E-content

M.Sc. Zoology (Semester II)
CC7- Biochemistry

Unit: 3.5

Free fatty acids: Synthesis and importance

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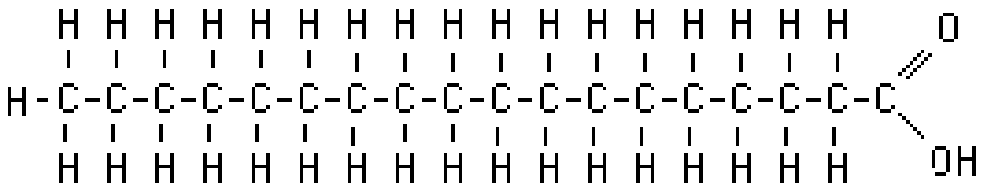
Introduction

A fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated

Fatty acids are the building blocks of the fat in our bodies and in the food we eat. During digestion, the body breaks down fats into fatty acids, which can then be absorbed into the blood.

Fatty acid molecules are usually joined together in groups of three, forming a molecule called a triglyceride.



Stearic acid, a saturated fatty acid

Overview of fatty acid biosynthesis

Occurs in the cytosol of certain animal tissues; e.g., liver and mammary gland. Also occurs in plants and bacteria.

Uses acetyl-CoA, NADPH as starting materials.

Produces a pool of palmitic acid (16:0) that can be further modified.

Fatty acids play several important roles:

1. Building blocks for phospholipids and glycolipids.

2. Target proteins to membranes.

3. High energy source of fuel.

4. Fatty acid derivatives are used as hormones and intracellular messengers.

Key players of Fatty Acid Biosynthesis

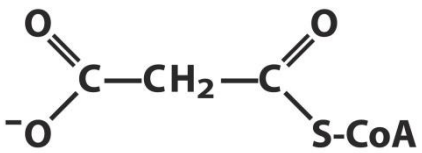
1. Malonyl-CoA

2. Fatty Acid Synthase

3. Acyl Carrier Protein

1. Malonyl-CoA: The intermediate molecule

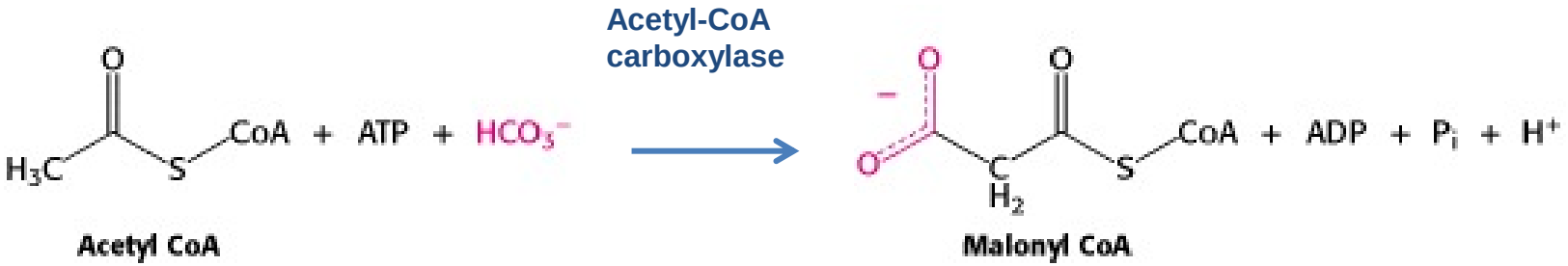
In fatty acid synthesis, acetyl-CoA is the direct precursor only of the methyl end of the growing fatty acid chain. All the other carbons come from the acetyl group of acetyl-CoA but only after it is modified to provide the actual substrate for fatty acid synthase, malonyl-CoA.



Malonyl-CoA

Malonyl-CoA contains a 3-carbon dicarboxylic acid, malonate, bound to Coenzyme A. Malonate is formed from acetyl-CoA by the addition of CO_2 using the biotin cofactor of the enzyme **acetyl-CoA carboxylase**.

Formation of malonyl-CoA is the **commitment step** for fatty acid synthesis, because malonyl-CoA has no metabolic role other than serving as a precursor to fatty acids.



Acetyl CoA

Malonyl CoA

Formation of malonyl-CoA

Acetyl-CoA carboxylase has three activities:

biotin carrier protein
biotin carboxylase
Transcarboxylase

Bicarbonate is phosphorylated, then picked up by biotin

Biotin swinging arm transfers CO_2 to acetyl-CoA

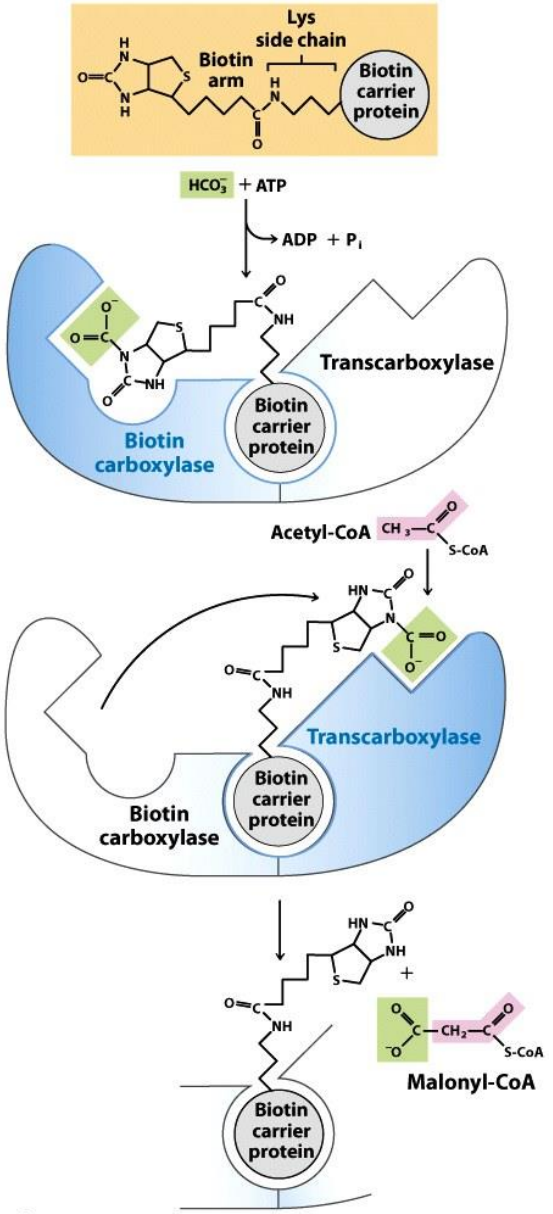
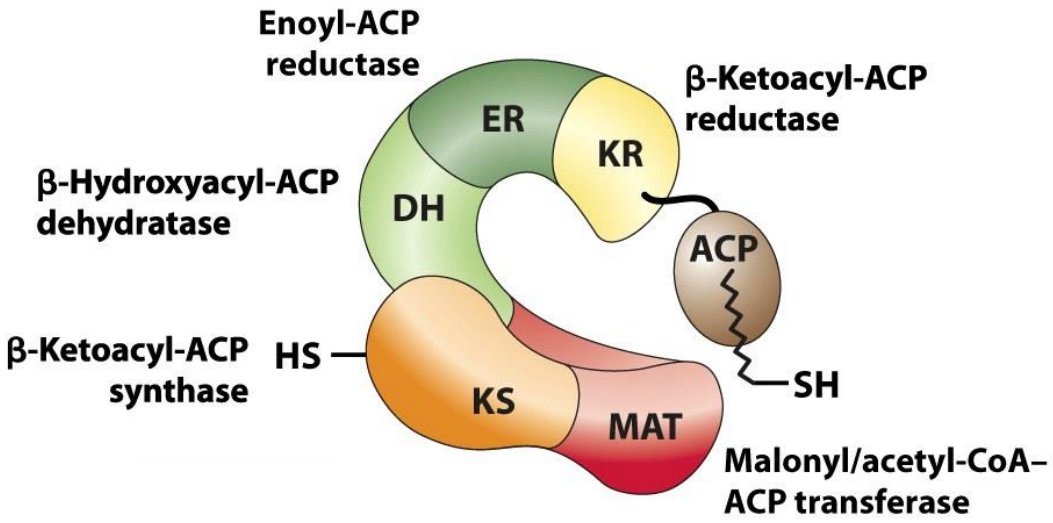


Figure 21-1
Lehninger Principles of Biochemistry, Fifth Edition

2. Fatty acid synthase (FAS)

The fatty acid synthases (FAS) of eukaryotes have the component enzymes linked in a large polypeptide chain.

Mammalian fatty acid synthase (FAS) is a large homodimeric multifunctional enzyme that regulates the *de novo* biosynthesis of long-chain fatty acids



Fatty acid synthase

This cytosolic enzyme catalyzes the formation of 16 carbon (C_{16}) palmitate, from acetyl-coenzyme A (acetyl-CoA) and malonyl-coenzyme A (malonyl-CoA) in the presence of NADPH.

