

Fatty Acid Biosynthesis

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Fatty acid biosynthesis is different from the β -oxidation degradation pathway.

1. Fatty acid biosynthesis occurs in the cytosol, β -oxidation occurs in the mitochondrial matrix.
2. All of the carbon atoms of fatty acids come from acetyl CoA.
3. Intermediates of fatty acid biosynthesis are covalently linked to an acyl carrier protein. In β -oxidation the fatty acids are attached to Coenzyme A.
4. In animals, the enzymes involved in fatty acid biosynthesis are contained in one long polypeptide chain, whereas the enzymes of β -oxidation are independent enzymes found in the matrix.
5. The oxidation/reduction reagents of fatty acid biosynthesis are $\text{NADP}^+/\text{NADPH}$ whereas the redox reagents of β -oxidation are NAD^+/NADH and FAD/FADH_2 .

The Design Strategy for Fatty Acid Biosynthesis.

- Fatty acids are constructed by the addition of two carbon units derived from acetyl-CoA.
- The acetate units are activated by the formation of malonyl-CoA at the expense of ATP.
- The driving force for the addition of two carbon units to the growing chain is the decarboxylation of malonyl-CoA.
- The chain elongation stops at palmitoyl-CoA.
- Other enzymes add double bonds or additional carbon atom to the carbon chain.

Fatty acid biosynthesis in the cytosol requires a sufficient concentration of NADPH and acetyl-CoA.

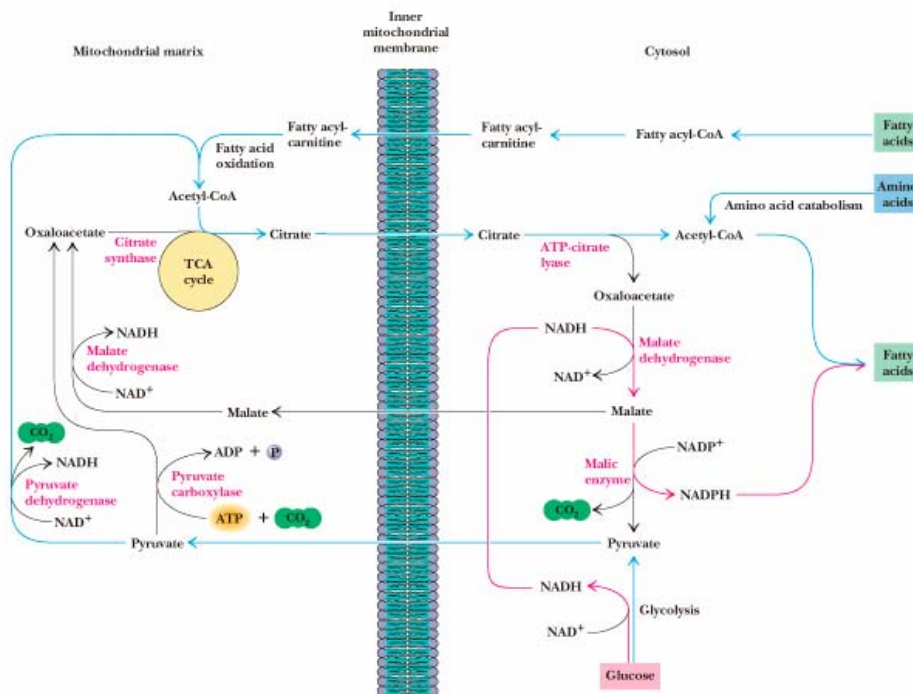
NADPH is generated in the cytosol by the pentose phosphate pathway, and by the malic enzyme which oxidizes malate into pyruvate and CO_2 , generating NADPH.

There are 3 principle ways of producing acetyl-CoA in the cytosol of the cell.

1. Amino acid degradation produces acetyl-CoA.
2. Fatty acid oxidation in the matrix of the mitochondria produces acetyl-CoA which is converted into citrate which is transported into the cytosol by the tricarboxylate transporter. ATP-citrate lyase converts citrate in the cytosol into acetyl-CoA.
3. Glycolysis generates pyruvate which can be carboxylated in the mitochondria into oxaloacetate and then converted into citrate which is transported into the cytosol by the translocase. ATP-citrate lyase converts citrate in the cytosol into acetyl-CoA.

