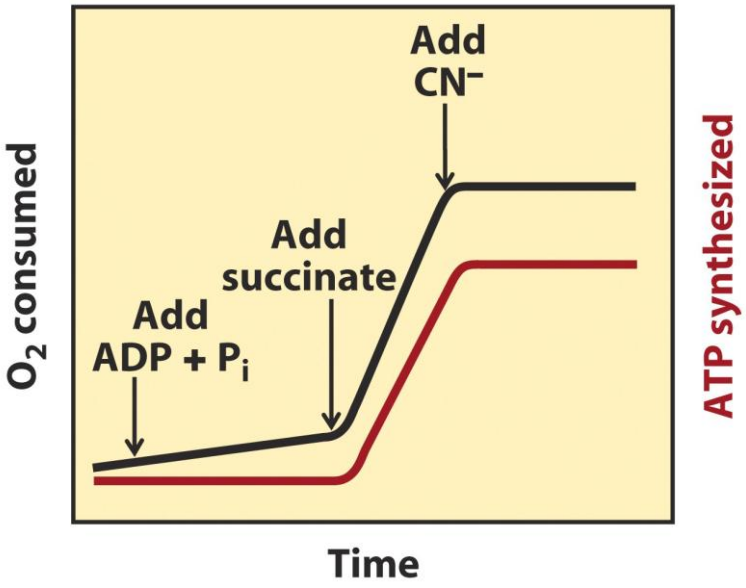
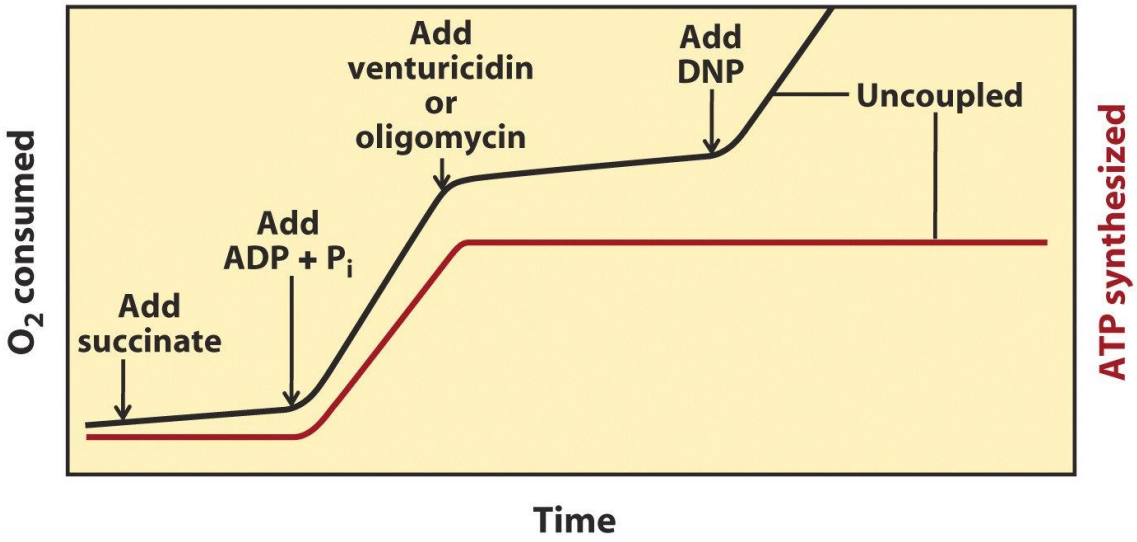
Bryan Krantz: University of California, Berkeley
MCB 102, Spring 2008, Metabolism Lecture 13
Reading: Chs. 19 & 21 of *Principles of Biochemistry*, "Oxidative Phosphorylation & Photophosphorylation." & "Lipid Biosynthesis."

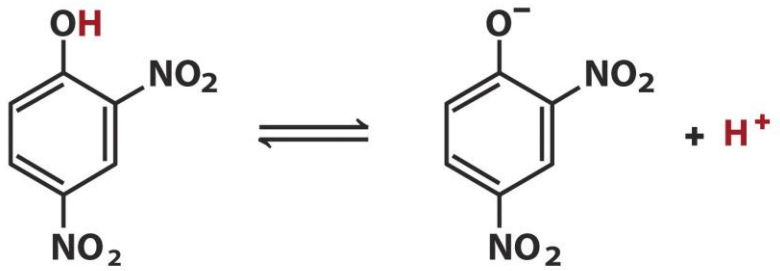
ATP SYNTHESIS VIA THE PMF

Uncouplers.

Let us consider the action of respiratory inhibitors and uncouplers, like DNP:

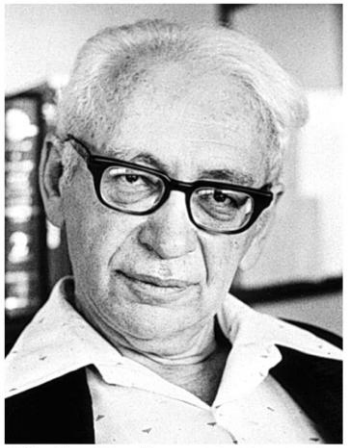






2,4-Dinitrophenol (DNP)

ATP SYNTHESIS AND PMF ARE COUPLED


Efraim Racker, 1913–1991

How? Peter Mitchell did not have the answer for this one. This is the last energy **transduction** step: *i.e.*, the transmembrane electrical/chemical potential (PMF) is converted to store the energy in the high energy phosphate ester linkage of ATP.

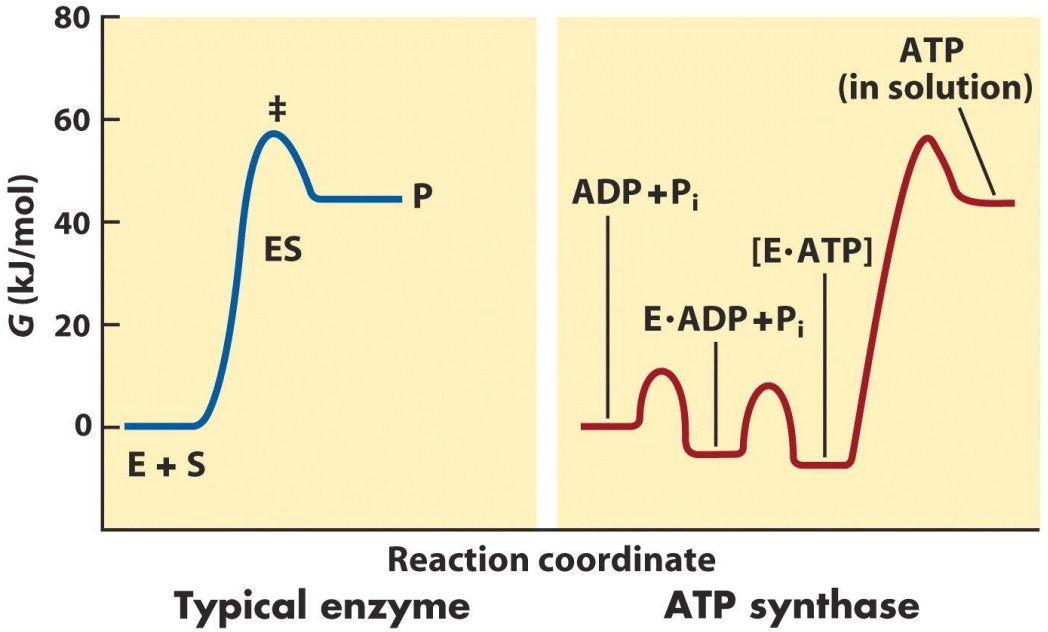
Efraim Racker purified the **F_oF_1 ATP synthase** component of the electron transport chain in the 1960s. Curious results were obtained.

