

LECTURE 13-FATTY ACID BIOSYNTHESIS

Friday, May 09, 2008
10:46 AM

Metabolism Lecture 13 — FATTY ACID & CHOLESTEROL BIOSYNTHESIS — Restricted for students enrolled in MCB102, UC Berkeley, Spring 2008 ONLY

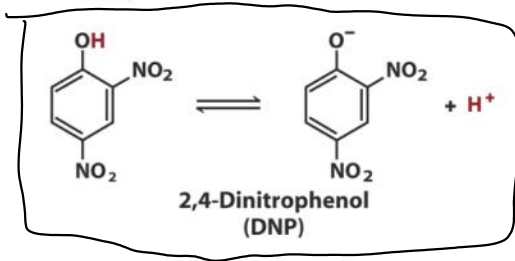
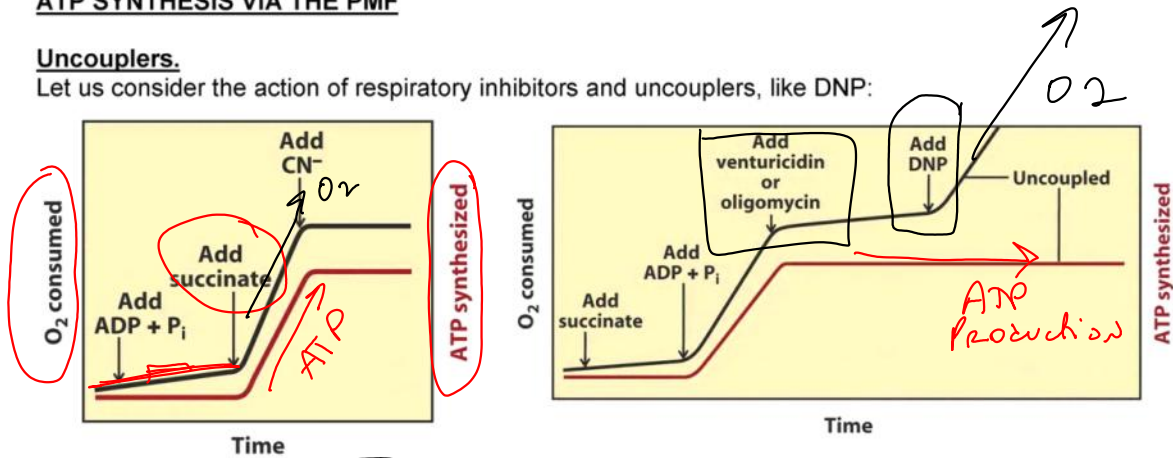
Bryan Krantz: University of California, Berkeley
MCB 102, Spring 2008, Metabolism Lecture 13

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:



Skunk cabbage

ATP SYNTHESIS AND PMF ARE COUPLED

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.



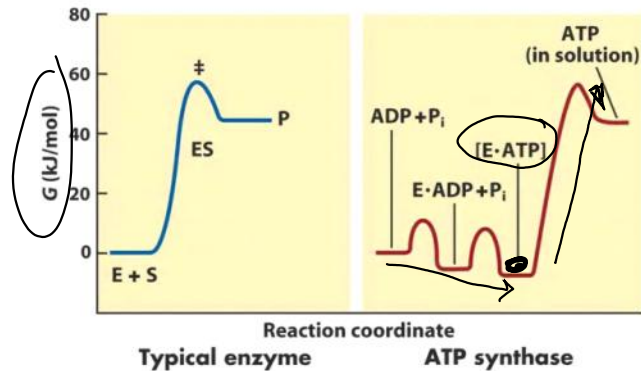
Efraim Racker, 1913-1991

Efraim Racker purified the **F₀F₁ 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 (F₁) was stripped off ATP synthesis was uncoupled.
- From this result they concluded, that F₀—the other membrane-embedded component—was a proton translocating pore.
- Adding back F₁ allowed the F₀ to be blocked and ATP synthesis could resume.