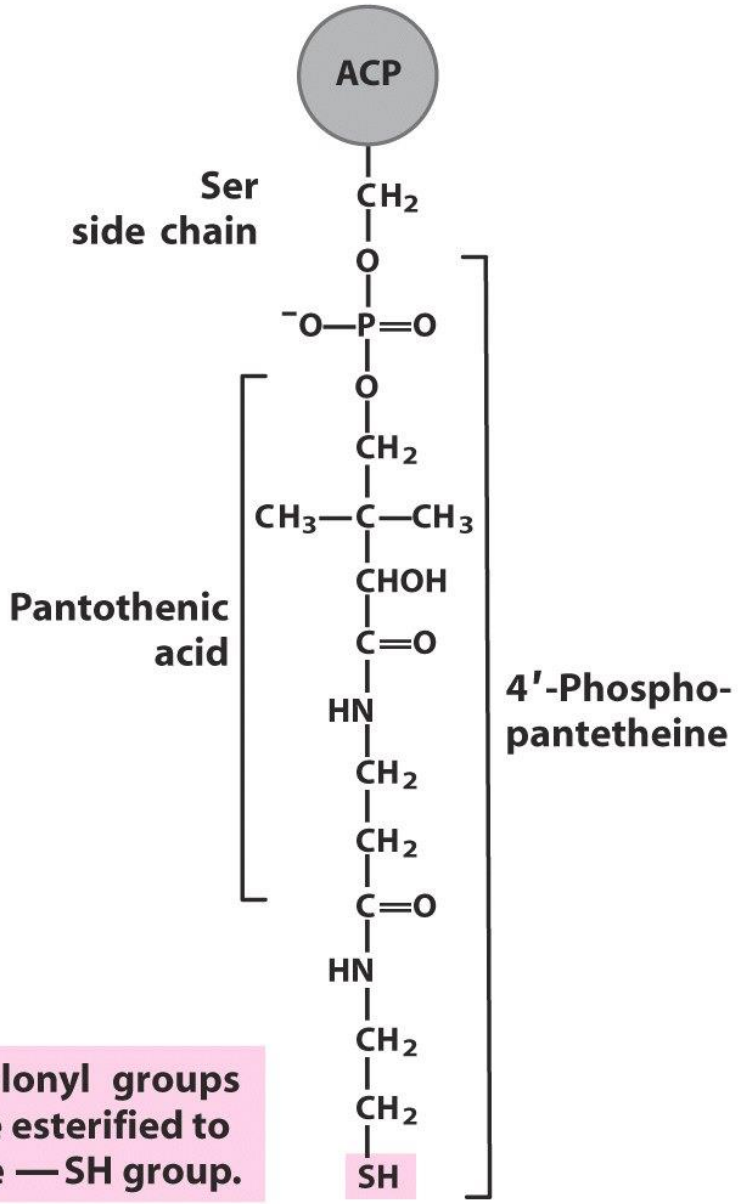
The FAS monomer (approximately 270 kDa) contains seven catalytic activities and from the N-terminus the order is beta-ketoacyl synthase (KS), acetyl/malonyl transacylase (MAT), beta-hydroxyacyl dehydratase (DH), enoyl reductase (ER), beta-ketoacyl reductase (KR), acyl carrier protein (ACP), and thioesterase (TE).

3. Acyl Carrier Protein

ACP, a single polypeptide chain of 77 residues, a part of the mammalian Fatty acid synthase, can be regarded as a giant prosthetic group, a "macro CoA."

The intermediates of fatty acid chain are linked to the sulfhydryl terminus of a phosphopantetheine group, which is, in turn, attached to a serine residue of the ACP.

ACP moves from one domain to next during the fatty acid chain initiation, elongation and termination events and facilitates the biosynthesis of fatty acids.



Main steps of Fatty Acid Synthesis

1. ***Initiation Stage***

Step 1: loading of acetyl-CoA onto fatty acid synthase
Step 2: loading of malonyl- CoA onto fatty acid synthase

2. ***Assembly Stage***

Step 1: Condensation
Step 2: Reduction
Step 3: Dehydration
Step 4: Reduction

3. ***Transfer to KS***

4. ***Next cycle begins***

1. Initiation Stage

Step 1: loading of acetyl-CoA onto fatty acid synthase

Before the condensation reactions that build up the fatty acid chain begin, the two thiol groups on the enzyme complex must be charged with the correct acyl groups. The two thiol groups are:

(KS)-Cys–SH
(ACP)-phophopantetheine –SH

Step 1: First, the acetyl group of acetyl-CoA is transferred to ACP in the reaction catalysed by the MAT. The acetyl group is then transferred to the Cys-SH group of the KS.

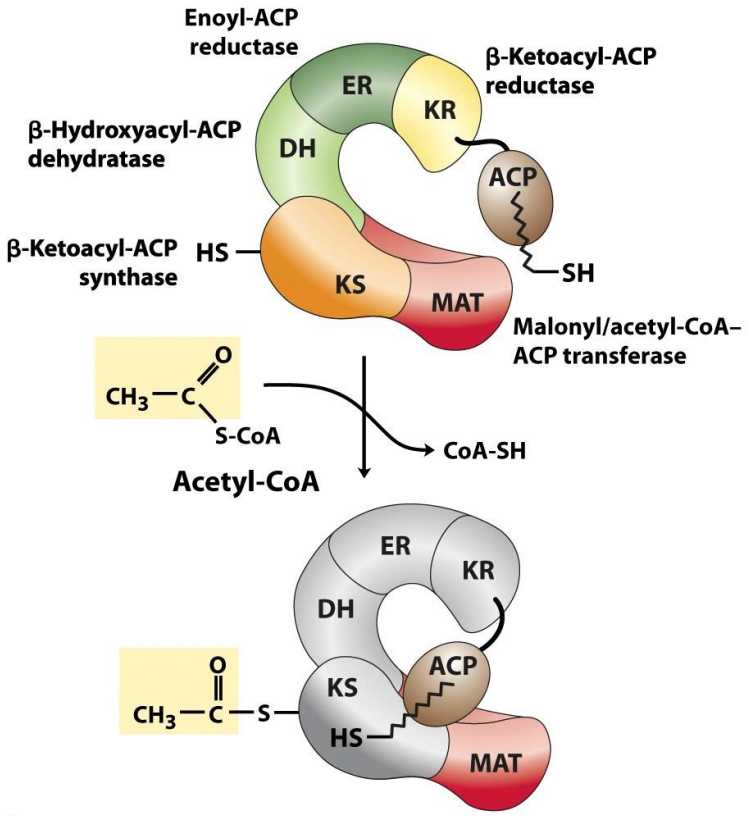


Figure 21-6 part 1
Lehninger Principles of Biochemistry, Fifth Edition

1. Initiation Stage

Step 2: loading of malonyl- CoA onto fatty acid synthase

The second reaction, transfer of the malonyl group from malonyl-CoA to the –SH group of ACP, is also catalysed by MAT.