- They could reconstitute the electron transport chain in liposome vesicles.
- When a water-soluble component (**F_1**) was stripped off ATP synthesis was uncoupled.
- From this result they concluded, that **F_o**—the other membrane-embedded component—was a proton translocating pore.
- Adding back **F_1** allowed the **F_o** to be blocked and ATP synthesis could resume.

F_oF_1 ATP synthase stabilizes the formation of ATP from ADP and Pi.

- The enzyme has a strong binding site for ATP.
- The ***K_{eq}***' for ATP formation in the enzyme bound state is ~1.

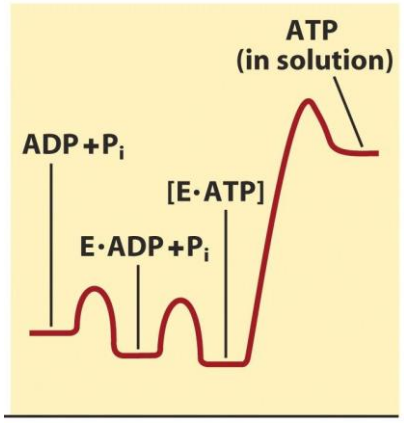
- The **E●ATP complex** is stable, and release of ATP will be the struggle.

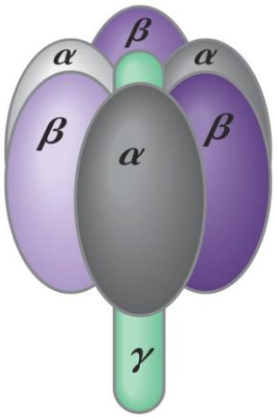


ATP RELEASE REQUIRES PMF

The energy diagram does not anticipate a means to get the ATP out of the **E●ATP** complex.

This is an uphill battle.





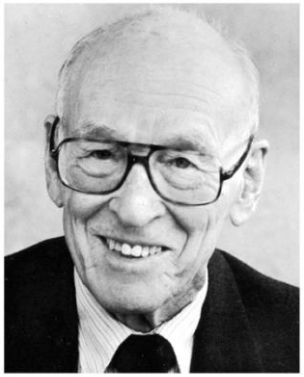
ATP synthase

Rotary Model for ATP Synthesis

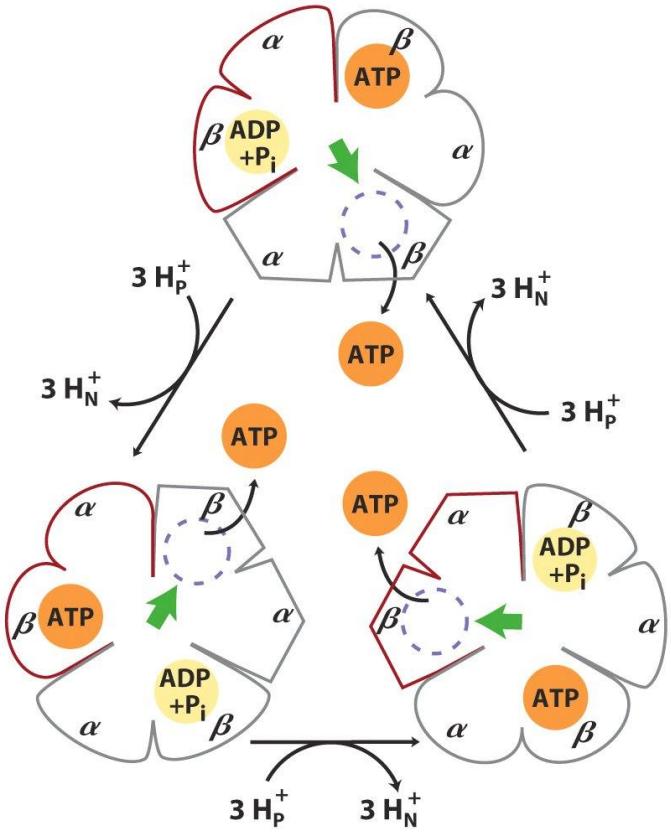
The **binding change mechanism** of energy coupling was proposed by **Paul Boyer**, who shared the Nobel Prize.

The model accounts for the existence of 3 catalytic sites in F1 with different affinities for ATP, ADP and Pi.

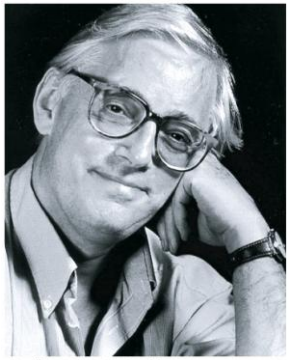
The F1Fo is really a rotary machine, driven into motion by the flow of protons down their PMF gradient.



