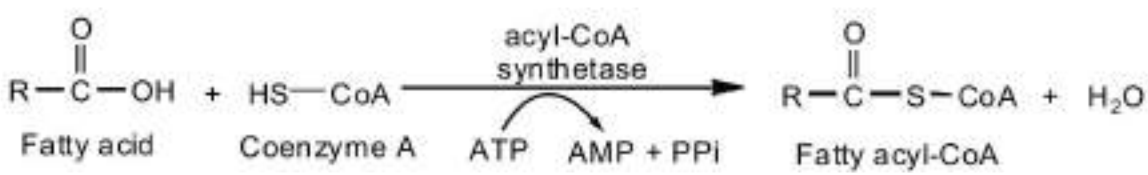
Fatty Acids metabolism (β-Oxidation)

The fatty acids cross the mitochondrial wall

The enzymes of fatty acid oxidation in animal cells are located in the mitochondrial matrix, as demonstrated in 1948 by Eugene P. Kennedy and Albert Lehninger. The fatty acids with chain lengths of **12 or fewer** carbons enter mitochondria without the help of membrane transporters. Those with **14 or more carbons**, which constitute the majority of the FFA obtained in the diet or released from adipose tissue, cannot pass directly through the mitochondrial membranes; they must first undergo the three enzymatic reactions of the carnitine shuttle.

The first reaction is catalyzed by a family of isozymes (different isozymes specific for fatty acids having short, intermediate, or long carbon chains) present in the **outer mitochondrial membrane**, the acyl-CoA synthetase (Thiokinase), which promote the general reaction:

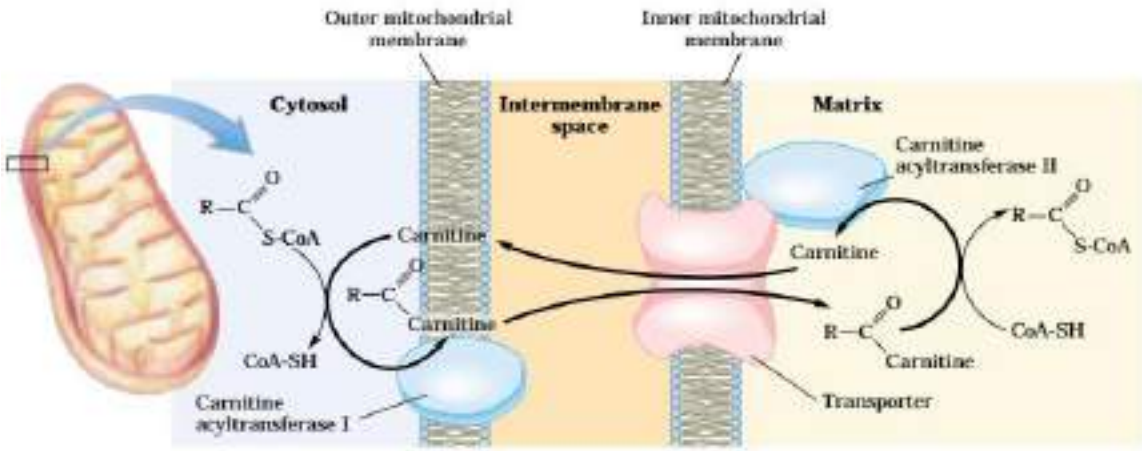


Thus, acyl-CoA synthetases catalyze the formation of a thioester linkage between the fatty acid carboxyl group and the thiol group of coenzyme A to yield a fatty acyl–CoA, coupled to the cleavage of ATP to AMP and PPi.

The fatty acid must be transported across the inner mitochondrial membrane that is impermeable to CoA. Therefore, a specialized carrier transports the long-chain acyl group from the cytosol into the mitochondrial matrix. This carrier is **carnitine**, and this rate-limiting transport process is called the carnitine shuttle.

