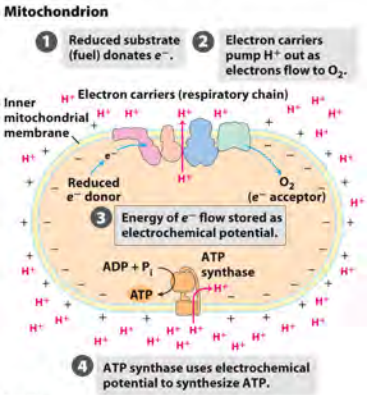
Phosphorylation

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

Peter Mitchell, 1920-1992

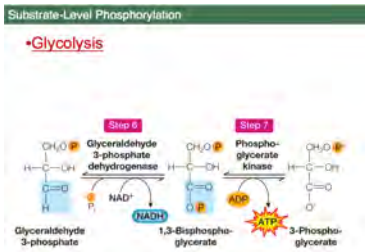
VS.



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

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

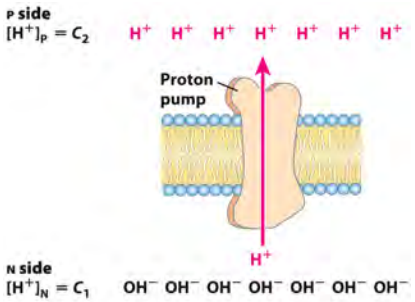
Chemiosmotic Theory

- 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.
- Energy needed to phosphorylate ADP is provided by the flow of protons down the electrochemical gradient. This can be calculated.
- The energy released by electron transport is used to transport protons against the electrochemical gradient. Primary and secondary transport principles are at work.

- 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

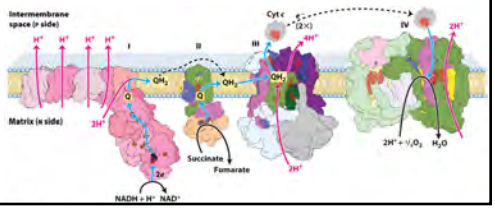


$$\Delta G = RT \ln (C_2/C_1) + ZF\Delta\psi$$
$$= 2.3RT\,\Delta pH + F\Delta\psi$$

$\Delta G' = 1.0 + 1.4 = 2.4$ 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) by one of three means:
  - actively transporting 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 (Complex I, II, & III)
    - reduction of oxygen (Complex IV)

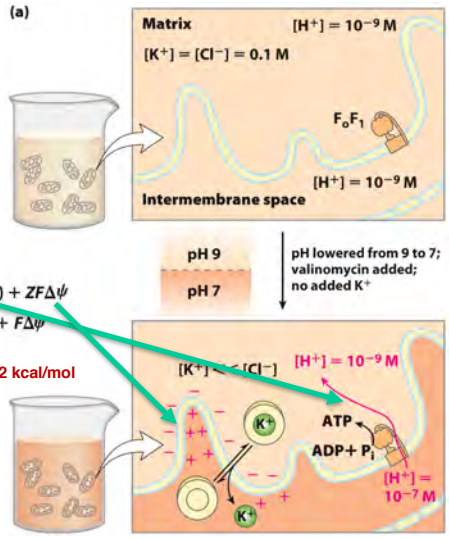


Phosphorylation

Chemiosmotic Theory

Mitchell's experiment:

Nobel Prize in Chemistry 1978



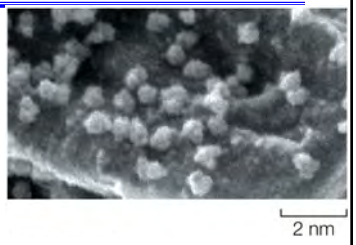
$$\Delta G = RT \ln (C_2/C_1) + ZF\Delta\psi$$
$$= 2.3RT\,\Delta pH + F\Delta\psi$$

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

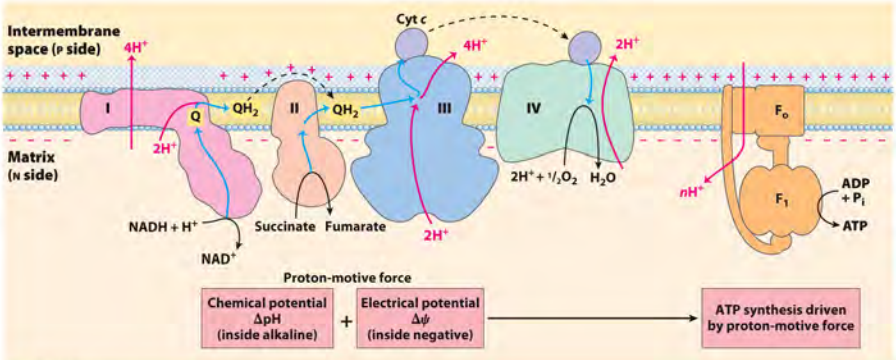
In the absence of electron transport, an artificial H^+ gradient is sufficient for ATP synthesis by mitochondria.