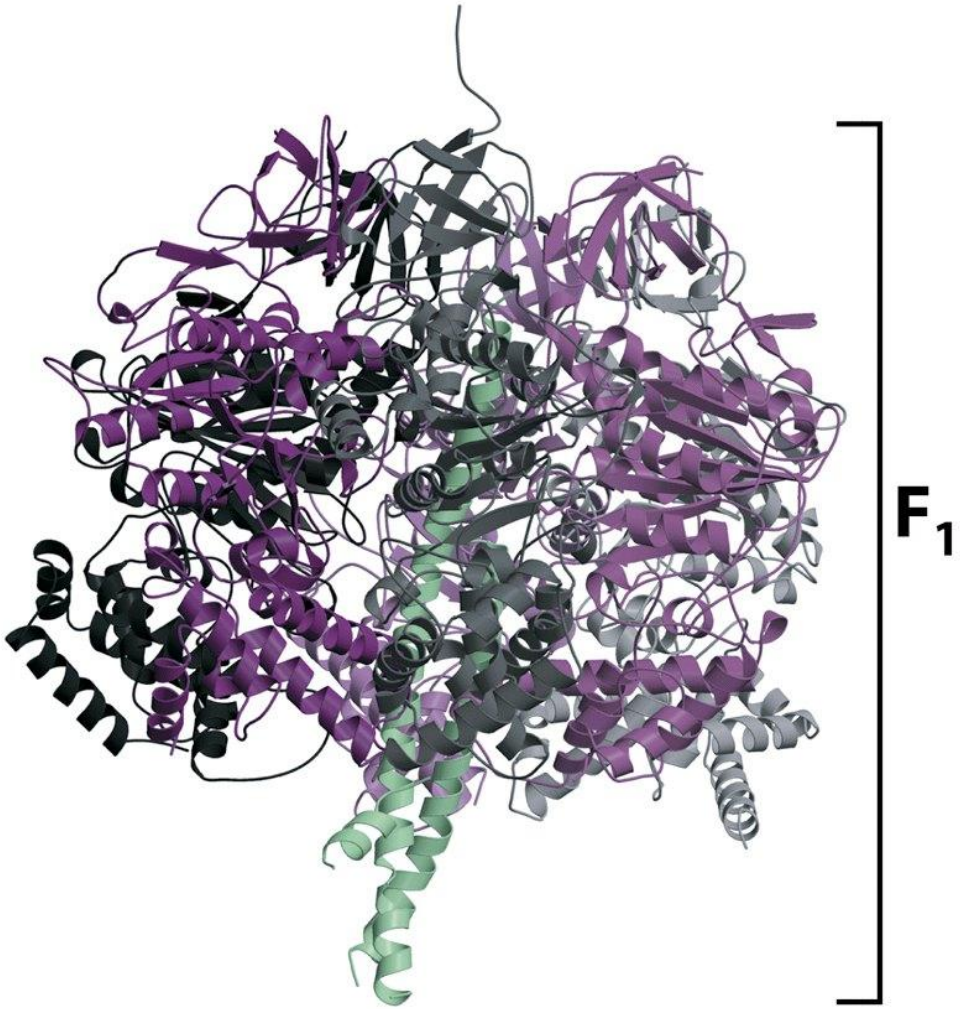
Paul Boyer

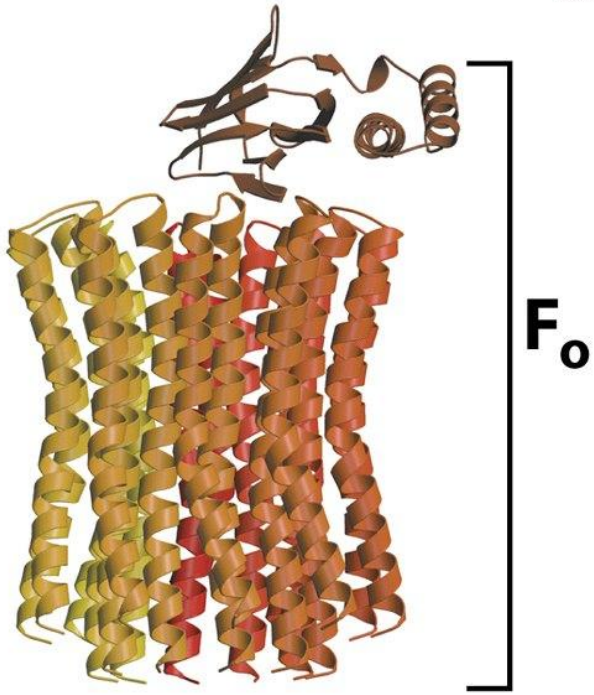


Crystal Structure F_oF_1 ATP Synthase.

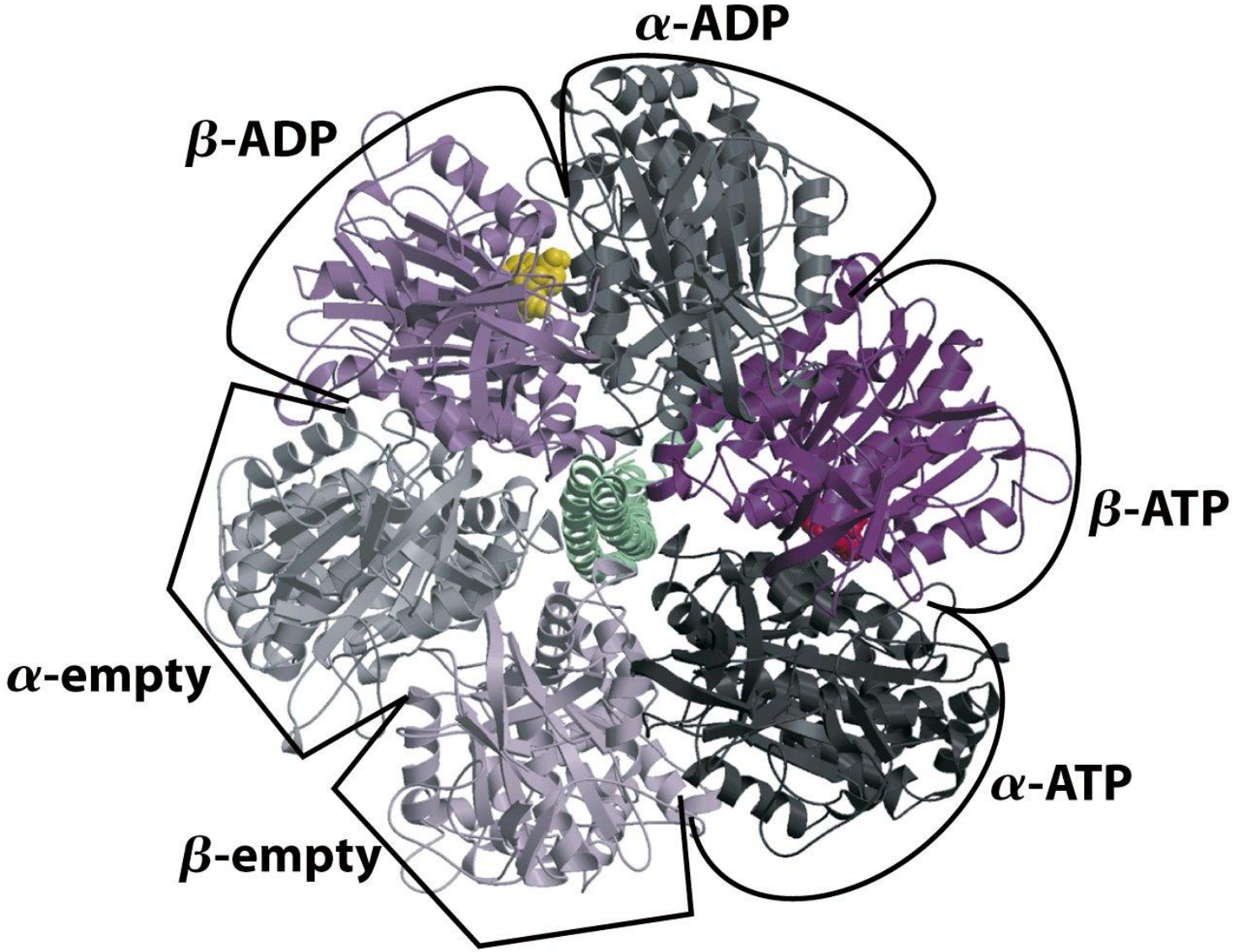

John E. Walker

John Walker determined the three-dimensional structure of F_oF_1 ATP Synthase, sharing the Nobel with Boyer.