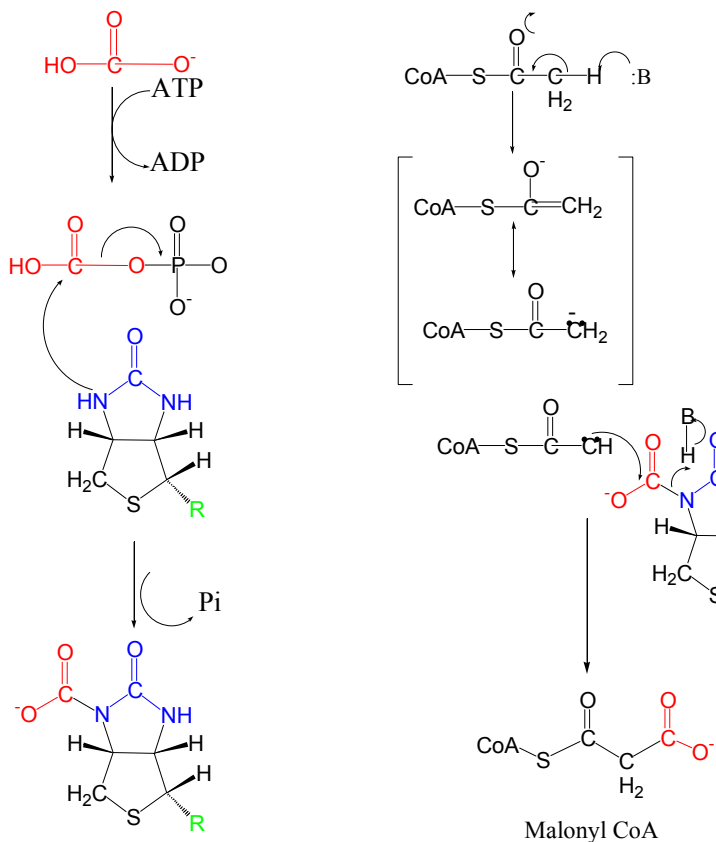
The acetyl-CoA formed by amino acid degradation is insufficient for fatty acid biosynthesis.

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Figure 25.1



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I. Acetyl-CoA Carboxylase (ACC)



Acetyl CoA molecules are the building blocks of fatty acid synthesis.

The acetyl CoA molecules need to be activated for fatty acid biosynthesis.

Acetyl CoA is carboxylated to form malonyl CoA by **acetyl CoA carboxylase** which is a biotin containing enzyme.

The carboxylation reaction is **irreversible** and is the **first committed step of fatty acid biosynthesis**.

The mechanism of this carboxylase is the same as pyruvate carboxylase and propionyl CoA carboxylase.

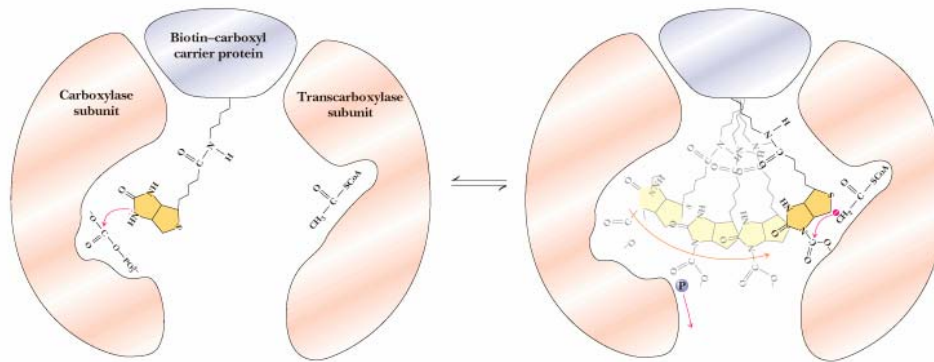
ATP is used to activate bicarbonate in the form of carboxyphosphate which leads to the carboxylation of biotin.

The activated CO₂ group is transferred to acetyl-CoA to form malonyl CoA.

Acetyl CoA carboxylase has three domains:

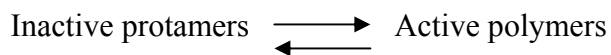
1. A biotin carboxyl group carrier protein.
2. Biotin carboxylase which adds CO₂ to biotin.
3. A transcarboxylase which transfers the CO₂ group from biotin to acetyl CoA to form malonyl CoA.