This way charged synthase complex, the acetyl and malonyl groups are activated for the chain-lengthening process.

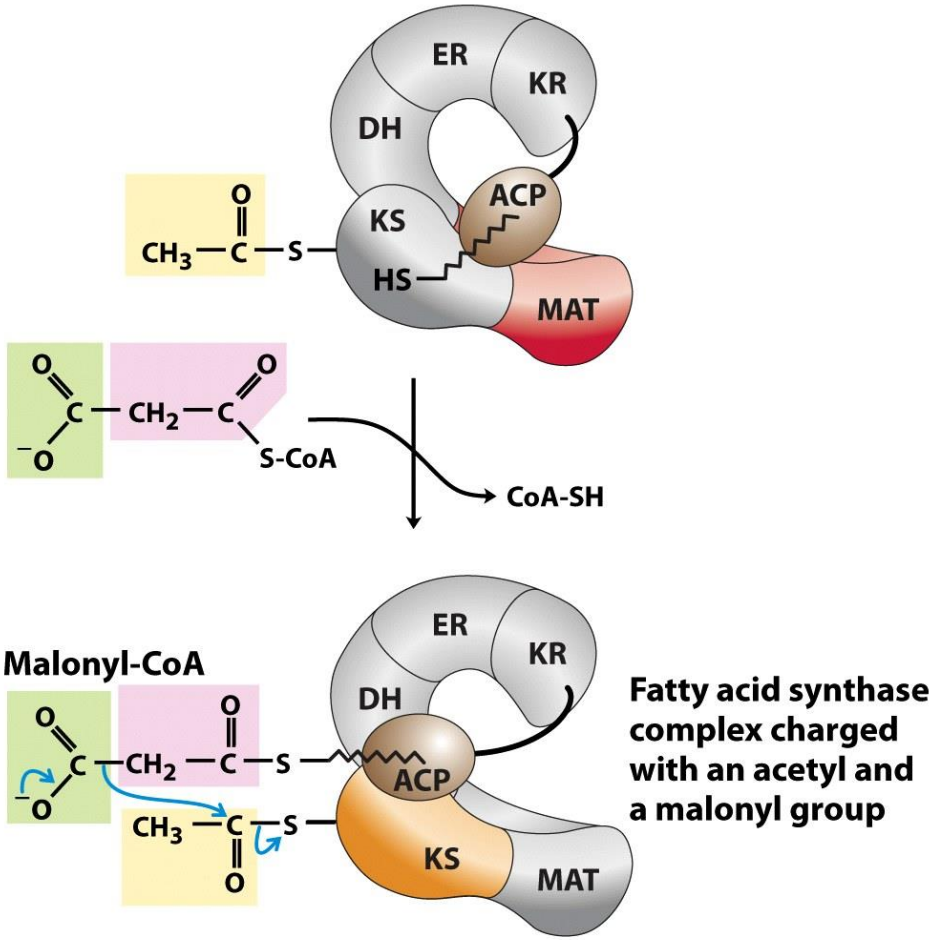


Figure 21-6 part 2
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2. Assembly Stage (4 steps)

Step 1: Condensation
Step 2: Reduction
Step 3: Dehydration
Step 4: Reduction of double bond

Step 1: Condensation

Reaction of malonyl group with acetyl group to form acetoacetyl- ACP

Loss of CO_2

Catalysed by β-ketoacyl-ACP synthase (KS)

The acetyl group is transferred from the Cys–SH group of the enzyme (KS) to the malonyl group on the –SH of ACP, becoming the methyl-terminal two-carbon unit of the new acetoacetyl group.