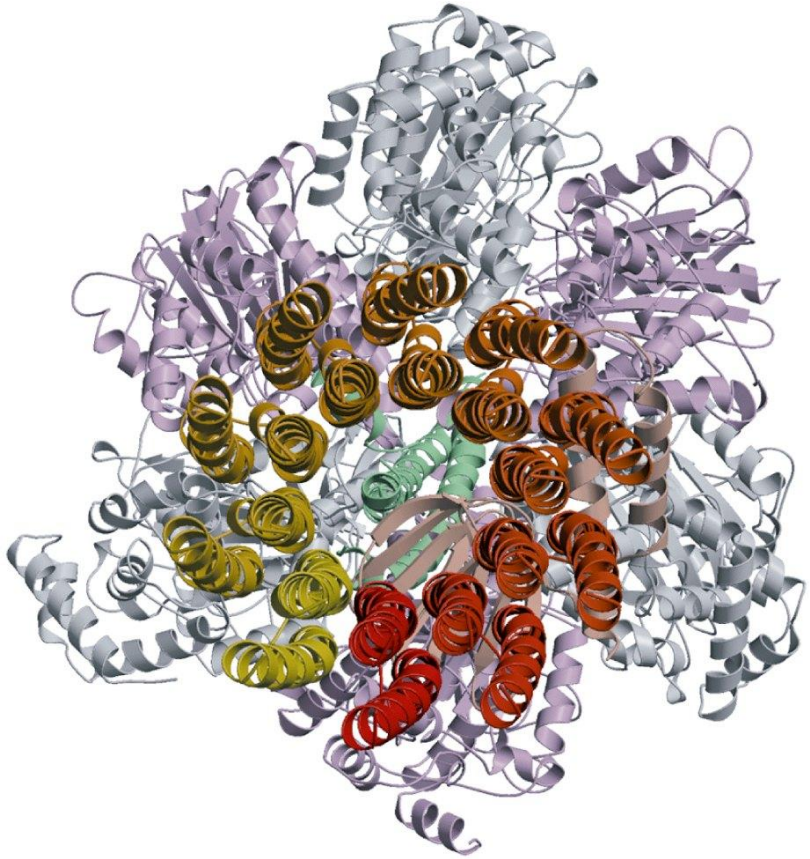


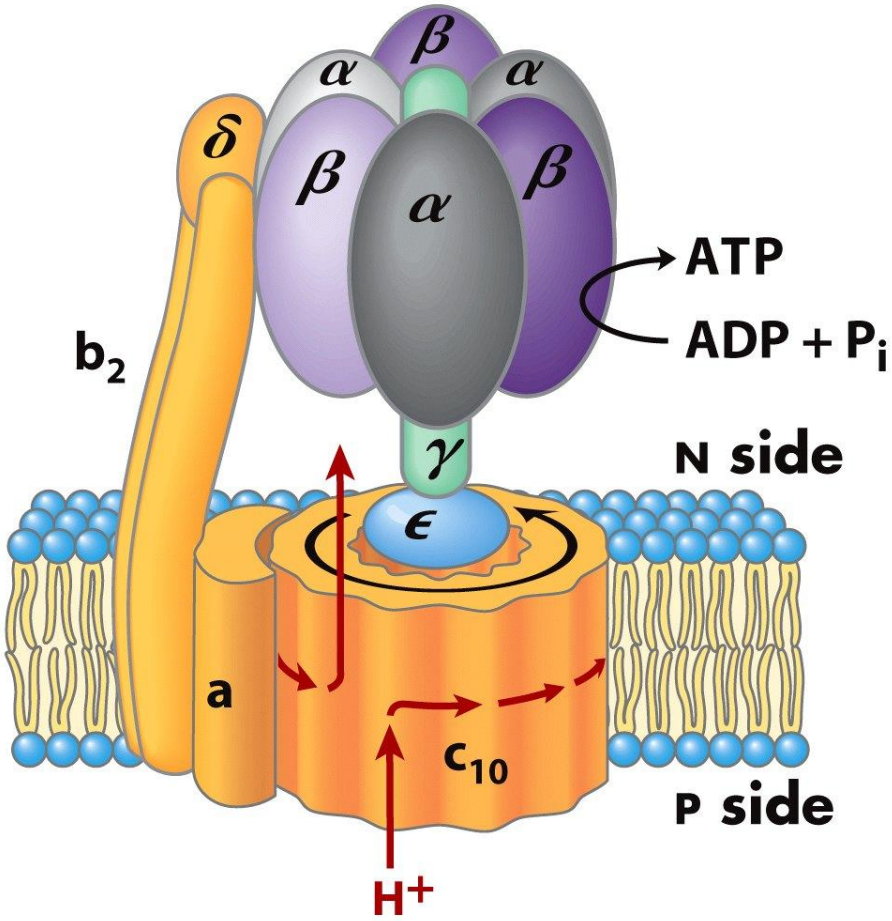


Structure of the 3 Binding Sites

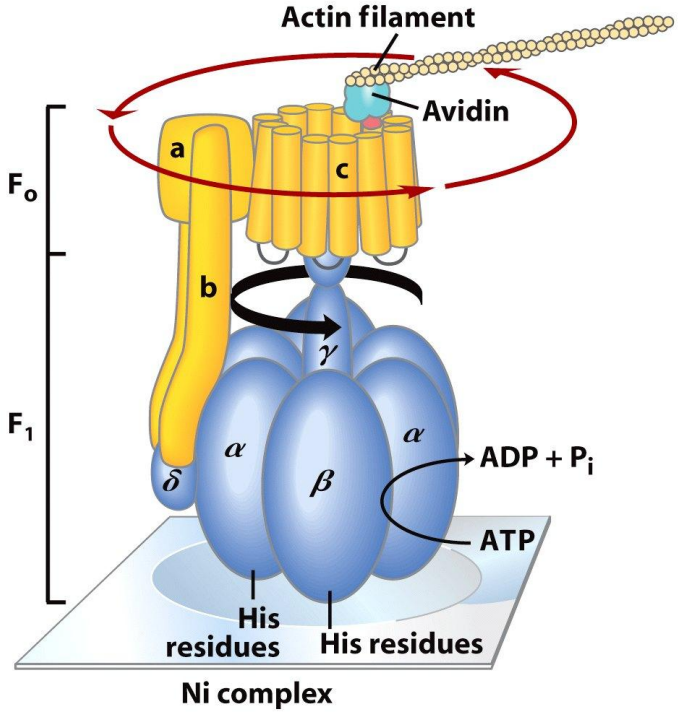


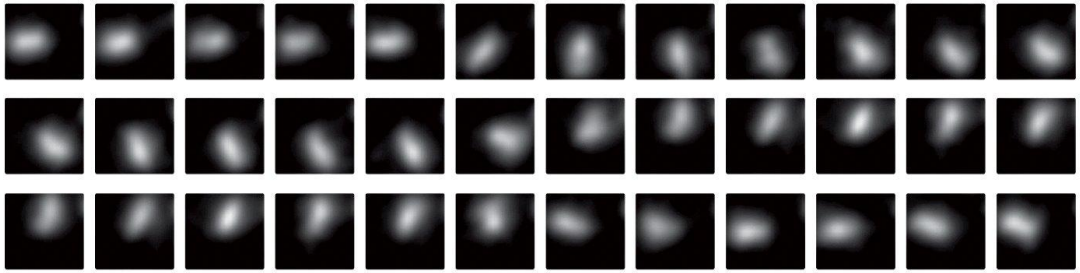
How the machine works.



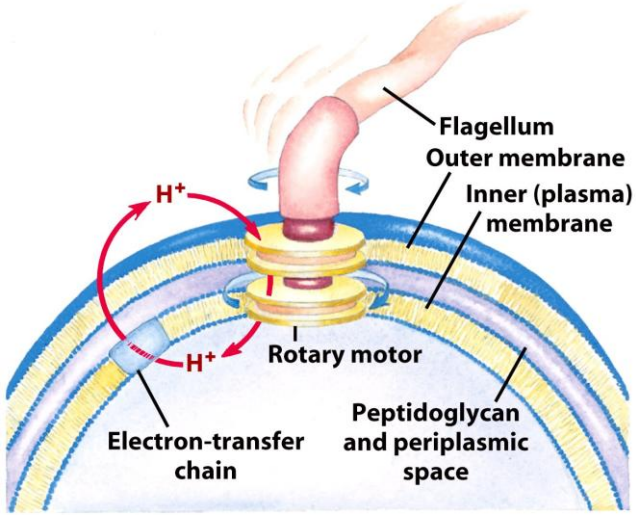


Experiment shows rotation could drive synthesis.