F₀F₁ ATP synthase stabilizes the formation of ATP from ADP and P_i.

- 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.

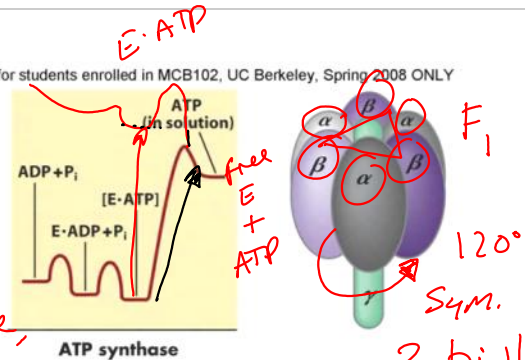


ATP RELEASE REQUIRES PMF

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

This is an uphill battle.

Proton Gradient (PMF) favors ATP release,



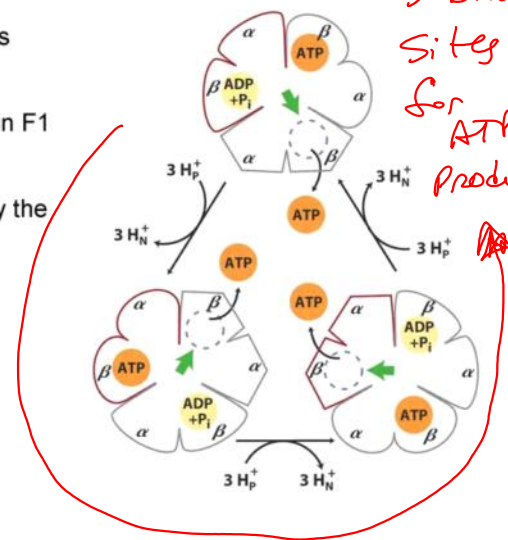
Rotary Model for ATP Synthesis

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.

3 binding sites for ATP production



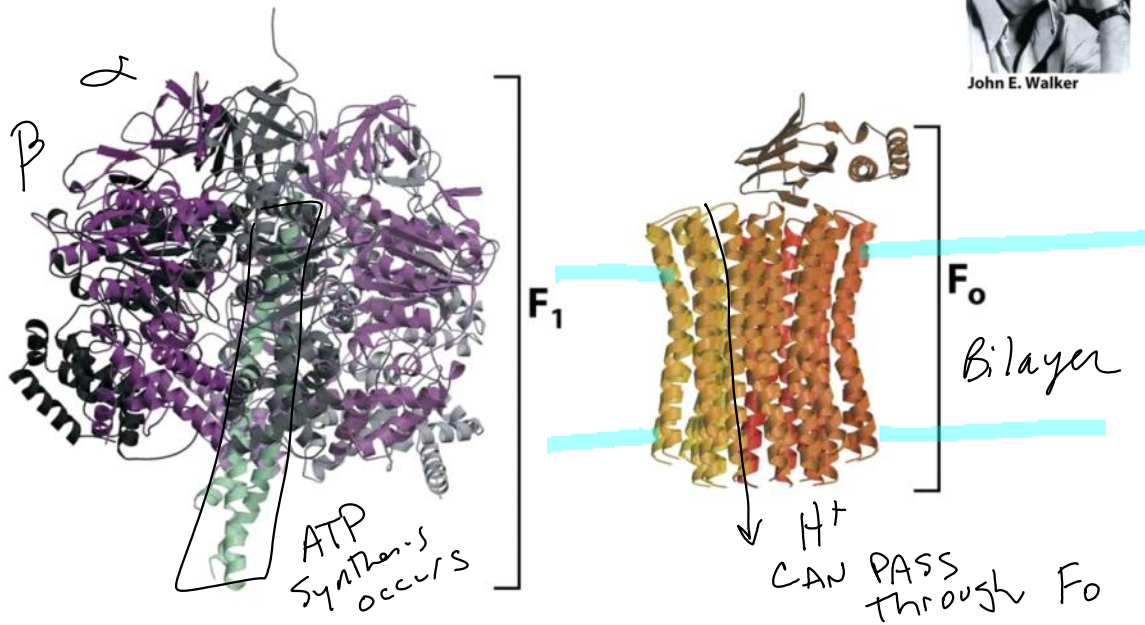
Paul Boyer

Crystal Structure F_0F_1 ATP Synthase.