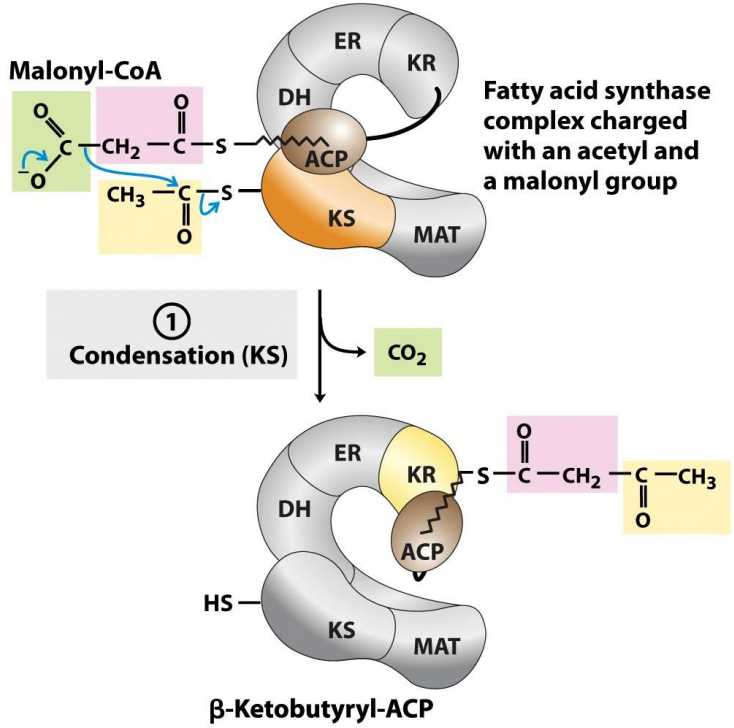

Figure 21-6 part 3
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Step 2: Reduction

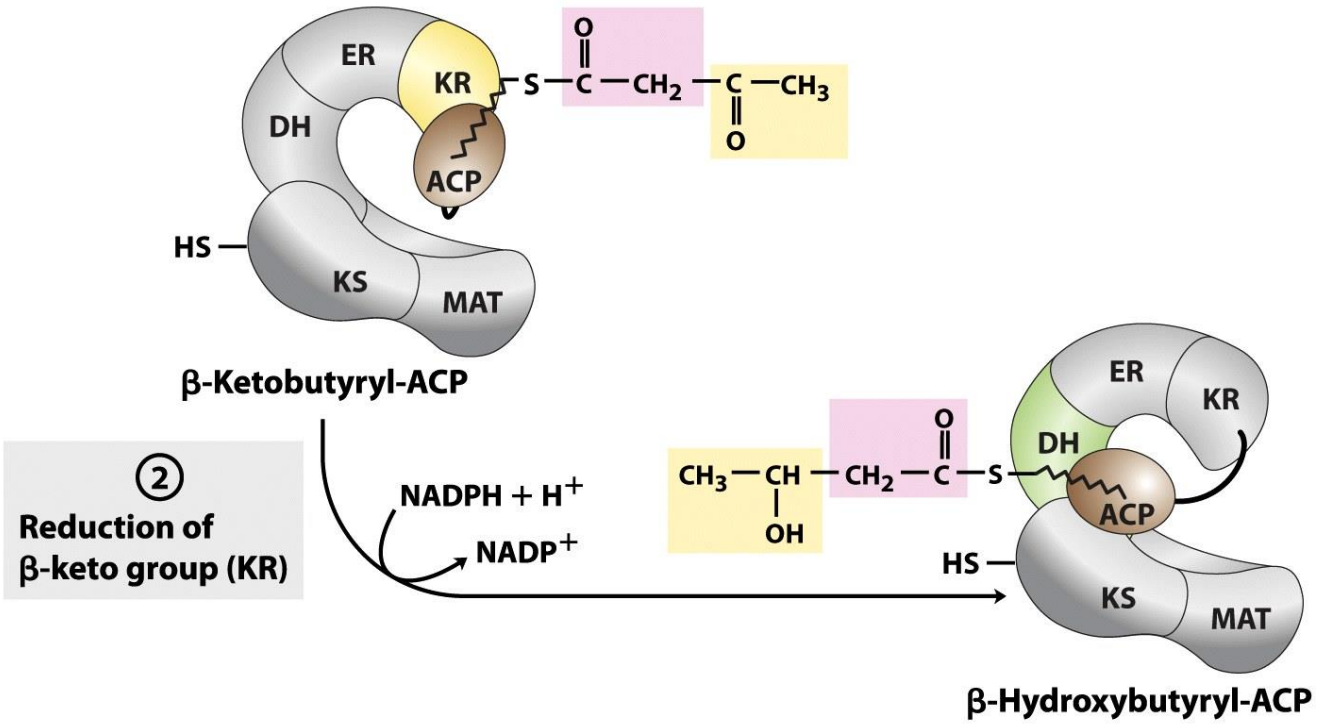

Figure 21-6 part 4
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The acetoacetyl-ACP formed in the condensation step now undergoes reduction of the carbonyl group at C-3 to form β-hydroxybutyryl-ACP.

The reaction is catalysed by β-ketoacyl-ACP reductase (KR) and the electron donor is NADPH.