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Figure 25.3



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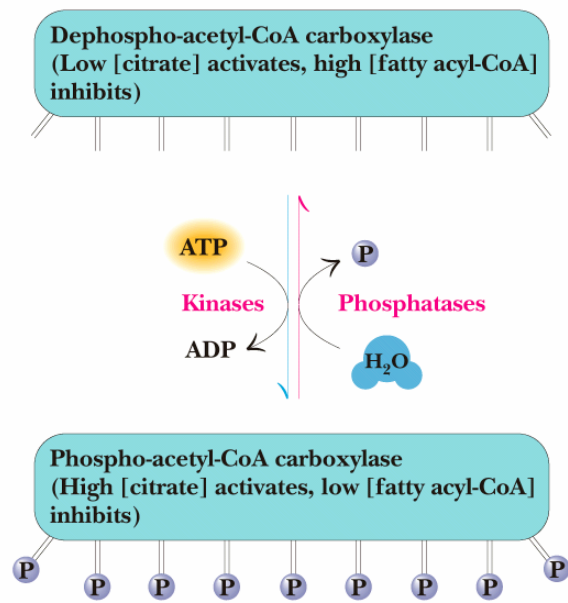
Because this is the first committed step of fatty acid biosynthesis, this acetyl CoA carboxylase (ACC) is allosterically regulated. In animals, ACC is a filamentous polymer composed of 230 kD protomers. Each protomer contains the biotin carboxyl carrier protein, the carboxylase and the transcarboxylase domains as well as allosteric regulatory sites. The polymeric form of this enzyme is active, the individual protomers are not. The activity of ACC is dependent of the equilibrium between the two forms of this enzyme.



The final product of fatty acid biosynthesis is palmitoyl CoA. There is an allosteric binding site for palmitoyl-CoA which shifts the equilibrium toward the inactive protamers.

Citrate which is a precursor for acetyl CoA formation in the cytosol is an allosteric activator of this enzyme. It binds to an allosteric binding site shifting the equilibrium towards the active polymers.

The regulatory effects of citrate and palmitoyl CoA are modulated by the phosphorylation state of acetyl-CoA carboxylase. The animal ACC enzyme has 8-10 potential phosphorylation sites on each protomer. Some of these phosphorylation sites are regulatory others are silent and have no effect on enzyme activity. Acetyl CoA carboxylase is phosphorylated by protein kinases and dephosphorylated by protein phosphatases.



The unphosphorylated ACC binds citrate with high affinity and thus becomes fully active at a very low citrate concentration.

The phosphorylated ACC enzyme has a greatly reduced affinity for citrate, so high concentrations of citrate are required to activate the enzyme.

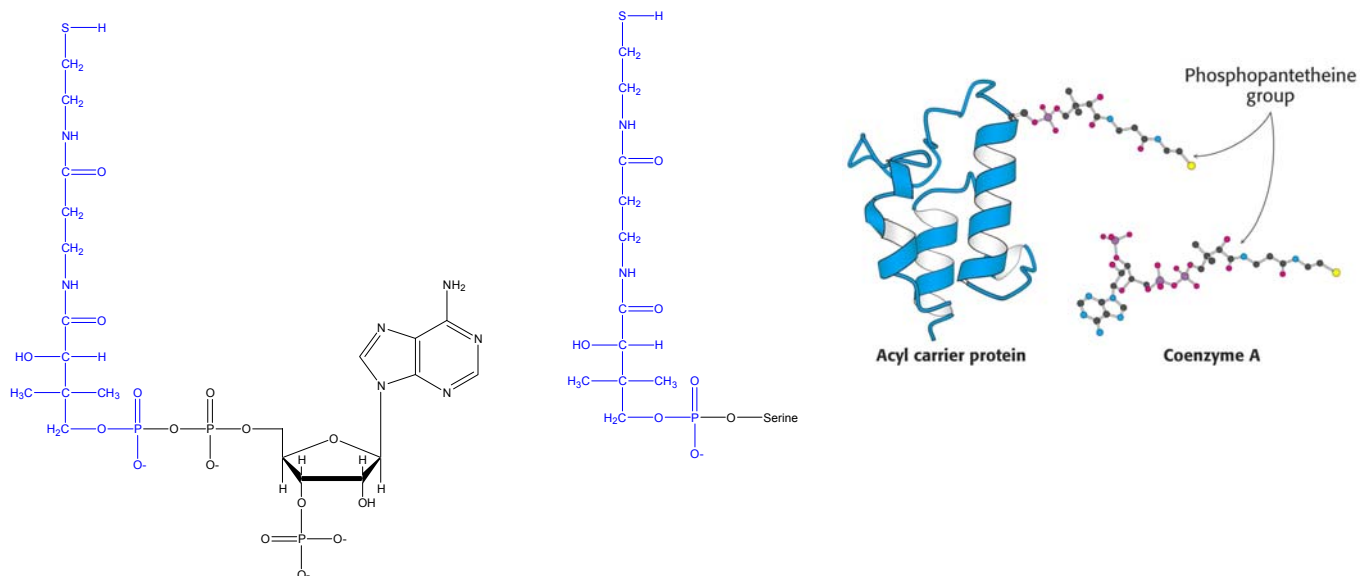
Palmitoyl CoA binds preferably to the phosphorylated form of the enzyme, so when the ACC enzyme is phosphorylated it takes a small concentration of palmitoyl CoA to inactivate it.