Phosphorylation

Chemiosmotic Theory for ATP Synthesis:



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



Phosphorylation

Chemiosmotic Theory

Chemiosmotic Energy Coupling Requires Membranes

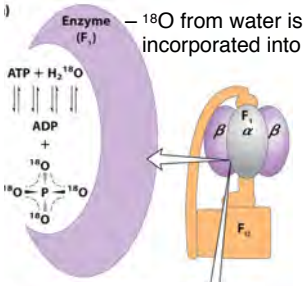
- The proton gradient needed for ATP synthesis can be stably established across a membrane that is impermeable to ions.
  - plasma membrane in bacteria
  - inner membrane in mitochondria
  - thylakoid membrane in chloroplasts
- The membrane must contain proteins that couple the "downhill" flow of electrons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



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

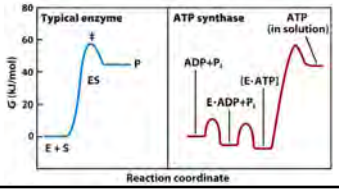
–Kinetic studies

$$\text{Enz-ATP} \rightleftharpoons \text{Enz-(ADP} + \text{P}_\text{i})$$

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

– Compare to >10^5 for in solution

–Structural studies



Phosphorylation

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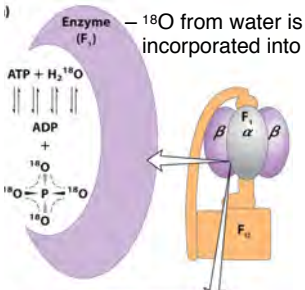

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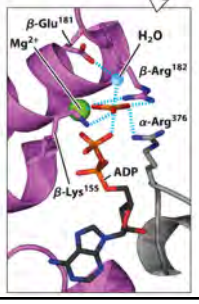
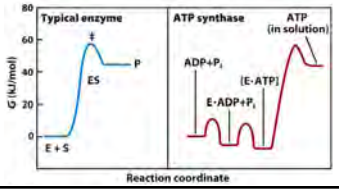
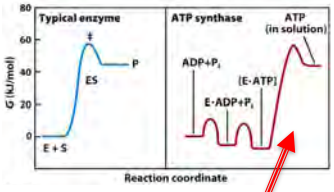




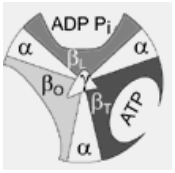
Phosphorylation

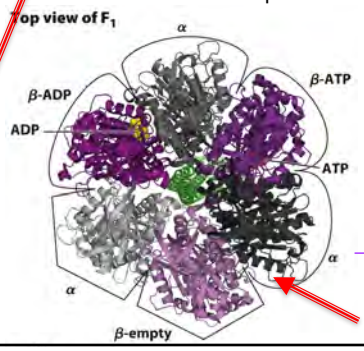
Mechanism: RELEASE



– 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





WHERE does the energy come from for the release?

WHAT drives the γ-subunits motion?