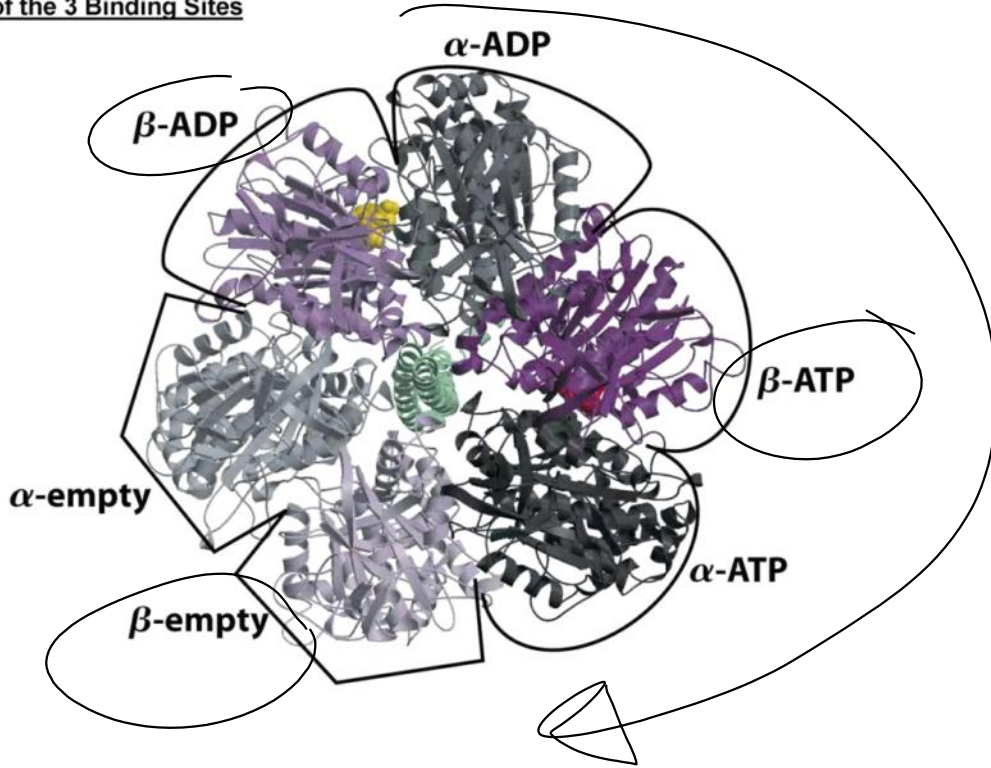
John Walker determined the three-dimensional structure of F_0F_1 ATP Synthase, sharing the Nobel with Boyer.