Step 3: Dehydration

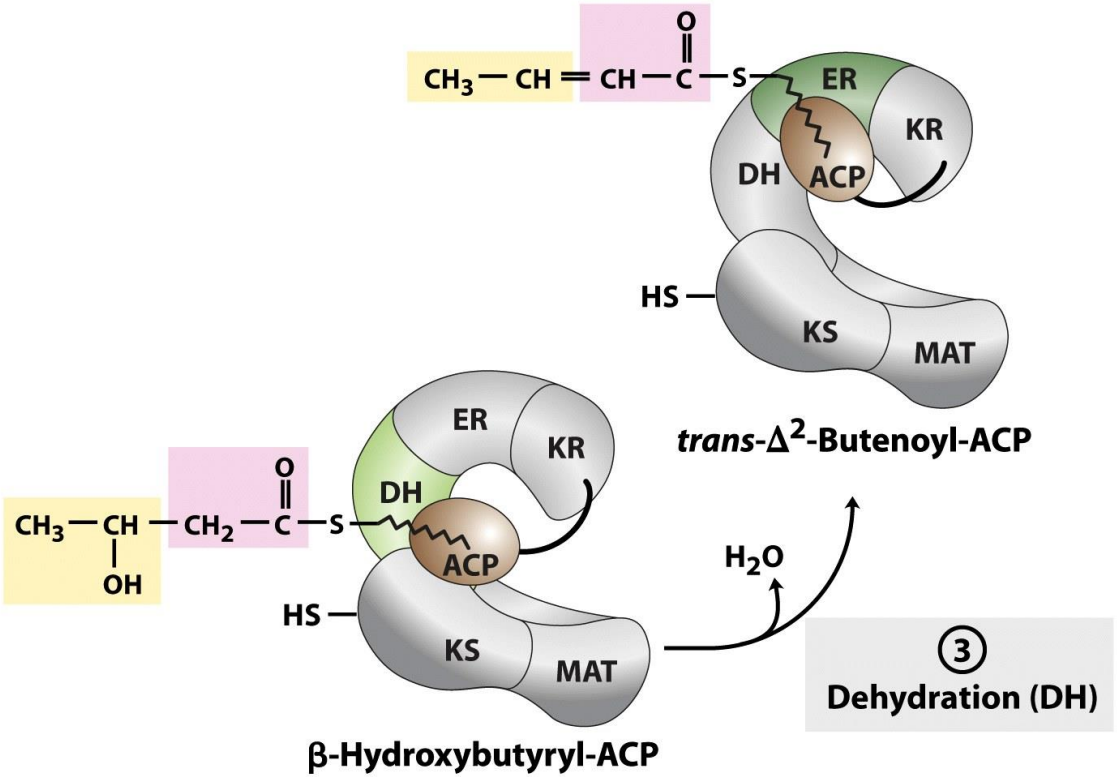

Figure 21-6 part 5
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The element of water are now removed from C-2 and C-3 of β-hydroxybutyryl-ACP to Yield a double bond in the product, trans-Δ^2-butenoyl-ACP.

The enzyme that catalysed this dehydration is β-hydroxyacyl-ACP-dehydratase (DH)

Step 4: Reduction of double bond

Finally, the double bond of nis reduced (saturated) to form butyryl-ACP by the action of enoyl-ACP reductase (ER).

NADPH is the electron donor in this step.

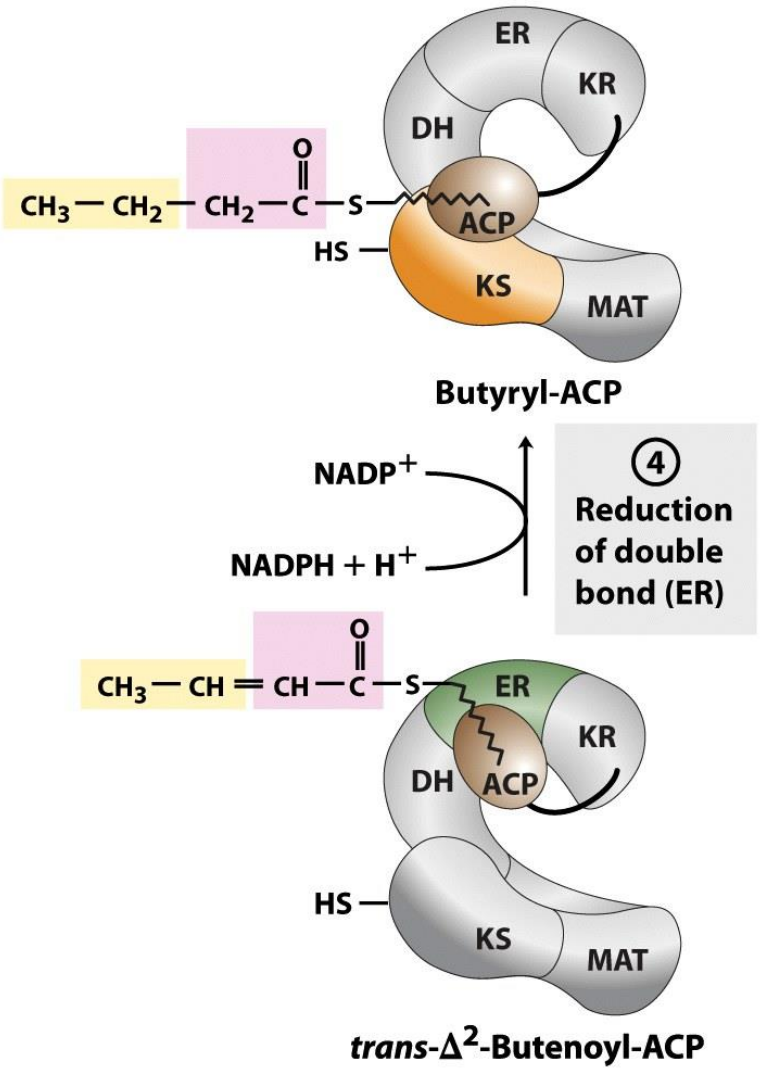

Figure 21-6 part 6
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3. Transfer to KS

The butryl group is now transferred from the phophopantetheine –SH group of ACP to the Cys-SH group of KS.