When the enzyme is dephosphorylated, the dephosphorylated enzyme has a low affinity for palmitoyl CoA and thus it takes a large

concentration of palmitoyl CoA to inactivate it.

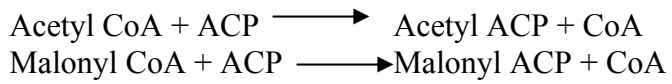
II Acyl Carrier Protein (ACP)

The intermediates of fatty acid biosynthesis are not linked to Coenzyme A. Rather the intermediates of fatty acid biosynthesis are linked to an acyl carrier protein. The acyl carrier protein is very similar to Coenzyme A in that it contains a phosphopantetheine prosthetic group. Coenzyme contains the phosphopantetheine group attached to an adenosine nucleotide. In the acyl carrier protein, the phosphopantetheine group is attached to a serine residue.

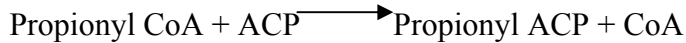


III. Elongation Phase of Fatty Acid Biosynthesis

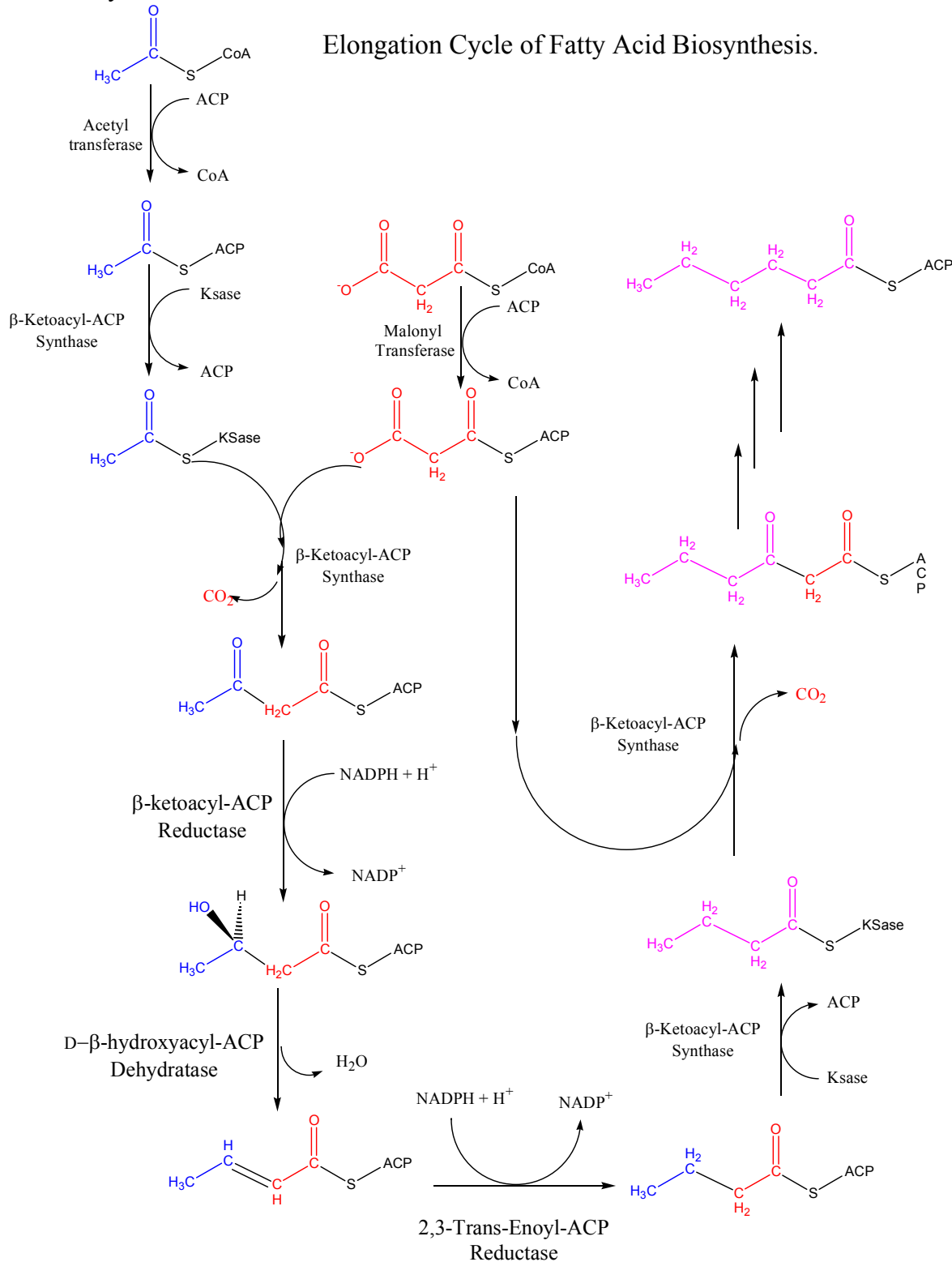
Before fatty acid biosynthesis begins, fatty acid synthetase must be primed with acetyl CoA. The elongation phase of fatty acid biosynthesis begins with the formation of acetyl ACP and malonyl ACP. Acetyl transacylase and malonyl transacylase catalyzes the reactions.