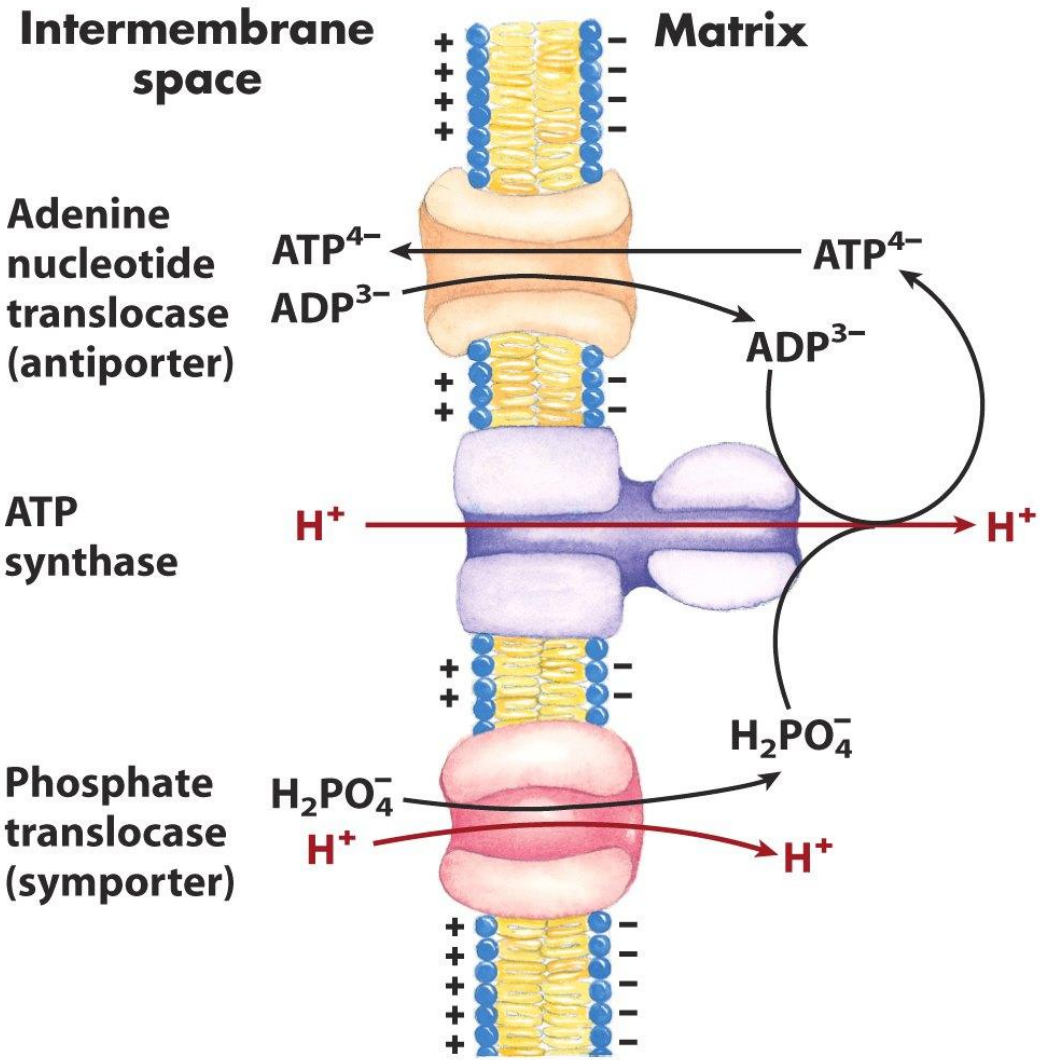




- Parallel example of the rotary mechanism occurs in the bacterial flagellum.



Final Bookkeeping on the H_P^+ → ATP conversion



FATTY ACID & CHOLESTEROL BIOSYNTHESIS

Fatty Acid Biosynthesis.

- We make fatty acids in our own bodies.
- If you avoid fatty foods in your diet, you still generate fat.
- Some fatty acids are essential in our diet.
- Fatty acid biosynthesis occurs mainly in the liver.

Compare and Contrast Fatty Acid Synthesis to β Oxidation

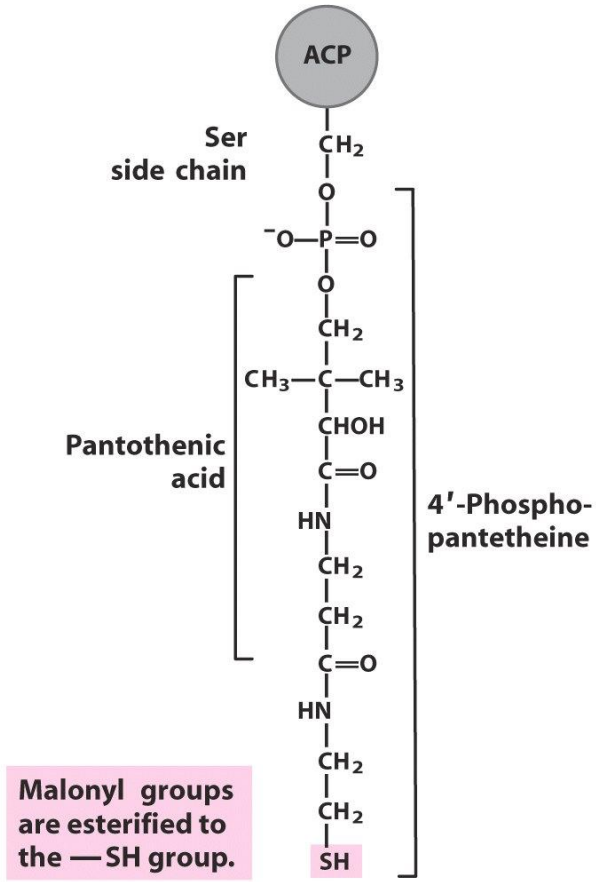
- The intermediates have a lot in common: like oxidation in reverse.
- While fatty acid degradation occurs in the mitochondrion, biosynthesis occurs in the cytosol.
- The second big difference is that the carrier of these fatty acyl chains was CoA in the beta-oxidation pathway. The carrier is a protein called **ACP (acyl carrier protein)** in the synthesis pathway.
- Synthesis requires an activated starting molecule, i.e., Malonyl-CoA, to join these discrete pieces together. Breakdown used only Acetyl-CoA.
- Another big difference between biosynthesis and degradation is that in biosynthesis, we use NADPH and not NADH.

ACP (acyl carrier protein)

- Analog of CoA.
- Uses the same essential vitamin (B5), **pantothenic acid**.
- Has the free thiol (-SH) group on the end.
- The other end is attached via a phosphate to a Ser residue in Acyl Carrier Protein.

Flexible prosthetic group can build the fatty chain, thus aiding in transfer reactions.

The growing fatty chain substrate can interact with many of the enzymes surrounding ACP.



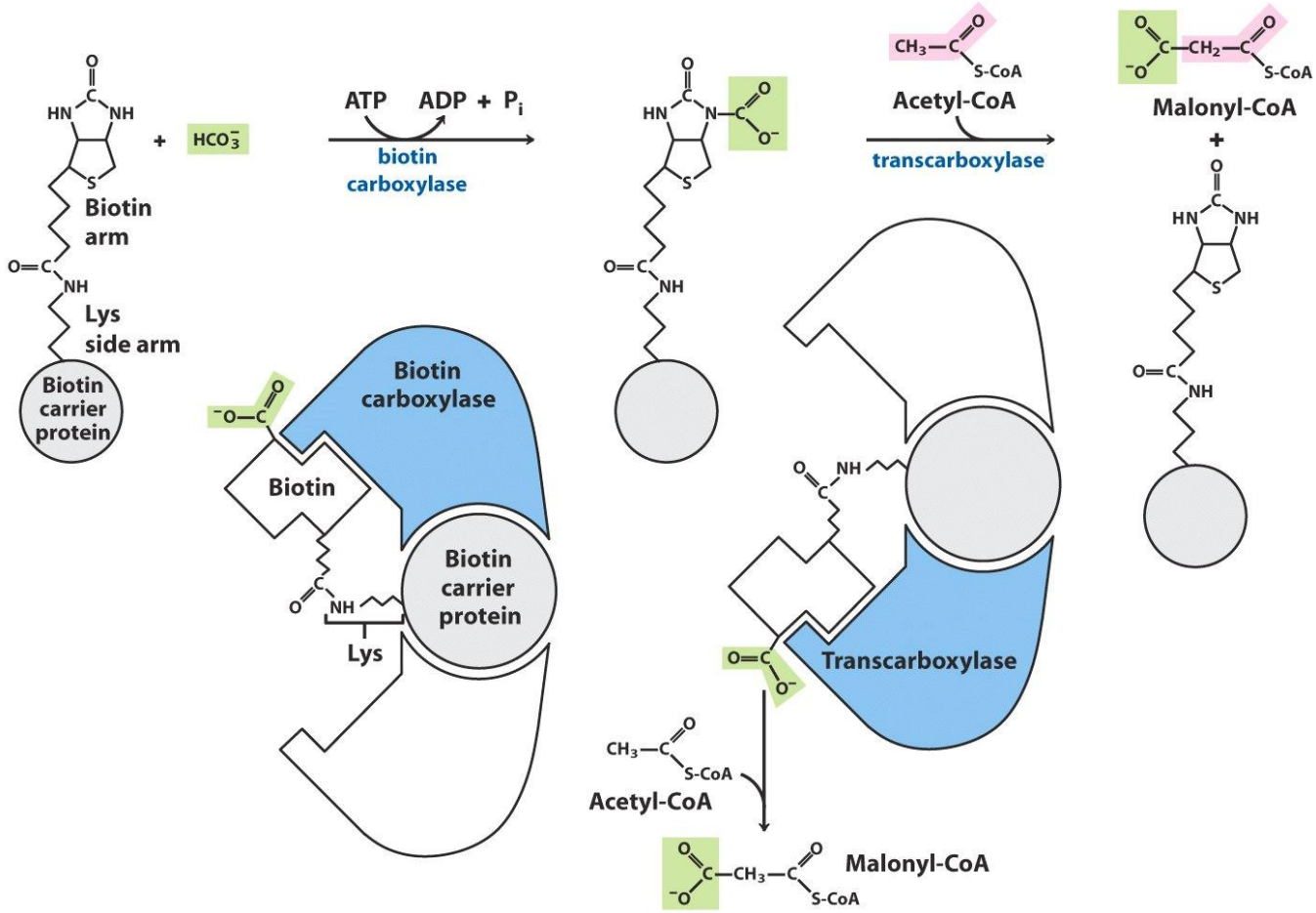
Biotin Carboxylase

You cannot simply reverse the thiolase reaction; it is strongly downhill.