After this the next round of four reaction cycle begins.

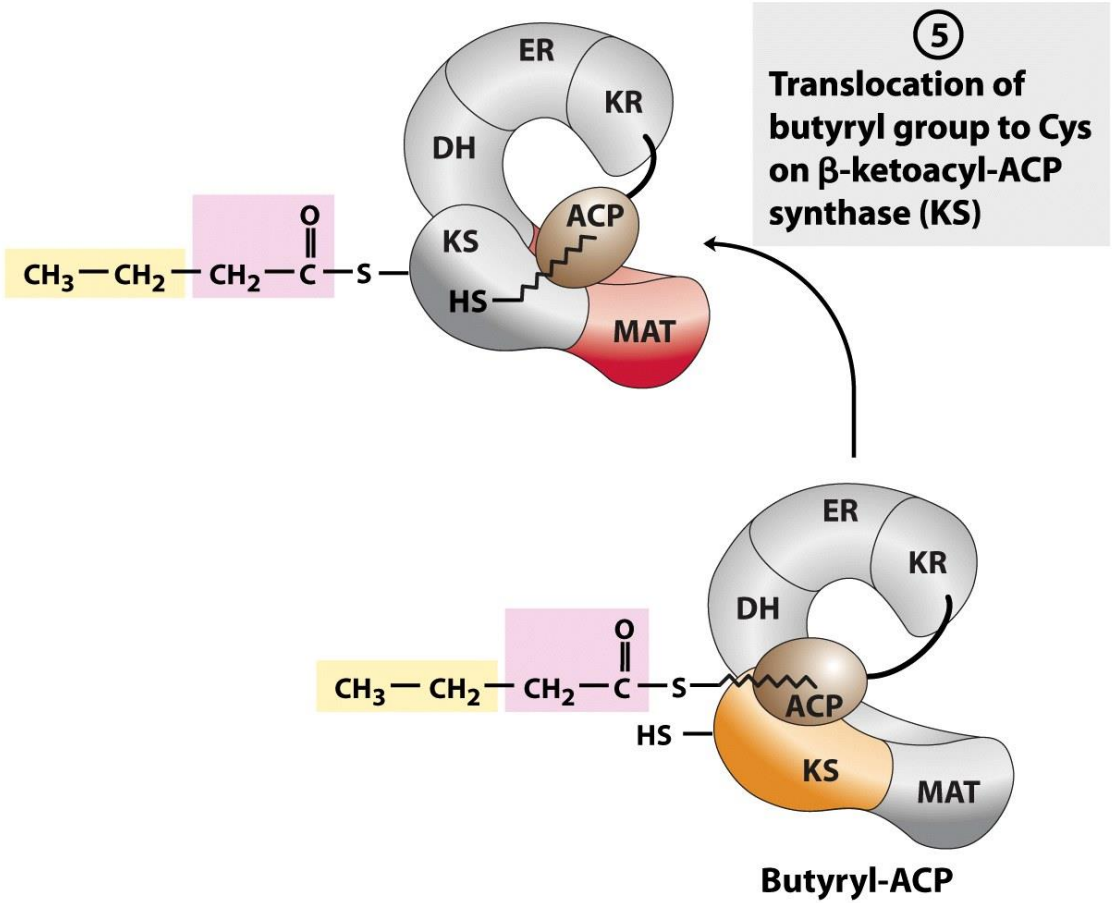

Figure 21-6 part 7
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4. Next cycle begins

Another malonyl group is linked to ACP to initiate next round of four reaction cycle.

The elongation cycle is repeated six more times, using malonyl–CoA each time, to produce palmityl–ACP.

The elongation cycles continue until C_{16}-acyl ACP is formed.

This intermediate is a good substrate for a thioesterase that hydrolyzes C_{16}-acyl ACP to yield palmitate and ACP.