First, the acyl group is transferred from CoA to carnitine by carnitine acyltransferase I (an enzyme of the outer mitochondrial membrane), This reaction forms acylcarnitine, and regenerates free CoA.

Second, the acylcarnitine is transported into the mitochondrial matrix in exchange for free carnitine by Carnitine acyltransferase II. Carnitine acyltransferase II is an enzyme of the inner mitochondrial membrane catalyzes the transfer of the acyl group from carnitine to CoA in the mitochondrial matrix, thus regenerating free carnitine.



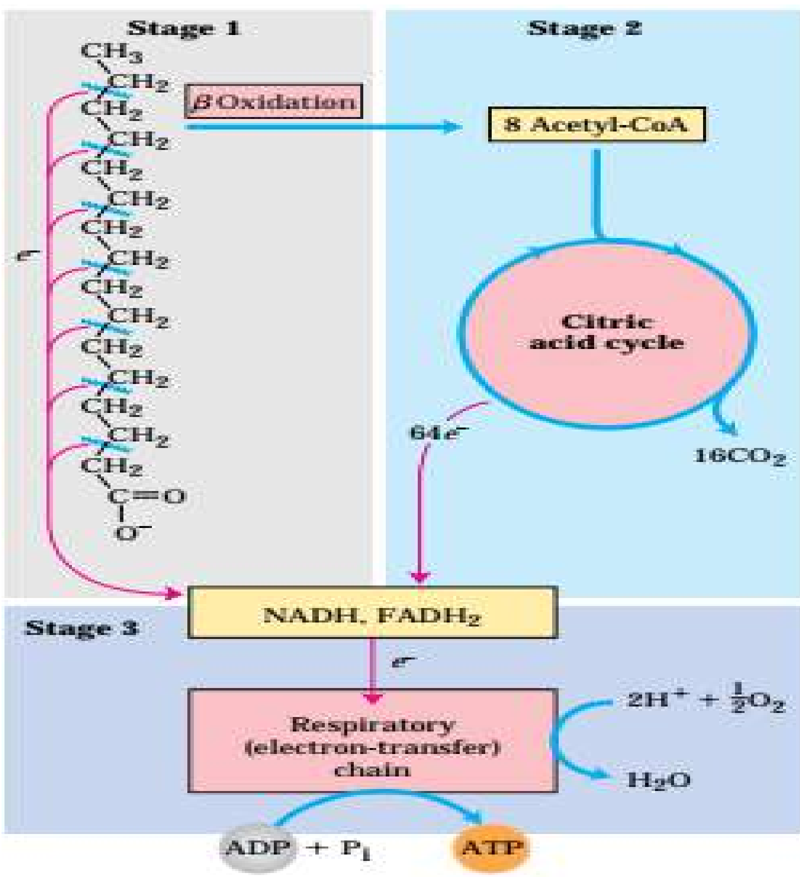
Fatty acid entry into mitochondria via the acyl-carnitine/carnitine transporter

β-Oxidation of fatty acids

The major pathway for catabolism of fatty acids is a mitochondrial pathway called β-oxidation, in which two-carbon fragments are successively removed from the carboxyl end of the fatty acyl CoA, producing acetyl CoA, NADH, and $FADH_2$.

As noted earlier, mitochondrial oxidation of fatty acids takes place in three stages. In the **first stage**, oxidation of fatty acids undergoes oxidative removal of successive two-carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain. For example, the 16- carbon palmitic acid (palmitate at pH 7) undergoes seven passes through the oxidative sequence, in each pass losing two carbons as acetyl-CoA. At the end of seven cycles the last two carbons of palmitate (originally C-15 and C-