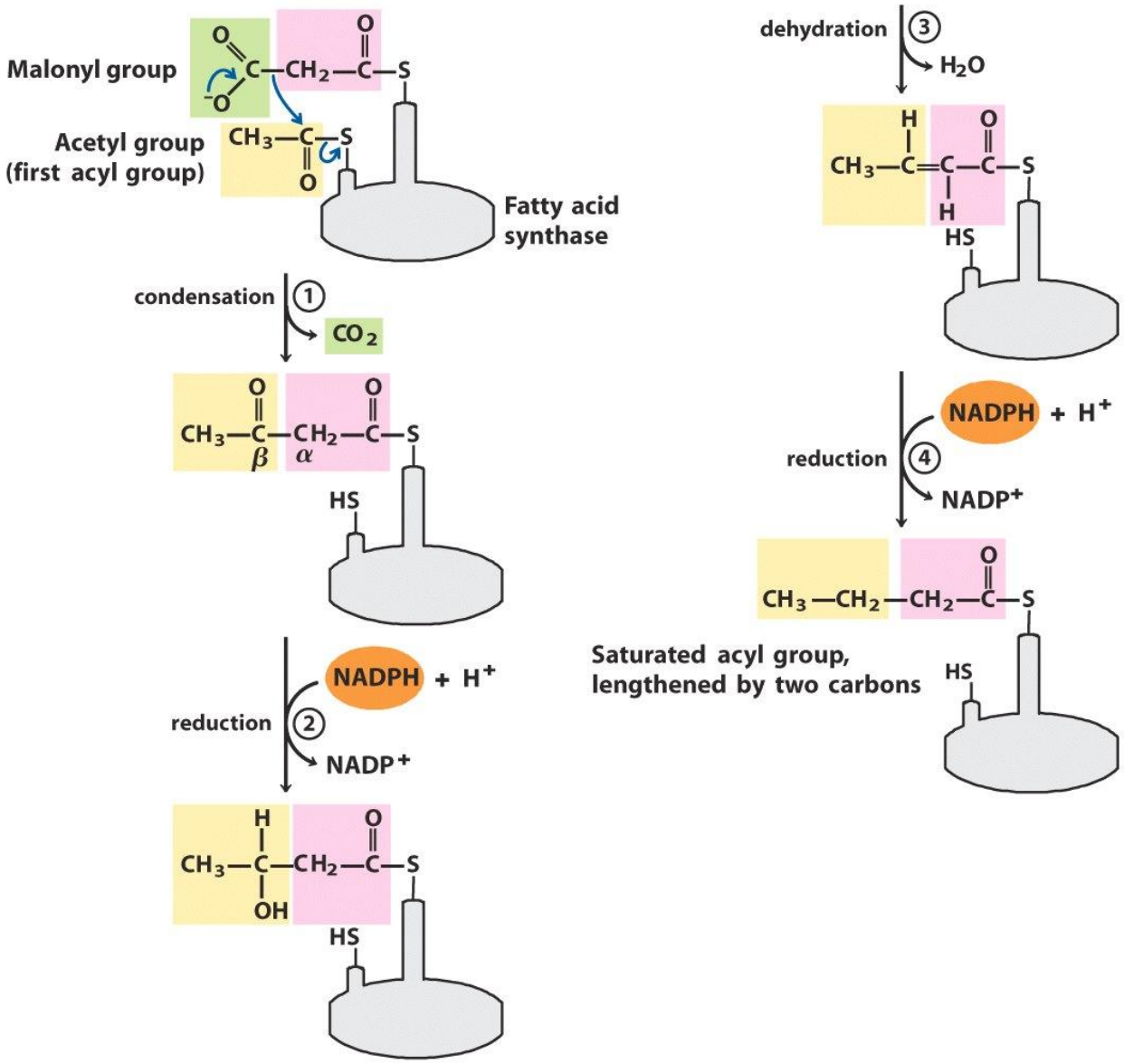
Acetyl-CoA + ATP + HCO_3^- → Malonyl-CoA + ADP

Mechanism. Biotin is required (like pyruvate carboxylase). Biotin is always used when you want to add a carboxyl (O=C=O$^-$) group onto something.

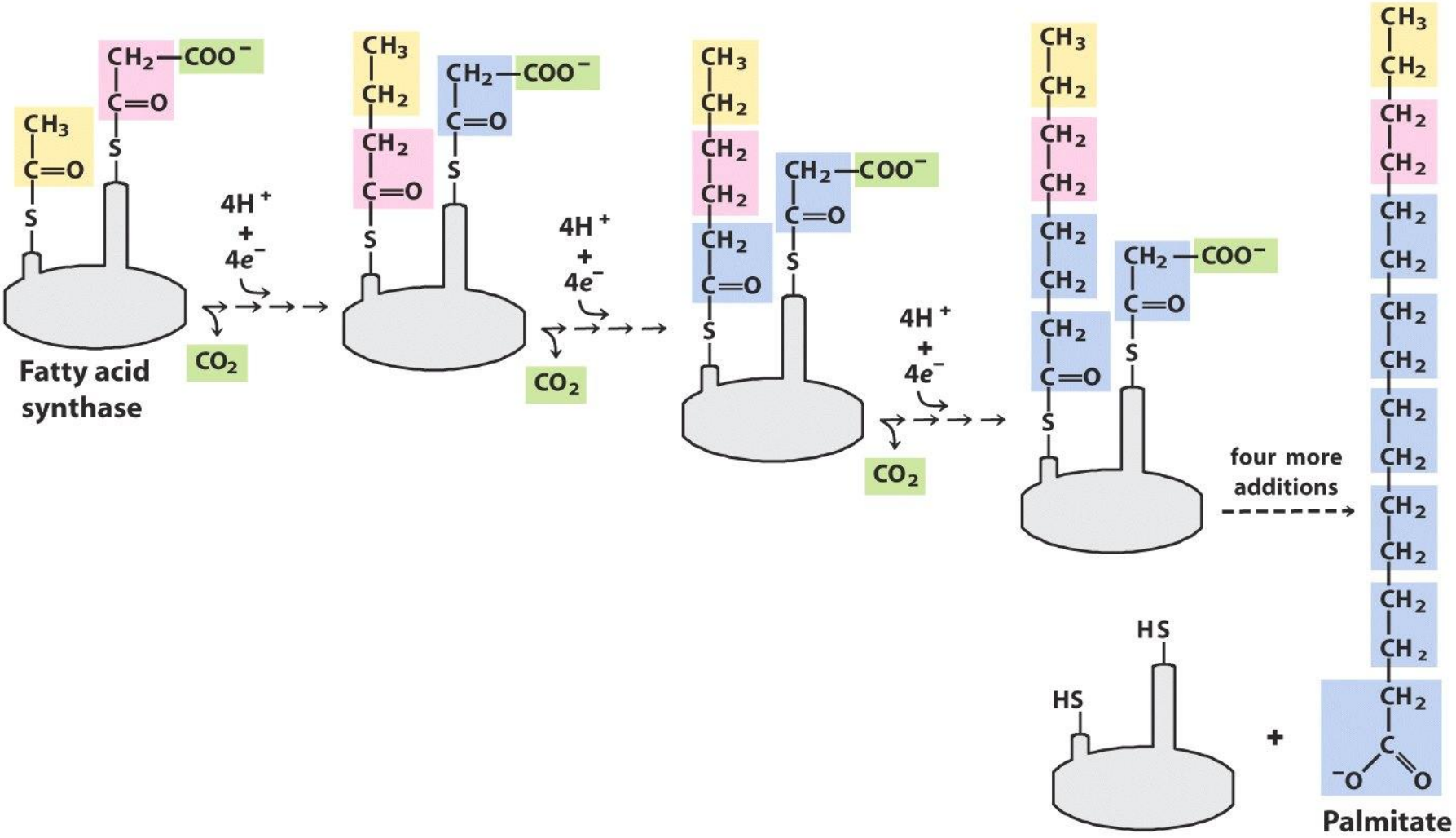
Energetics. Bicarbonate ion was activated by ATP.