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

Phosphorylation

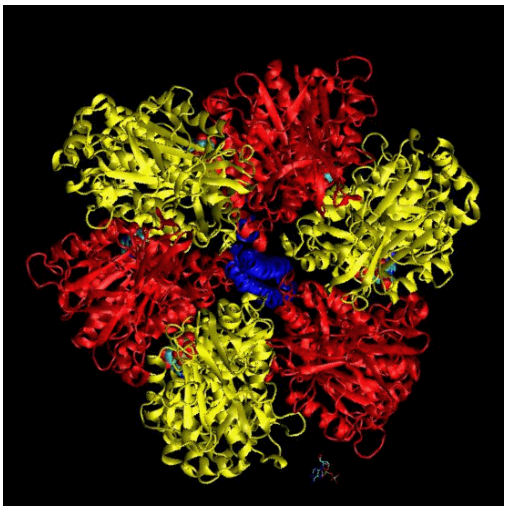
Mechanism

Binding-Change Model

Paul Boyer

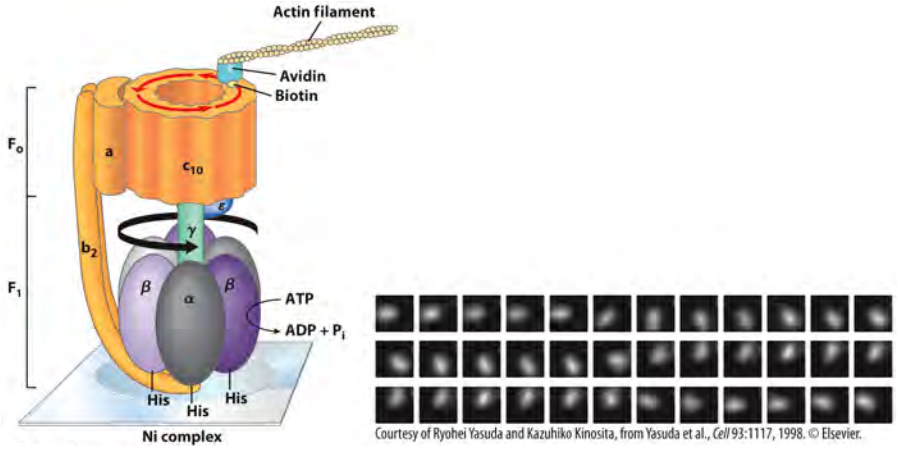
John E. Walker

Nobel Prize in Chemistry 1997



Phosphorylation

Experimental evidence for the rotary/binding-change mechanism



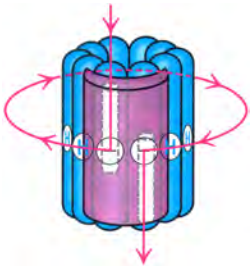
Courtesy of Ryohei Yasuda and Kazuhiko Kinosita, from Yasuda et al., *Cell* 93:1117, 1998. © Elsevier.

ATP Synthase moving a bead

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

Phosphorylation

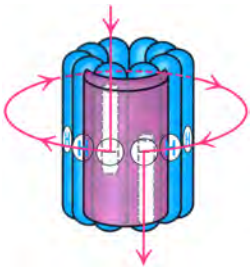
Mechanism

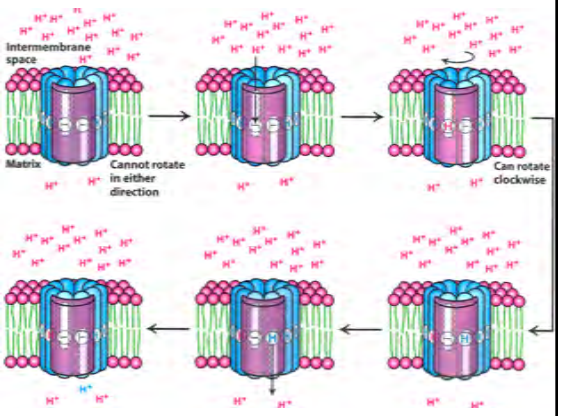


- Protons in excess outside the matrix forces them on a specific Asp residue in the C-subunit.
- But, it must enter through the **a-subunit**.
- Once bound, it causes a conformational change to rotate the c-ring and make available another proton binding site.
- Protons can't get access to the matrix 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





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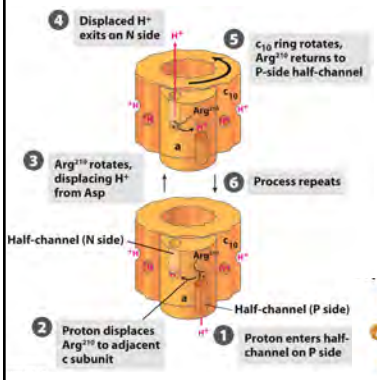
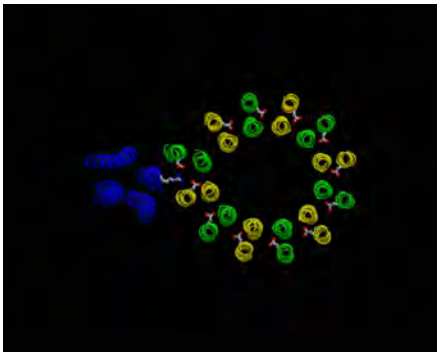
Phosphorylation