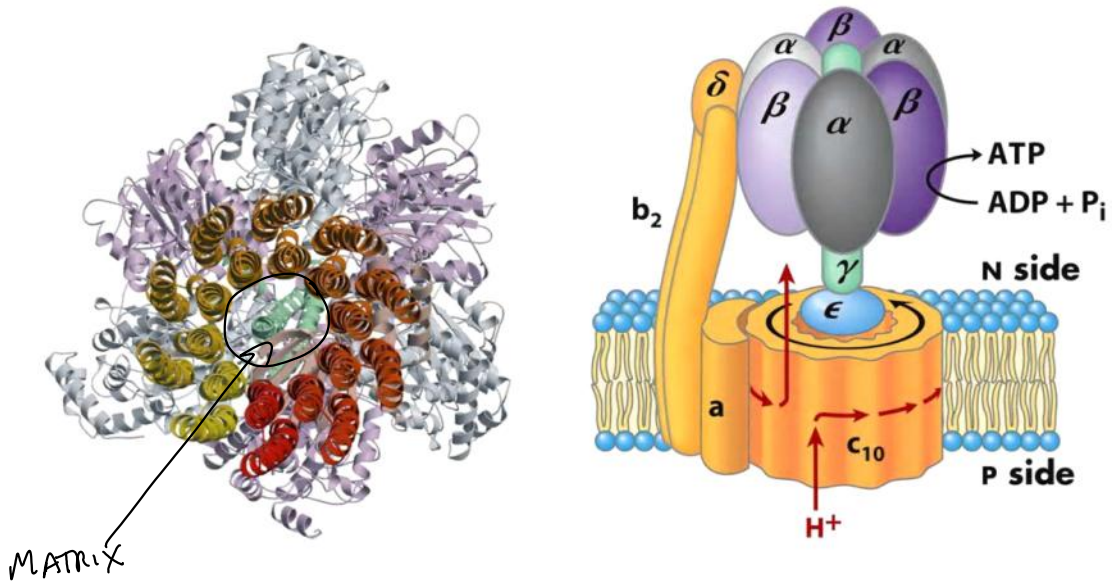
John E. Walker



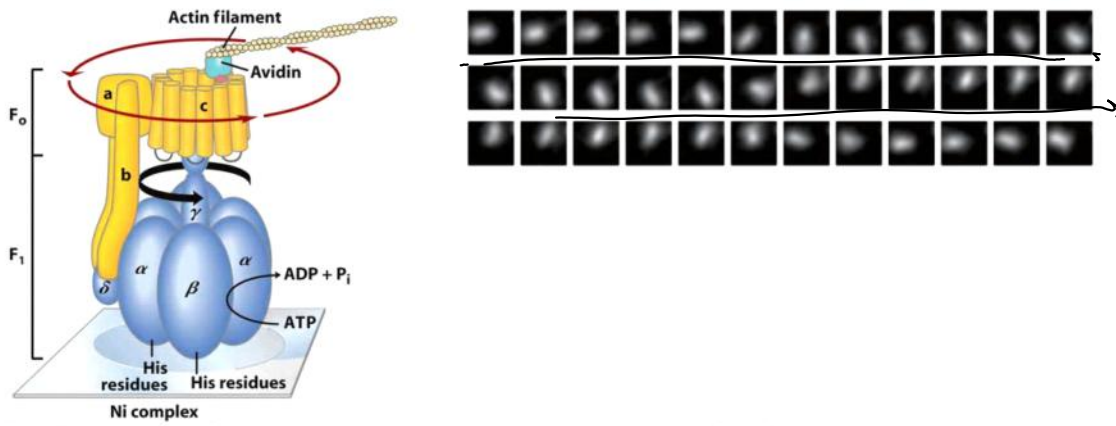
Structure of the 3 Binding Sites



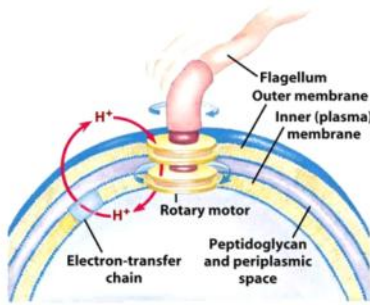
How the machine works.



Experiment shows rotation could drive synthesis.