16) remain as acetyl-CoA. The overall result is the conversion of the 16-carbon chain of palmitate to eight two-carbon acetyl groups of acetyl-CoA molecules. Formation of each acetyl-CoA requires removal of four hydrogen atoms (two pairs of electrons and four H) from the fatty acyl moiety by dehydrogenases. In the **second stage** of fatty acid oxidation, the acetyl groups of acetyl-CoA are oxidized to CO2 in the citric acid cycle, which also takes place in the mitochondrial matrix. Acetyl-CoA derived from fatty acids thus enters a final common pathway of oxidation with the acetyl-CoA derived from glucose via glycolysis and pyruvate oxidation. The first two stages of fatty acid oxidation produce the reduced electron carriers NADH and FADH2, which in the **third stage** donate electrons to the mitochondrial respiratory chain, through which the electrons pass to oxygen with the concomitant phosphorylation of ADP to ATP. The energy released by fatty acid oxidation is thus conserved as ATP.

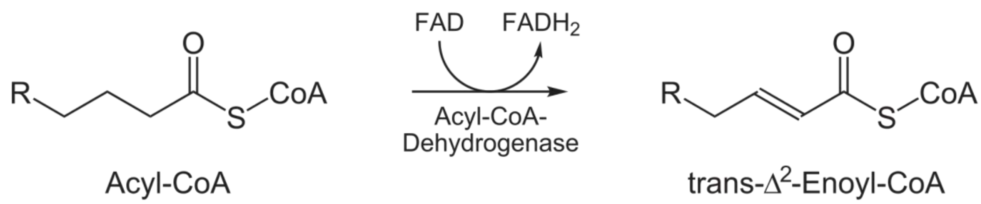


Stages of fatty acid oxidation

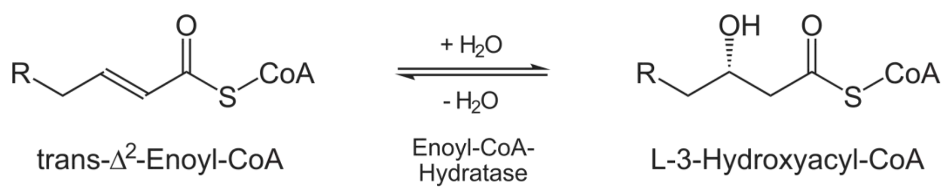
The β-Oxidation of Saturated Fatty Acids

Once the fatty acid is inside the mitochondrial matrix, beta-oxidation occurs by cleaving two carbons every cycle to form acetyl-CoA. The process consists of 4 steps.

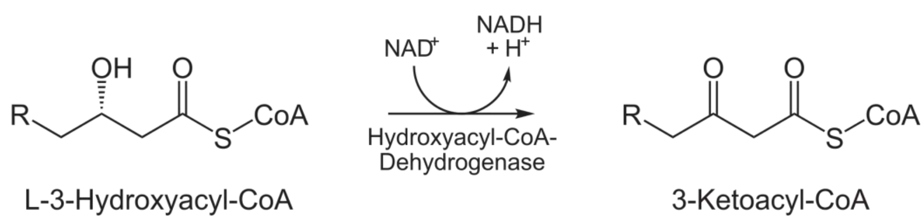
1. A long-chain fatty acid is dehydrogenated to create a trans double bond between C2 and C3. This is catalyzed by acyl CoA dehydrogenase to produce trans-delta 2-enoyl CoA. It uses FAD as an electron acceptor and it is reduced to $FADH_2$.



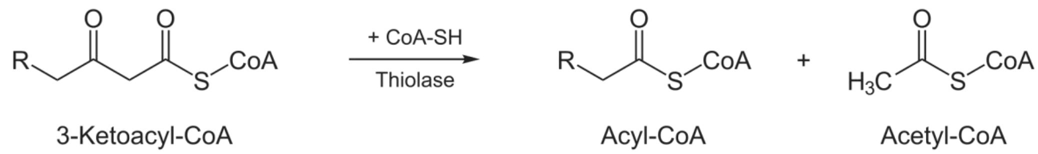
2. Trans-delta2-enoyl CoA is hydrated at the double bond to produce L-3-hydroxyacyl CoA by enoyl-CoA hydratase.