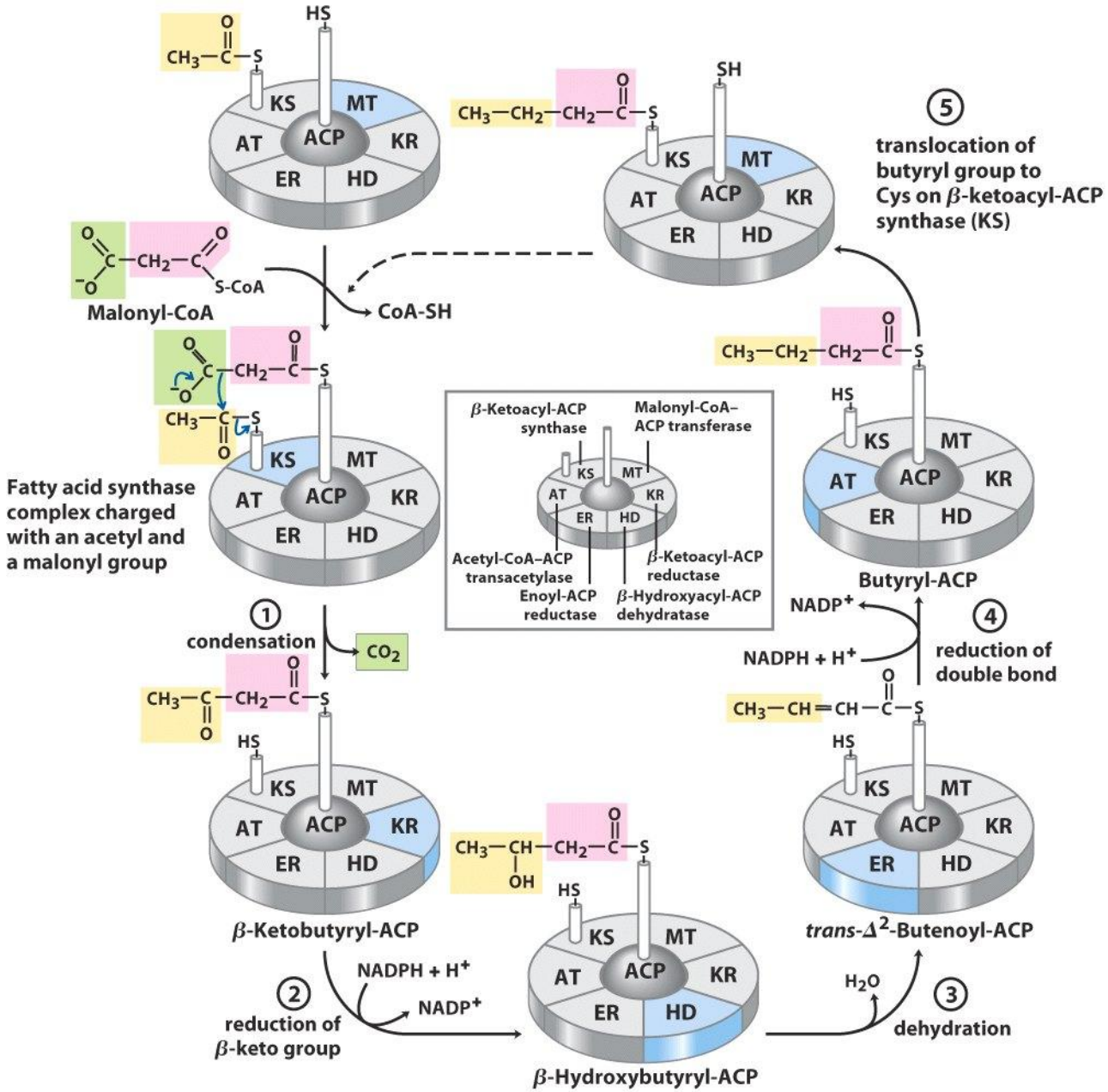
Fatty acid synthesis steps



Chain elongation



Fatty Acid Synthase Enzyme

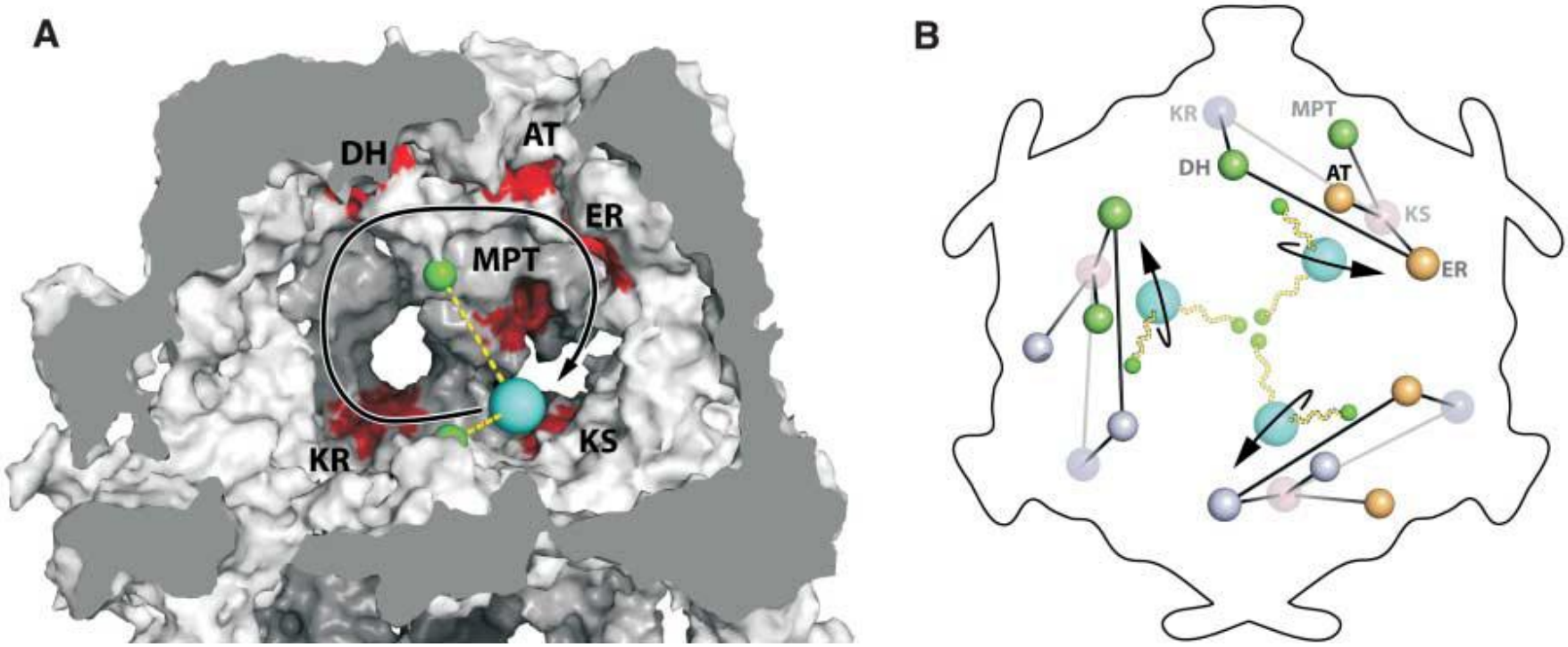


Fatty Acid Synthase Structure.