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



Final Bookkeeping on the $H_p^+ \rightarrow ATP$ conversion

NADH

Complex I	4 H^+
Complex III	4 H^+
Complex IV	2 H^+

10 H^+

3 H^+ for ATP Synthase
 1 H^+ for P_i

4 H^+ / ATP

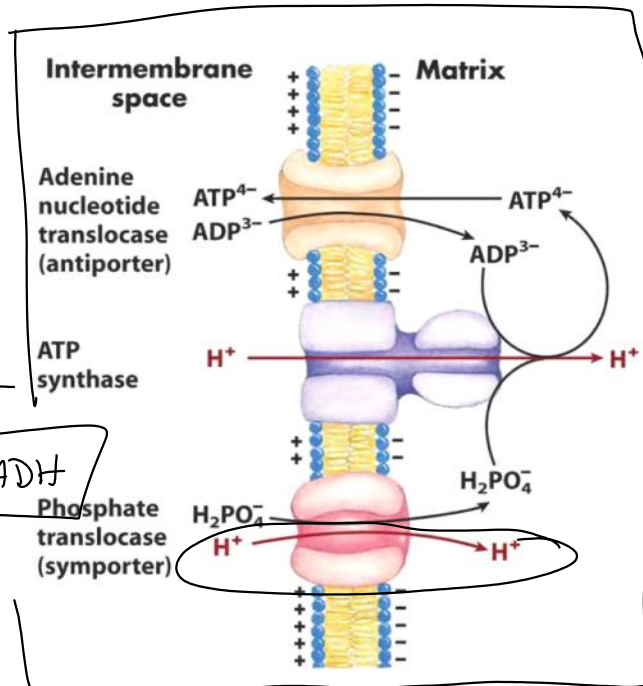
$$10/4 = 2.5 \text{ ATP/NADH}$$

~~FADH₂~~

Complex II	0
Complex III	4
Complex IV	2

6

$$6/4 = 1.5 \text{ ATP/FADH}_2$$



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.

Synthesis → fatty acids
Acetyl-CoA ↻
β-oxidation ↻

Compare and Contrast Fatty Acid Synthesis to β Oxidation

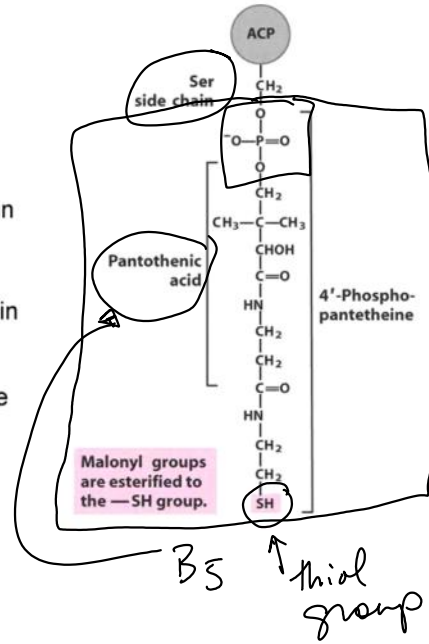
- The intermediates have a lot in common: like oxidation in reverse.
- While fatty acid degradation occurs in the mitochondrion, biosynthesis occurs in the cytosol.
- The second big difference is that the carrier of these fatty acyl chains was CoA in the beta-oxidation pathway. The carrier is a protein 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

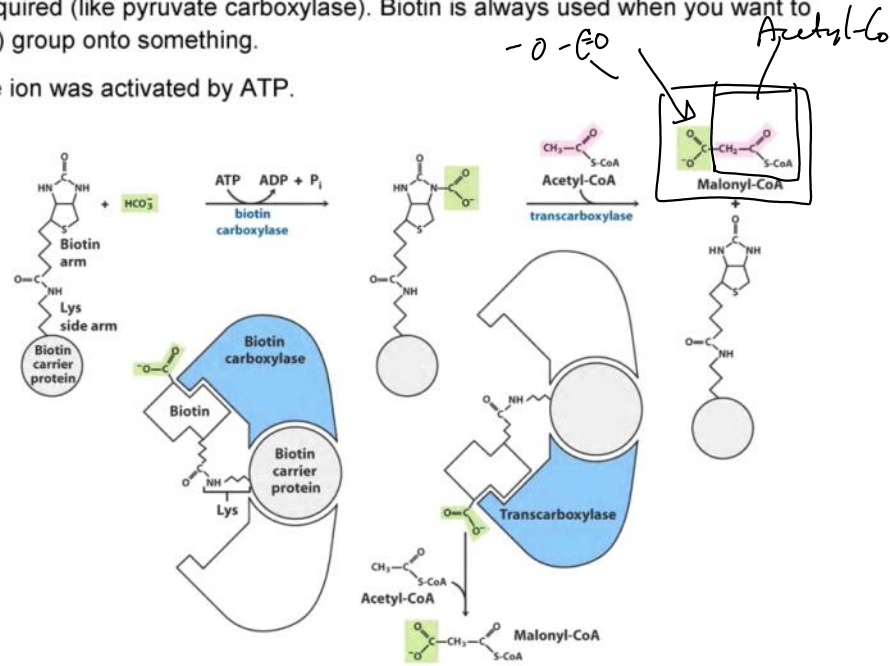
You cannot simply reverse the thiolase reaction; it is strongly downhill.