Acetyl transacylase will also catalyze the following reaction at a much slower rate:



This reaction allows for the synthesis of fatty acid chains of odd length. Malonyl transacylase is specific for malonyl CoA.

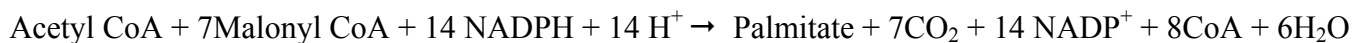


Once acetyl KSase and malonyl ACP have been formed, elongation can begin. First the acetyl group of acetyl ACP is transferred to a sulfhydryl residue of ketoacyl-ACP synthase also known as acyl-malonyl ACP condensing enzyme. The decarboxylation of malonyl ACP generates an enolate anion which is a good nucleophile that attacks the carbonyl of thioester of acetyl-S-KSase to form acetoacetyl ACP. The exergonic decarboxylation reaction drives the condensation reaction. The CO₂ group added to acetyl CoA by acetyl CoA carboxylase is given up in this reaction. In effect this reaction is driven by ATP. ATP was used to activate bicarbonate in the acetyl CoA carboxylase reaction. The free energy was conserved in the malonyl CoA and released upon decarboxylation to drive the synthesis of acetoacetyl CoA.

The next three reactions are the reverse of β-oxidation. First the ketone is reduced to the alcohol by the enzyme β-ketoacyl ACP reductase. This reaction differs from the reverse of the β-oxidation reaction in that the D-isomer rather than the L-isomer is formed and NADPH is the reducing agent rather than NADH. The alcohol is dehydrated D-β-hydroxyacyl ACP dehydratase to form crotonyl ACP which is reduced by enoyl-ACP reductase to form butyryl ACP. This step is also different than the mere reversal of β-oxidation in that NADPH is the reducing agent instead of FADH₂.

In the next round of fatty acid biosynthesis, butyryl ACP is transferred to the sulfhydryl group of β-ketoacyl-ACP synthase. The decarboxylation of a second molecule of malonyl CoA generates another nucleophile that attacks the carbonyl of the thioester of butyryl-S-KSase to form a C₆-β-ketoacyl ACP which is then reduced to the β-hydroxyacyl ACP, dehydrated to form the C₆-Δ²-enoyl-ACP which is then reduced to form the C₆-acyl-ACP which is then transferred to the sulfhydryl group of β-ketoacyl-ACP synthase, condensed with a third malonyl CoA etc. Etc. Etc., until palmitoyl ACP is formed. A thioesterase hydrolyzes the thioester bond of palmitoyl ACP to yield palmitate and ACP.

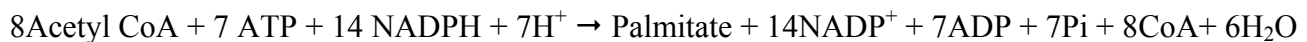
The overall reaction is shown below:



The formation of 7 malonyl CoA requires:



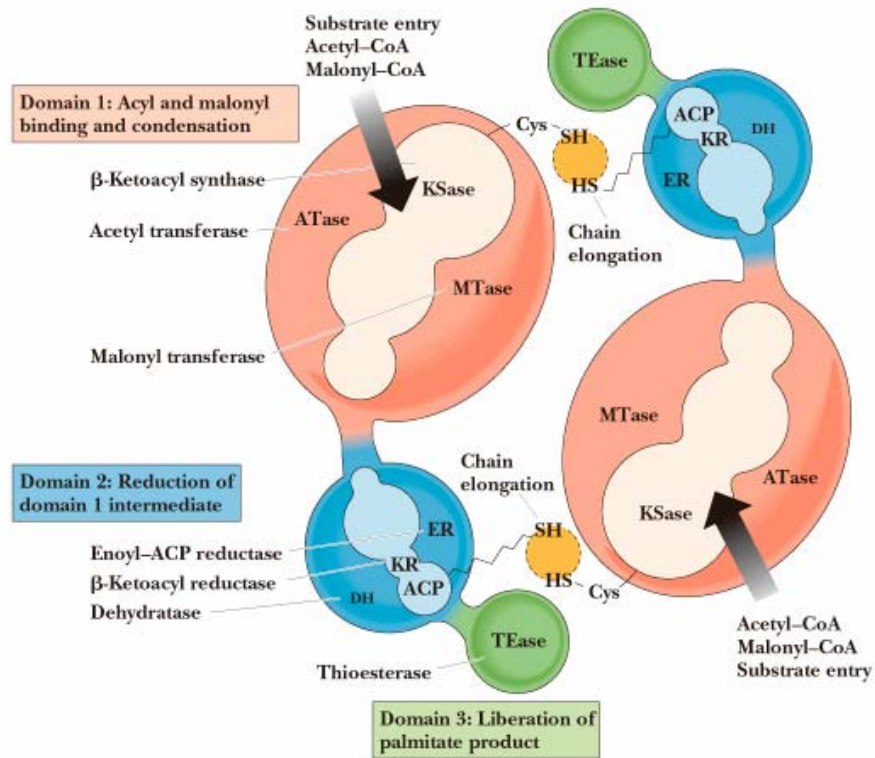
Combining these two steps:



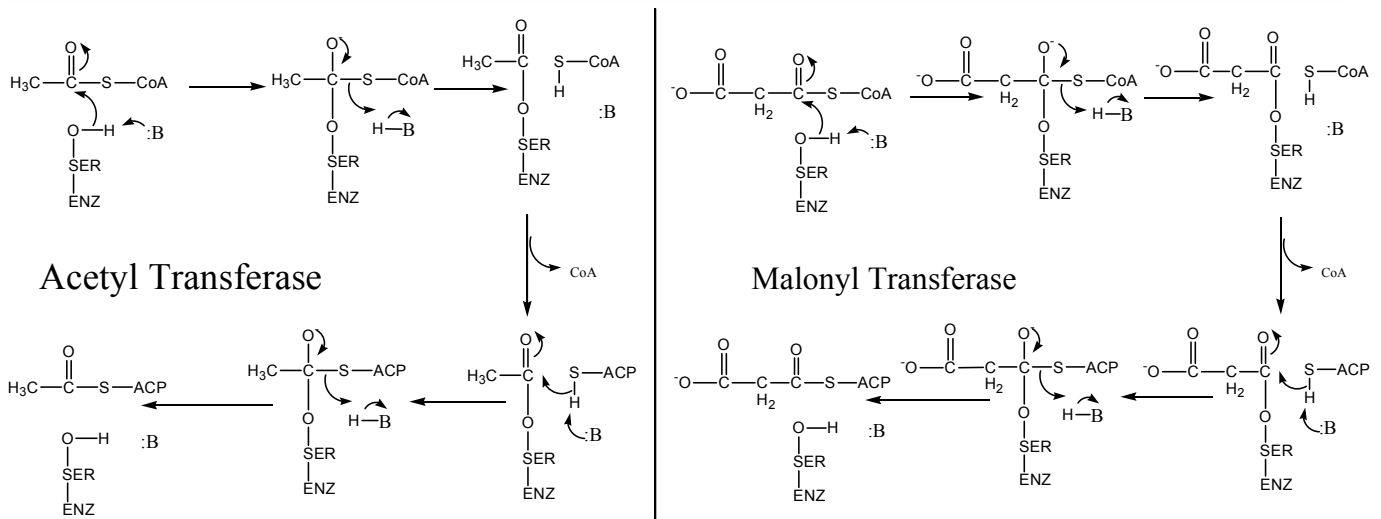
IV. The Fatty Acid Synthase Multienzyme Complex of Eukaryotes.

In contrast to bacterial fatty acid biosynthesis, Eukaryotes fatty acid synthase is a multienzyme complex contained in 2 different polypeptide chains. The α subunit is 213 kD, the β subunit is 203 kD. Animal fatty acid synthase complexes are dimers of αβ subunits. The separate activities of each dimer of αβ subunits are shown in the figure below. The α subunits contain the β-ketoacyl-ACP synthase (KSase) domain and the β-ketoacyl reductase domain. The β subunit contains the acetyl transferase domain, the malonyl transferase domain, the β-hydroxyacyl dehydrogenase domain and the enoyl reductase domain.