3. L-3-hydroxyacyl CoA is dehydrogenated again to create 3-ketoacyl CoA by 3-hydroxyacyl CoA dehydrogenase. This enzyme uses NAD as an electron acceptor.



4. Thiolysis occurs between C2 and C3 (alpha and beta carbons) of 3-ketoacyl CoA. Thiolase enzyme catalyzes the reaction when a new molecule of coenzyme A breaks the bond by nucleophilic attack on C3. This releases the first two carbon units, as acetyl CoA, and a fatty acyl CoA minus two carbons. The process continues until all of the carbons in the fatty acid are turned into acetyl CoA.



To completely oxidize the 18-carbon fatty acid above, 8 cycles of beta-oxidation have to occur. This will produce:

9 acetyl-CoAs

8 NADH

8 $FADH_2$

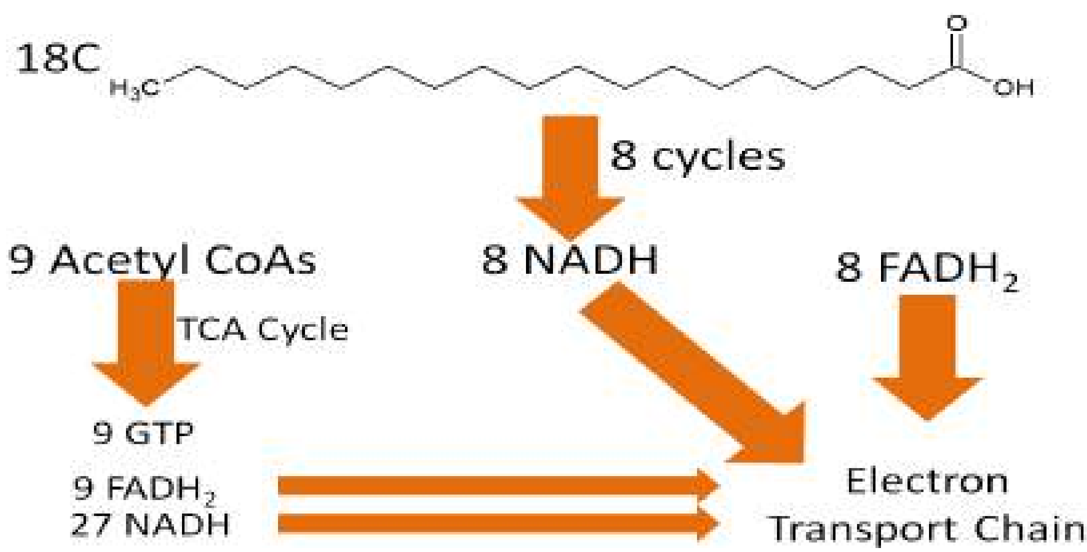
Those 9 acetyl-CoAs can continue into the citric acid cycle, where they can produce:

9 GTP

9 $FADH_2$

27 NADH

The products of the complete oxidation of a fatty acid are shown below.



Adding up the NADH and $FADH_2$, the electron transport chain ATP production from beta-oxidation and the citric acid cycle looks like this:

NADH

8 (beta-oxidation) + 27 (TCA) = 35 NADH X 2.5 ATP/NADH = 87.5 ATP

FADH2

8 (beta-oxidation) + 9 (TCA) = 17 FADH2 X 1.5 ATP/FADH2 = 25.5 ATP

GTP

9 GTP = 9 ATP

Total ATP from complete oxidation of an 18 carbon fatty acid:

87.5 + 25.5 + 9 = 122 ATP

Subtract 2 ATP (ATP–>AMP) required for activation of the fatty acid:

122-2 = 120 Net ATP

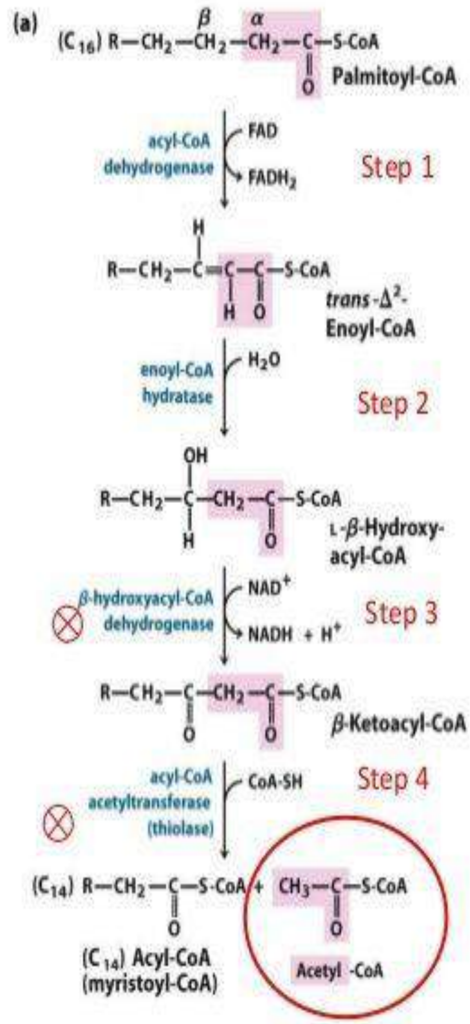
4 Steps of β-oxidation

1. Dehydrogenation of the fatty acyl-CoA to make a trans double bond between α and β carbon.
   - Short, medium, and long chain acyl-CoAdehydrogenases
   - e^- removed transferred to FAD
2. Hydration of the double bond

1. Dehydrogenation of the β-hydroxyl group to a ketone

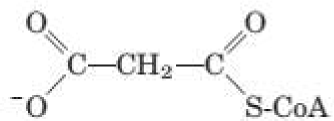
   - e^- removed transferred to NAD^+

1. Acylation – addition of CoA and production of acetyl-CoA



Biosynthesis of Fatty Acids

After the discovery that fatty acid oxidation takes place by the oxidative removal of successive two-carbon (acetyl-CoA) unit, biochemists thought the biosynthesis of fatty acids might proceed by a simple reversal of the same enzymatic steps. However, as they were to find out, fatty acid biosynthesis and breakdown occur by different pathways, are catalyzed by different sets of enzymes, and take place in different parts of the cell. Moreover, biosynthesis requires the participation of a three-carbon intermediate, malonyl-CoA, that is not involved in fatty acid breakdown.



Malonyl-CoA

Fatty Acid Synthesis Proceeds in a Repeating Reaction Sequence

The long carbon chains of fatty acids are assembled in a repeating four-step sequence (Fig. 1). A saturated acyl group produced by this set of reactions becomes the substrate for subsequent condensation with an activated malonyl group. With each passage through the cycle, the fatty acyl chain is extended by two carbons. When the chain length reaches 16 carbons, the product (palmitate, 16:0) leaves the cycle.

