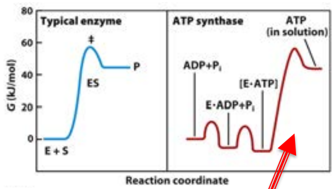
– Compare to >10^5 for in solution (from $\Delta G'^\circ$ = +7.3 kcal/mol)

–Structural studies



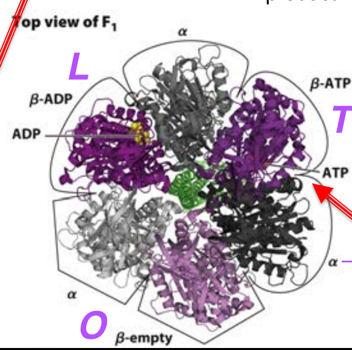
Phosphorylation

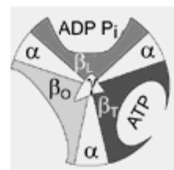
Mechanism: RELEASE



–MORE Structural studies

- Hexamer arranged in three $\alpha\beta$ dimers
- Dimers can exist in three different conformations:
  - open: empty
  - loose: binding ADP and P_i
  - tight: catalyzes ATP formation and binds product





T=tight
O=open
L=loose

Called the Binding-Change Model

WHERE does the energy come from for the release?

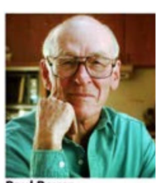
– This is where ALL the energy of PMF is used to release ATP!

(in Δconf. from tight to open; thus, releasing ATP)

Phosphorylation

Mechanism

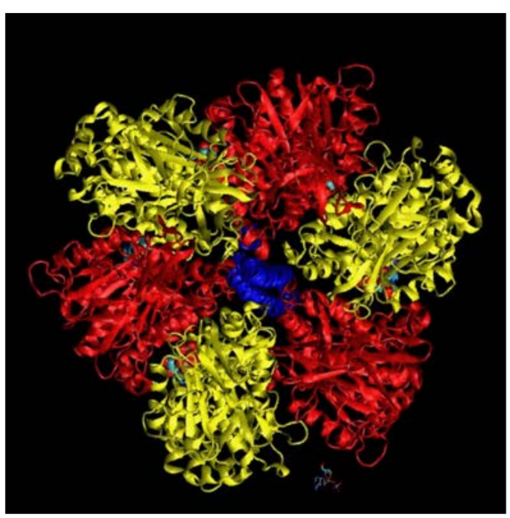
Binding-Change Model



Paul Boyer

John E. Walker

Nobel Prize in Chemistry 1997



WHAT drives the γ-subunit's motion?

ATP cannot be released from one site unless and until ADP and P_i are bound at the other………..the γ-subunit cannot move if the α/β-dimer is in the open conformation: without substrates IT ALL GRINDS TO A HALT!