The subunits are arranged in a head to tail fashion that allows the first domain of one subunit of fatty acid synthase to interact with the second and third domains of the other subunits.



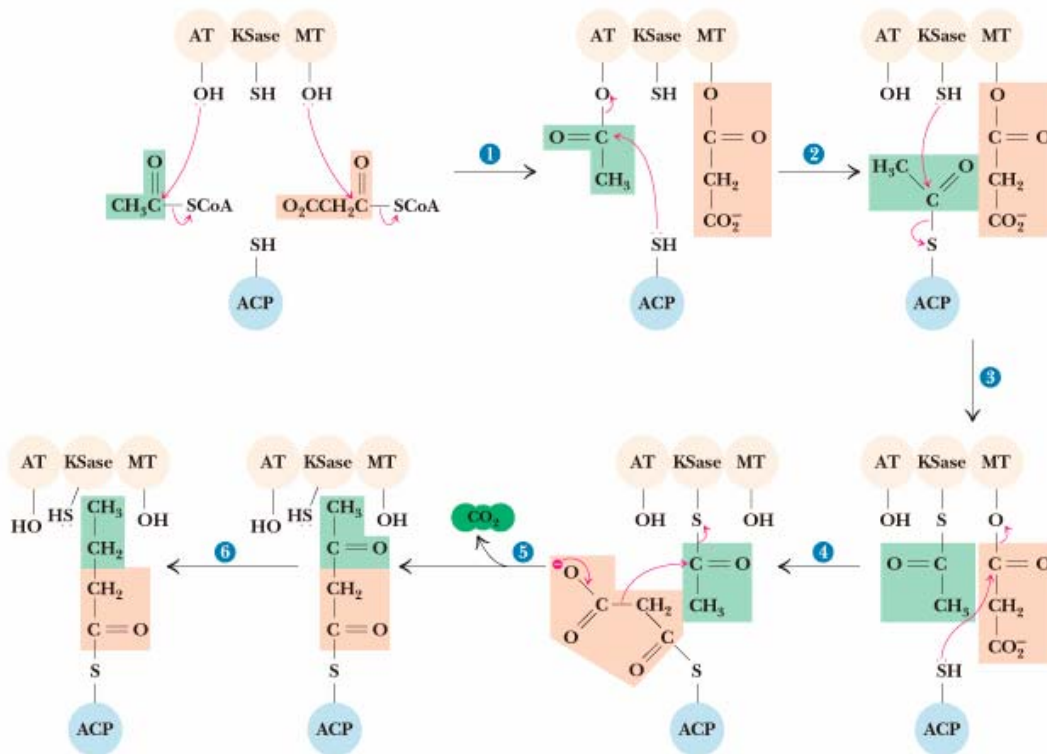
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The first step of the fatty acid synthase reaction is the formation of acetyl-O-enzyme intermediate between an acetyl group of acetyl CoA and an active site serine residue of the acetyl transferase domain as shown above on the left. In a similar manner, a malonyl-O-enzyme intermediate is formed between malonyl CoA and an active site serine residue of the malonyl transferase domain as shown above on the right.

The next step is the transfer of the acetyl group to the sulfhydryl of the acyl carrier protein (ACP). This acyl group is then transferred one more time to a cysteine residue of β -ketoacyl-ACP synthase as shown below. This frees the acyl carrier protein to acquire the malonyl group from the malonyl transferase. The next step is the condensation reaction in which decarboxylation of the malonyl-ACP generates a highly reactive nucleophile that attacks the carbonyl of acetyl-S-KSase.