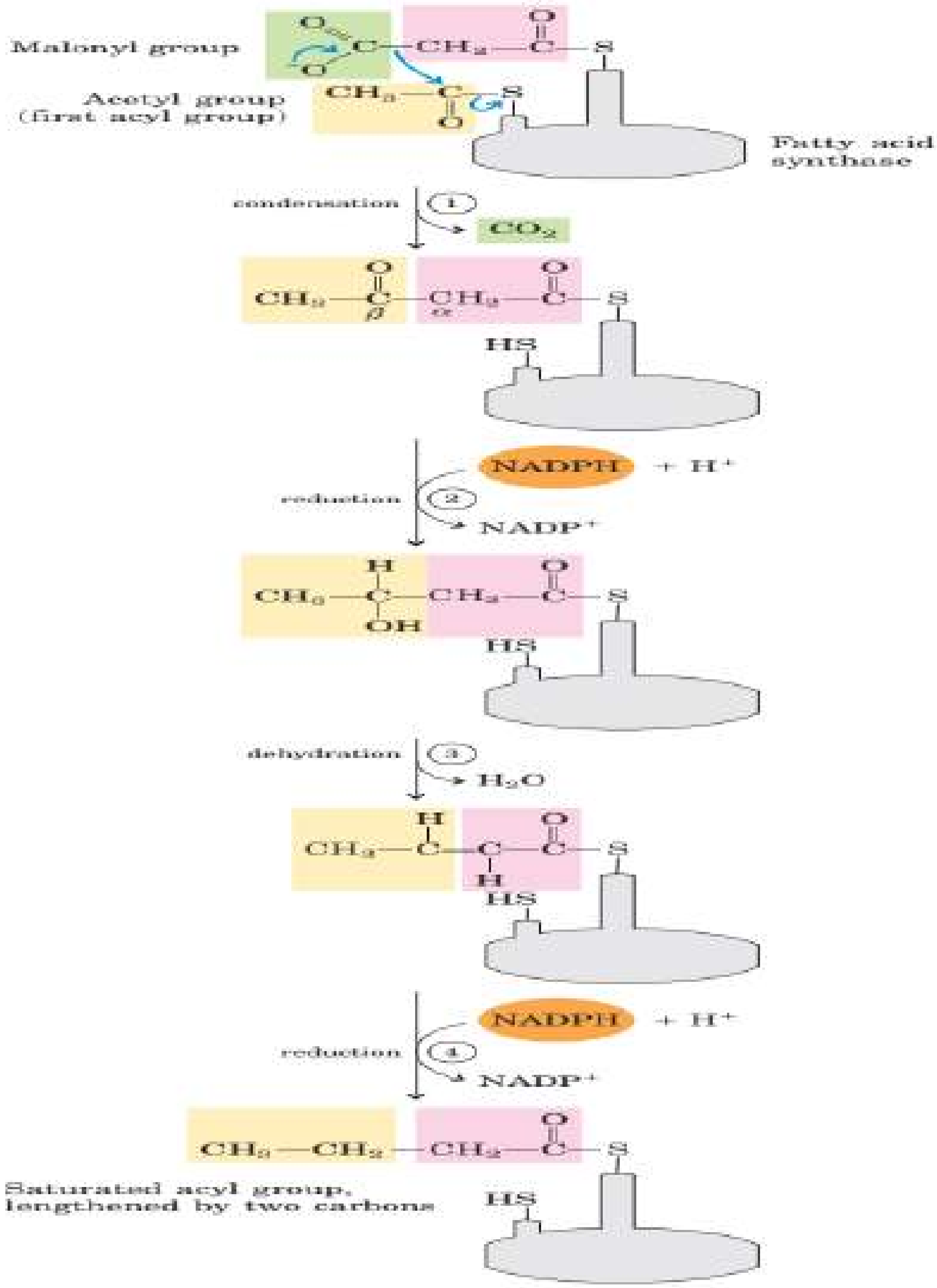


Figure 2: Addition of two carbons to a growing fatty acyl chain: a four-step sequence.