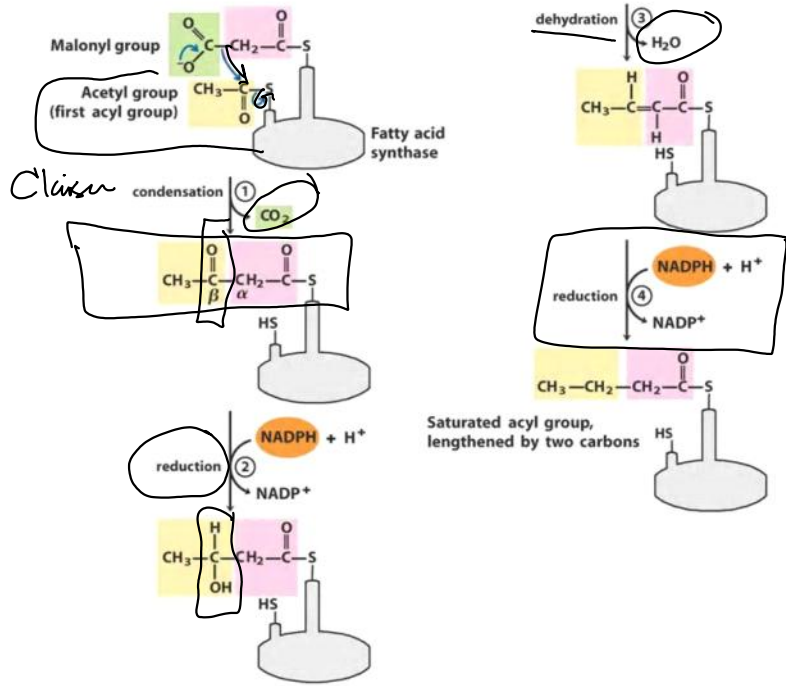
Mechanism. Biotin is required (like pyruvate carboxylase). Biotin is always used when you want to add a carboxyl (O=C=O) group onto something.

Energetics. Bicarbonate ion was activated by ATP.