The First Cycle. Acetyl transferase (AT) called acetyl-CoA transacetylase puts acetyl-CoA on KS. Then Malonyl-CoA transferase (MT); Ketoacyl-ACP synthase (KS); Ketoacyl reductase (KR); Dehydratase (HD); Enoyl reductase (ER).

Each Cycle Thereafter. AT then transfers the growing acyl group from the top of the ACP onto the sulfhydryl site onto KS; Malonyl-CoA transferase (MT); Ketoacyl-ACP synthase (KS); Ketoacyl reductase (KR); Dehydratase (HD); Enoyl reductase (ER).

Structure follows the reaction mechanism in clock-wise circle!



Energy Requirements.

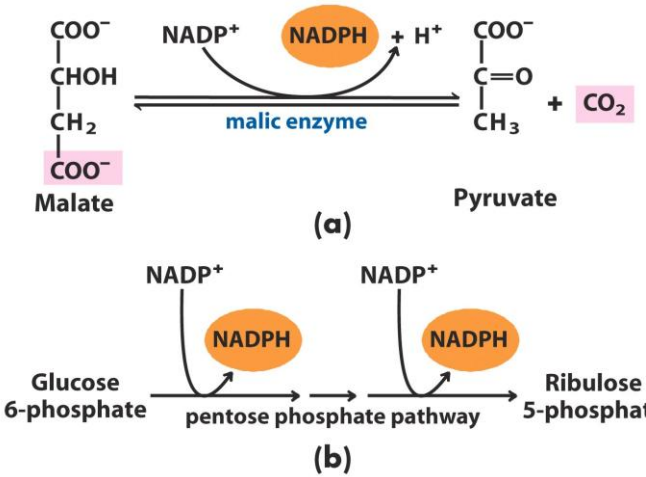
For C_{16} palmitic acid starting with Acetyl-CoA and a generous pool of NADPH:

7 ATP to charge Acetyl-CoA → Malonyl-CoA

14 NADPH molecules for the reductions of the C=C double bonds and C=O ketone.

A lot of energy iss needed to make a fatty acid.

You need NADPH. *Why? How?*



When Acetyl-CoA is Limiting. It is even more expensive because in order to make malonyl-CoA, you have to start from acetyl-CoA. Acetyl-CoA comes from pyruvate dehydrogenase and fatty acid oxidation. Pyruvate dehydrogenase is used to prevent futile cycling (of course).

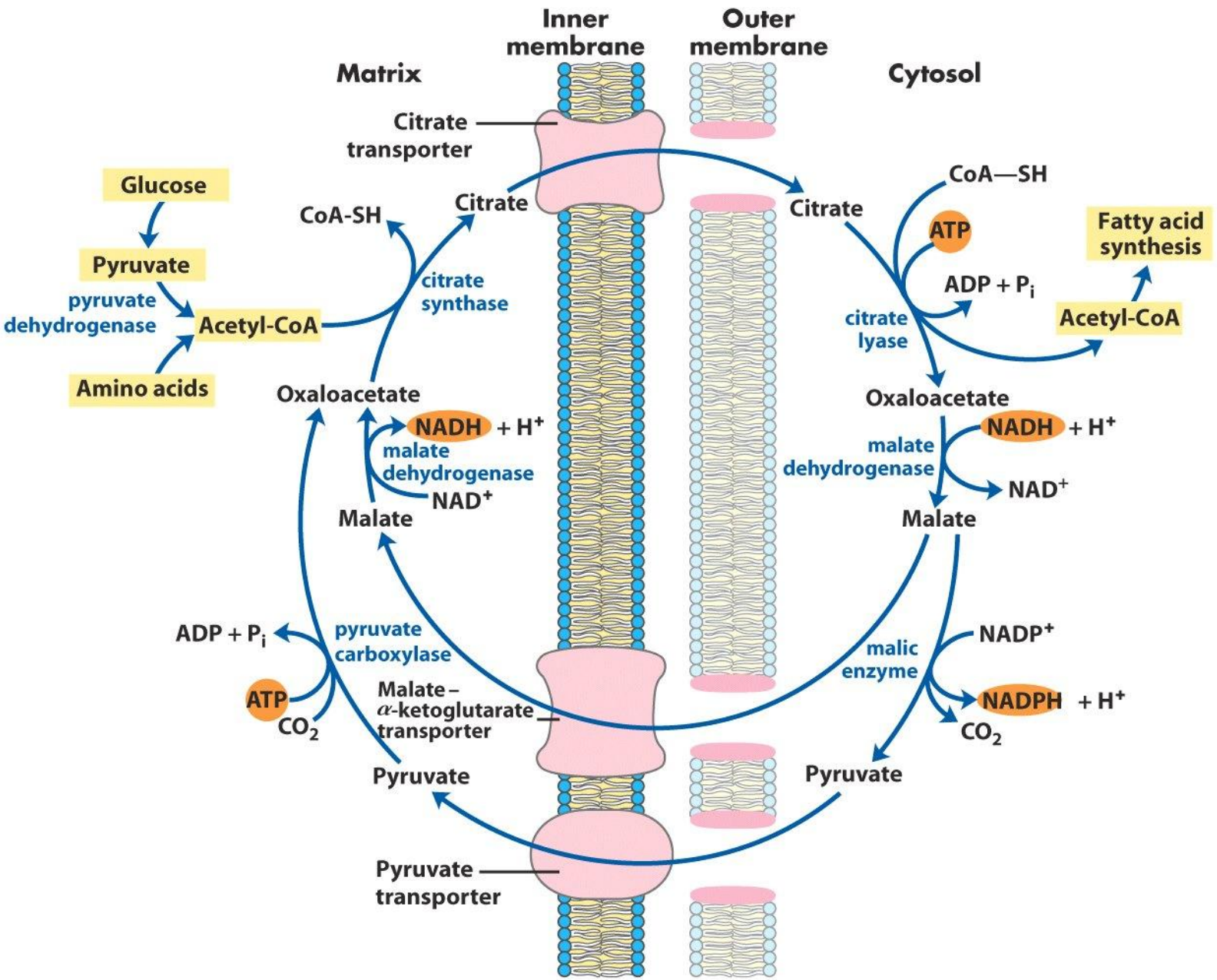
Pyruvate dehydrogenase is mainly in the mitochondrion.

Acetyl-CoA is made in the mitochondrion.

Citrate must be pumped out of mitochondria and cleaved using 1 ATP.

***So starting from citrate the process is more expensive by 1 ATP / Acetyl-CoA.

So there are a lot of transport steps summarized in the following figure.


Inner membrane
Outer membrane
Matrix
Cytosol
Citrate transporter
Glucose
Pyruvate
pyruvate dehydrogenase
Acetyl-CoA
Amino acids
CoA-SH
Citrate
citrate synthase
Oxaloacetate
NADH + H+
malate dehydrogenase
NAD+
Malate
ADP + Pi
pyruvate carboxylase
ATP
CO2
Malate – α-ketoglutarate transporter
Pyruvate
Pyruvate transporter
Citrate
CoA—SH
ATP
ADP + Pi
citrate lyase
Fatty acid synthesis
Acetyl-CoA
Oxaloacetate
NADH + H+
malate dehydrogenase
NAD+
Malate
NADP+
malic enzyme
NADPH + H+
CO2
Pyruvate