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Figure 25.11



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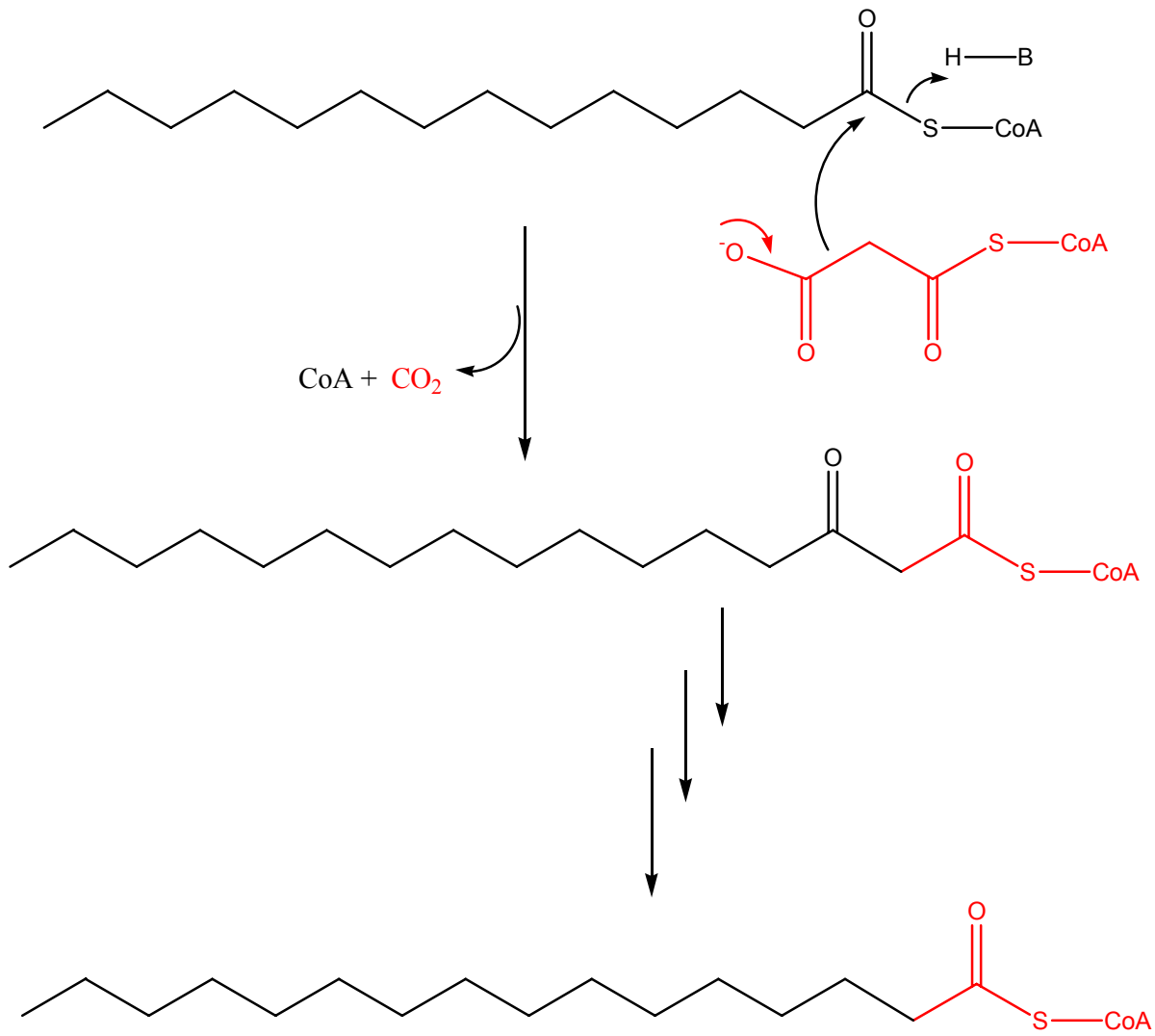
The next three steps are the reduction of the carbonyl to the alcohol, the dehydration and the reduction of the alkene to form saturated butyryl-ACP. A second malonyl group is transferred from malonyl CoA to the active site serine of malonyl transferase. The butyryl-ACP is then transferred to the cysteine residue of KSase. This frees the acyl carrier protein to acquire the malonyl group from the malonyl transferase. The next step is the condensation reaction in which decarboxylation of the malonyl-ACP generates a highly reactive nucleophile that attacks the carbonyl of butyryl-S-KSase. This cycle continues until palmitoyl ACP is formed. The thioester bond is hydrolyzed by thioesterase to form palmitate.

V. Elongation of Palmitate

Palmitate is the primary product of fatty acid synthase. Fatty acids with shorter chains can be generated by releasing the fatty acid before reaching 16 carbons of length. Fatty acids longer than 16 carbon atoms require special elongation reactions which occur either in the mitochondria or at the surface of the endoplasmic reticulum.

The elongation reaction that occurs in the endoplasmic reticulum is similar to fatty acid synthesis. As shown on the next page.

Elongation in the ER



The mitochondrial elongation reaction involves the addition and subsequent reduction of acetyl groups. The condensation reaction is catalyzed by thiolase. The reaction is the reverse of the thiolase reaction in β -oxidation.

Mitochondrial Elongation