*Pyruvate
carboxylase*



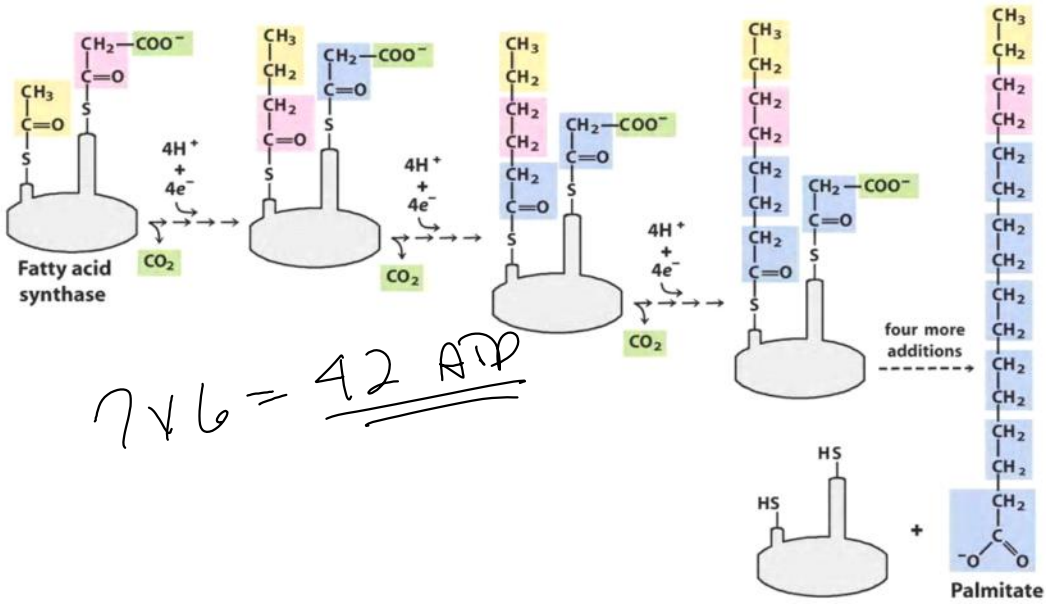
Fatty acid synthesis steps



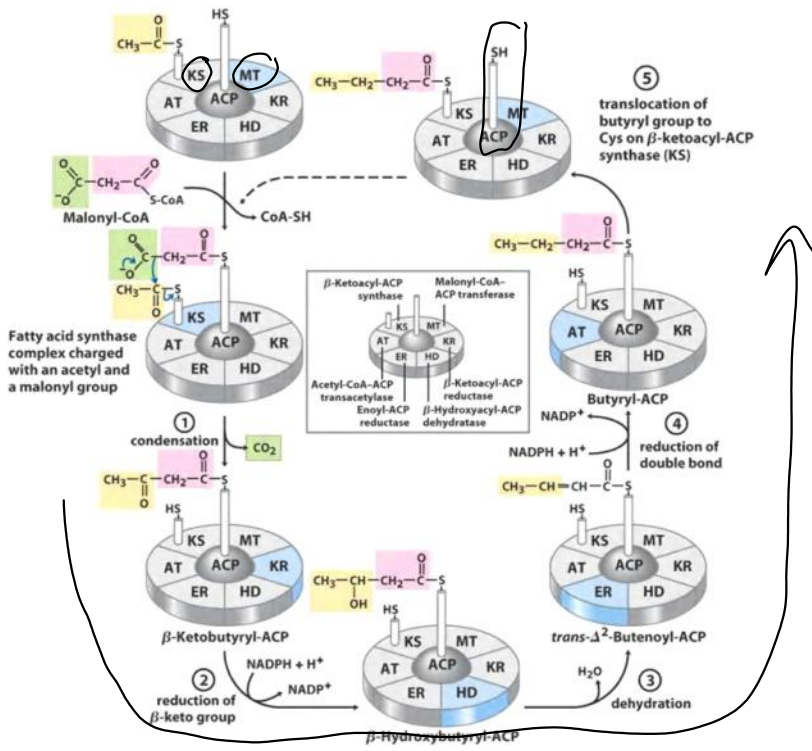
1 ATP
Acetyl-CoA
Malonyl-CoA
2 NADPH

6 ATP per cycle

Chain elongation



Fatty Acid Synthase Enzyme

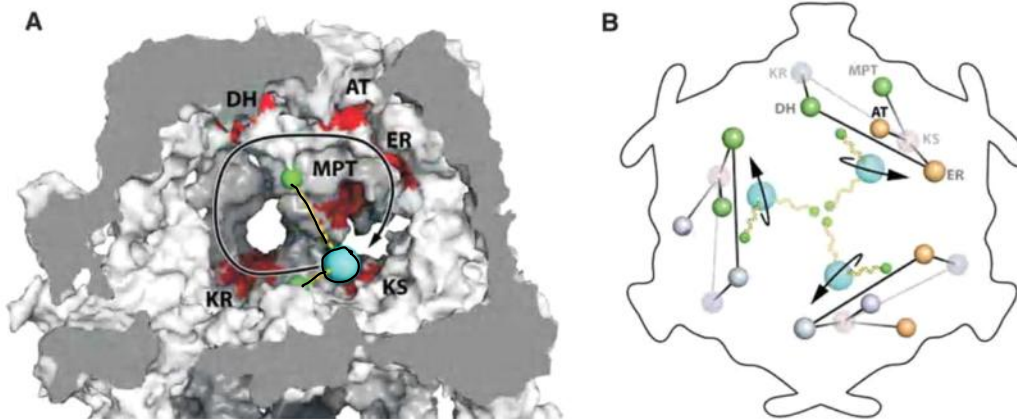


Fatty Acid Synthase Structure.

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.

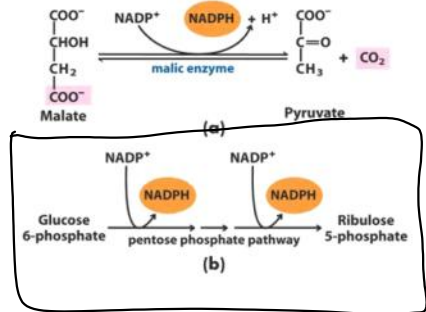