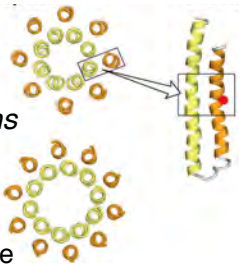
Mechanism





E. coli

H. sapiens



S. cerevisiae

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

Phosphorylation

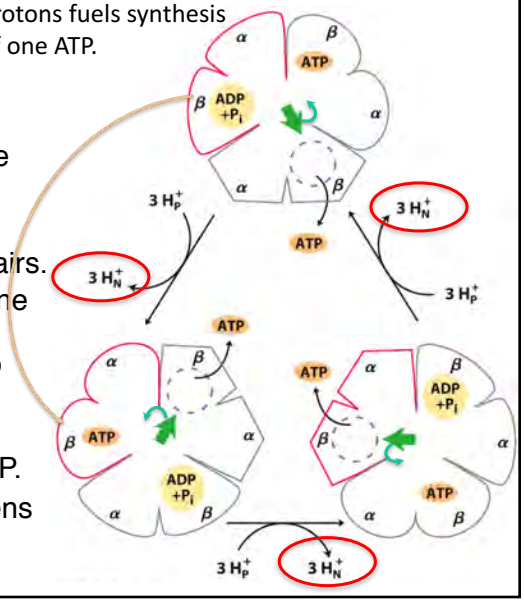
Mechanism

Coupling Proton Translocation to ATP Synthesis

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

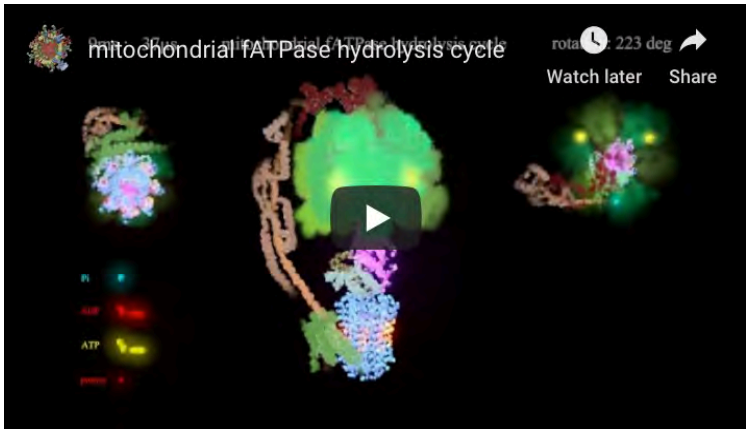
- Proton translocation causes a rotation of the F_0 subunit and the central shaft γ.
- This causes a conformational change within all the three $\alpha\beta$ pairs.
- The conformational change in one of the three pairs promotes condensation of ADP and P_i into ATP.
- The conformational change in another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

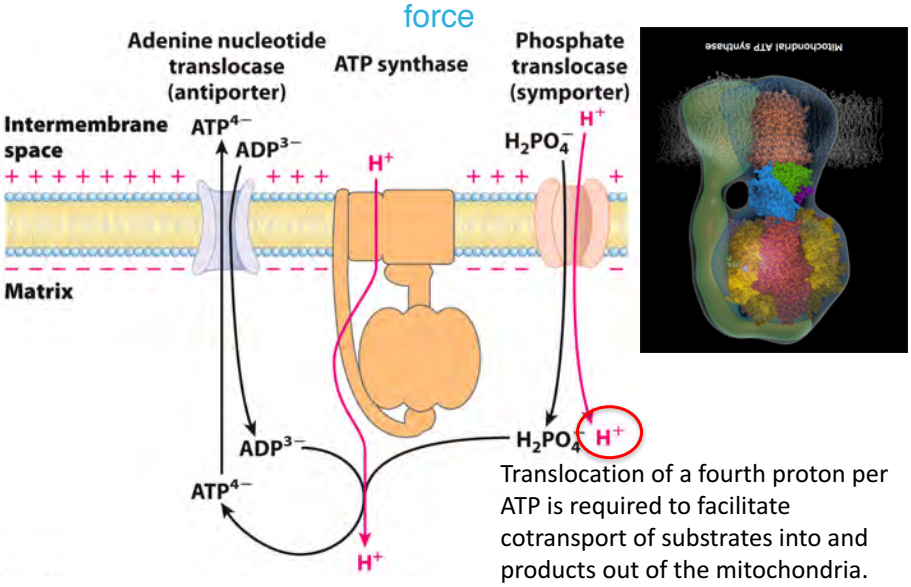


Animation of ATP Synthase

(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

- 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

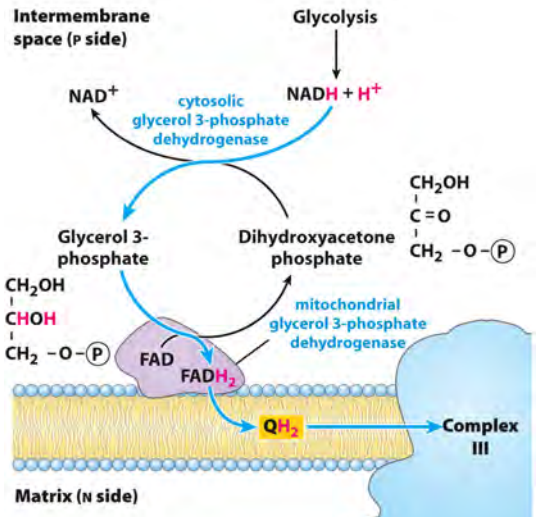
Net Production of ATP by Oxidation of Glucose

- 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.

Oxidative Phosphorylation

Regulation

- Primarily regulated by substrate availability
  - NAD^+ and ADP/P_i
- Inhibition of OxPhos leads to accumulation of NADH.
  - causes feedback inhibition cascade up to PFK-1 in Glycolysis
- 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)