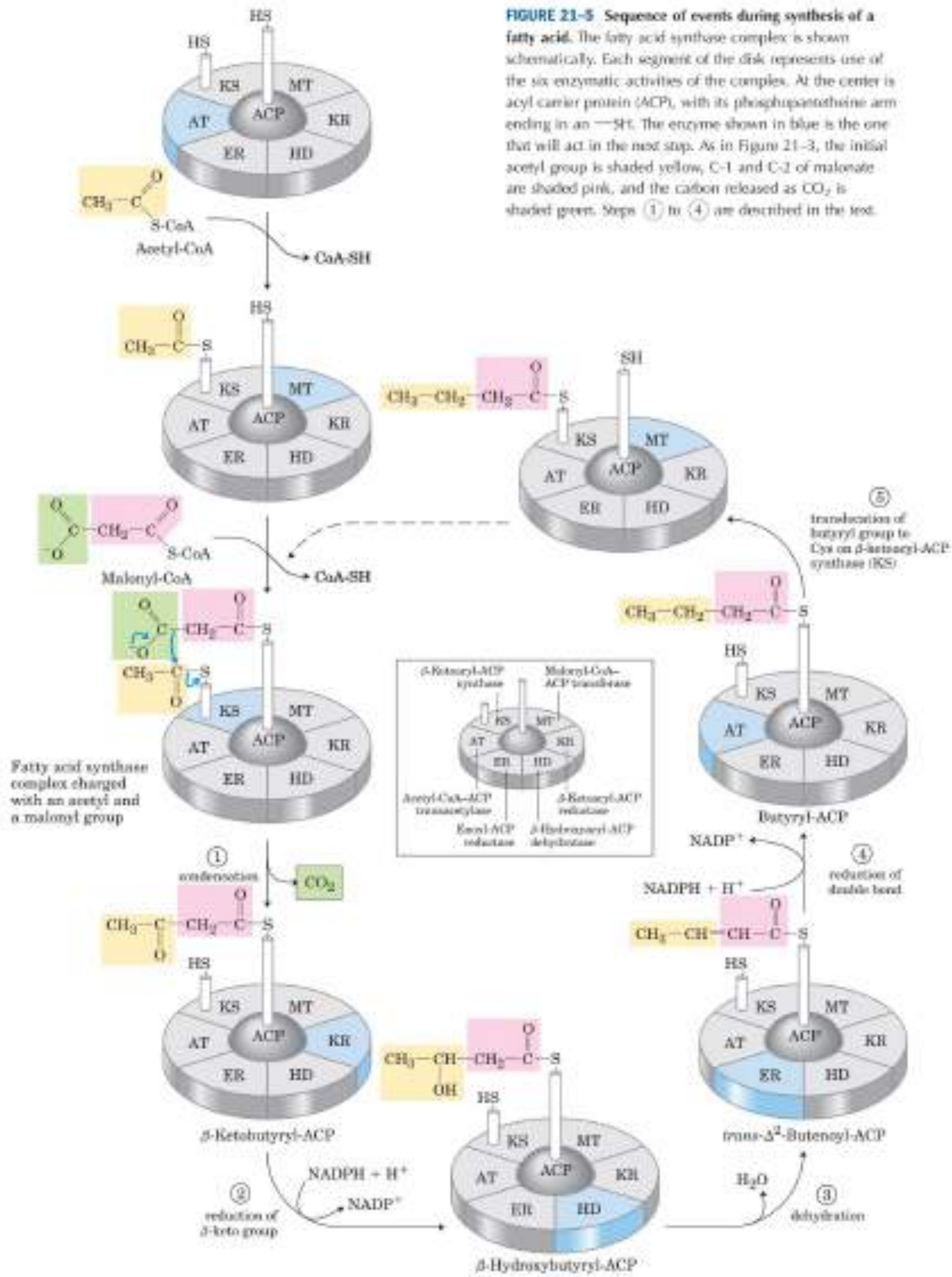


FIGURE 21–5 Sequence of events during synthesis of a fatty acid. The fatty acid synthase complex is shown schematically. Each segment of the disk represents one of the six enzymatic activities of the complex. At the center is acyl carrier protein (ACP), with its phosphopantetheine arm ending in an —SH. The enzyme shown in blue is the one that will act in the next step. As in Figure 21–3, the initial acetyl group is shaded yellow, C-1 and C-2 of malonate are shaded pink, and the carbon released as CO_2 is shaded green. Steps ① to ④ are described in the text.