For C_{16} palmitic acid starting with Acetyl-CoA and a generous pool of NADPH:

7 ATP to charge Acetyl-CoA → Malonyl-CoA

14 NADPH molecules for the reductions of the C=C double bonds and C=O ketone. → -04

A lot of energy is 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).

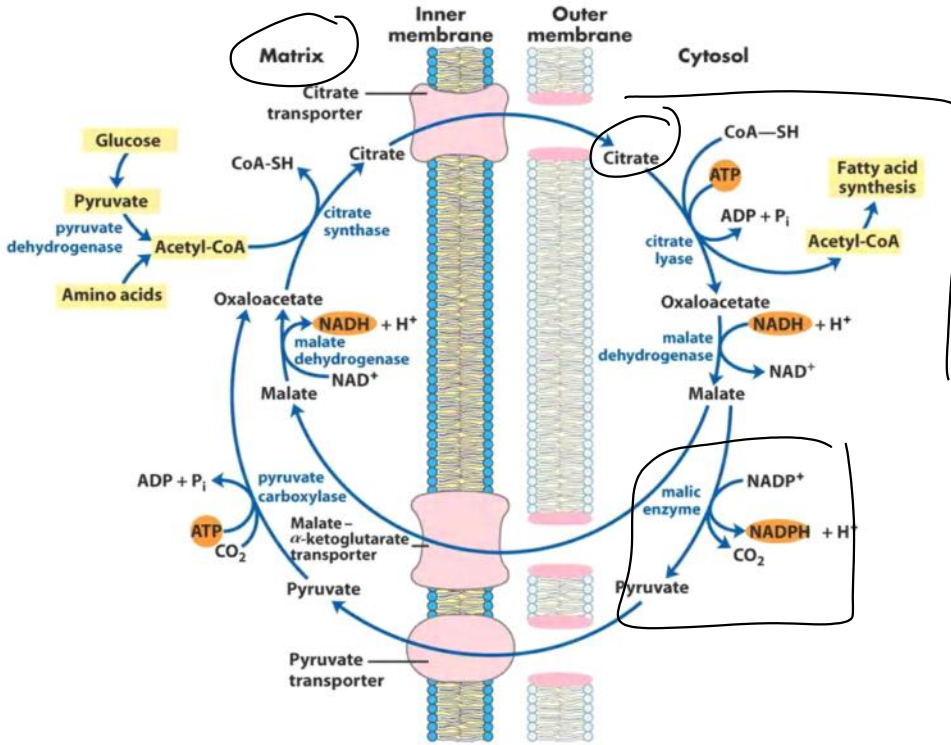
Pyruvate dehydrogenase is mainly in the mitochondrion.

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.



Need
Acetyl-Co
NADPH