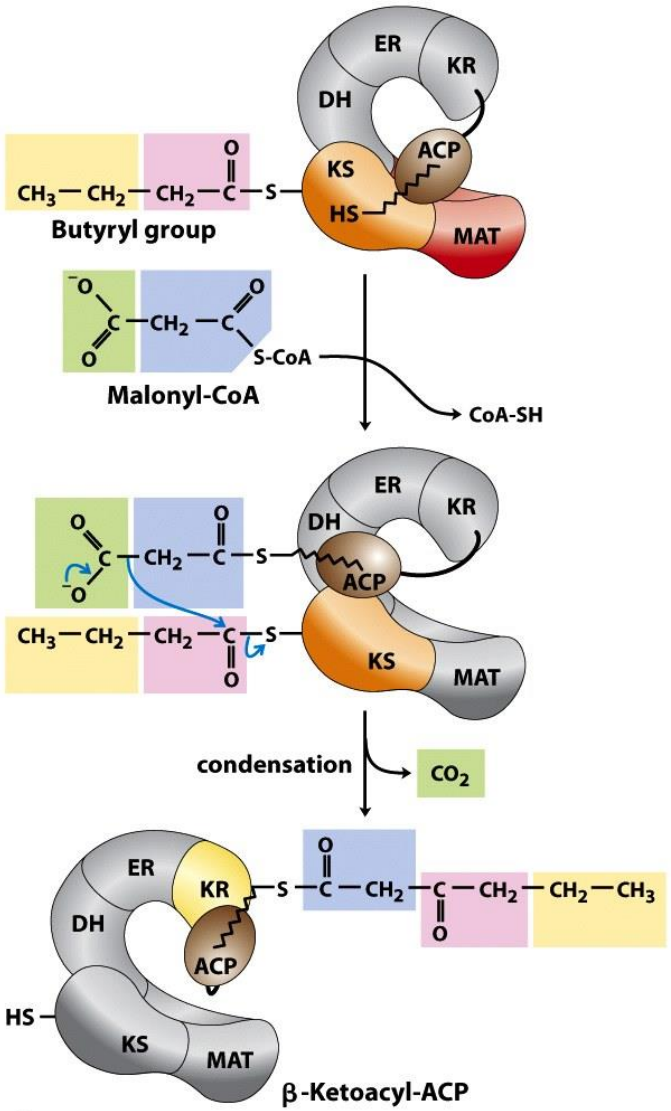

Figure 21-7
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The Stoichiometry of Fatty Acid Synthesis

The stoichiometry of the synthesis of palmitate is

$$\text{Acetyl CoA} + 7\ \text{malonyl CoA} + 14\ \text{NADPH} + 20\ \text{H}^+ \longrightarrow \text{palmitate} + 7\ \text{CO}_2 + 14\ \text{NADP}^+ + 8\ \text{CoA} + 6\ \text{H}_2\text{O}$$

The equation for the synthesis of the malonyl CoA used in the previous reaction is

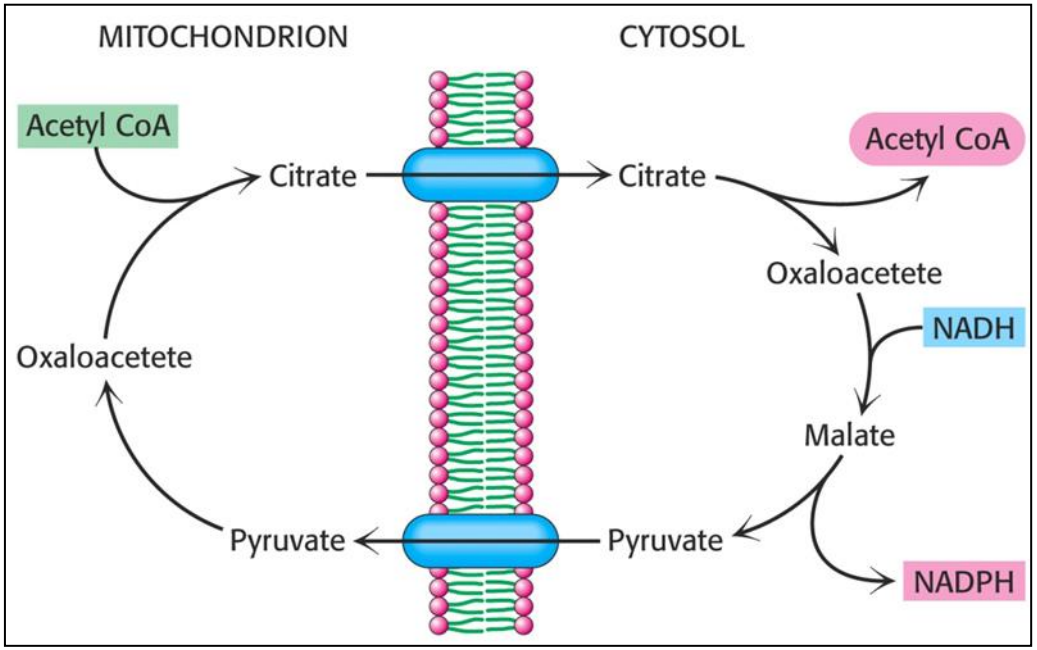
$$7\ \text{Acetyl CoA} + 7\ \text{CO}_2 + 7\ \text{ATP} \longrightarrow 7\ \text{malonyl CoA} + 7\ \text{ADP} + 7\ \text{P}_i + 14\ \text{H}^+$$

Hence, the overall stoichiometry for the synthesis of palmitate is

$$8\ \text{Acetyl CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 6\ \text{H}^+ \longrightarrow \text{palmitate} + 14\ \text{NADP}^+ + 8\ \text{CoA} + 6\ \text{H}_2\text{O} + 7\ \text{ADP} + 7\ \text{P}_i$$

Citrate Shuttle

The synthesis of palmitate requires the input of 8 molecules of acetyl CoA, 14 molecules of NADPH, and 7 molecules of ATP.
Fatty acids are synthesized in the cytosol, whereas acetyl CoA is formed from pyruvate in mitochondria. Hence, acetyl CoA must be transferred from mitochondria to the cytosol. Mitochondria, however, are not readily permeable to acetyl CoA.



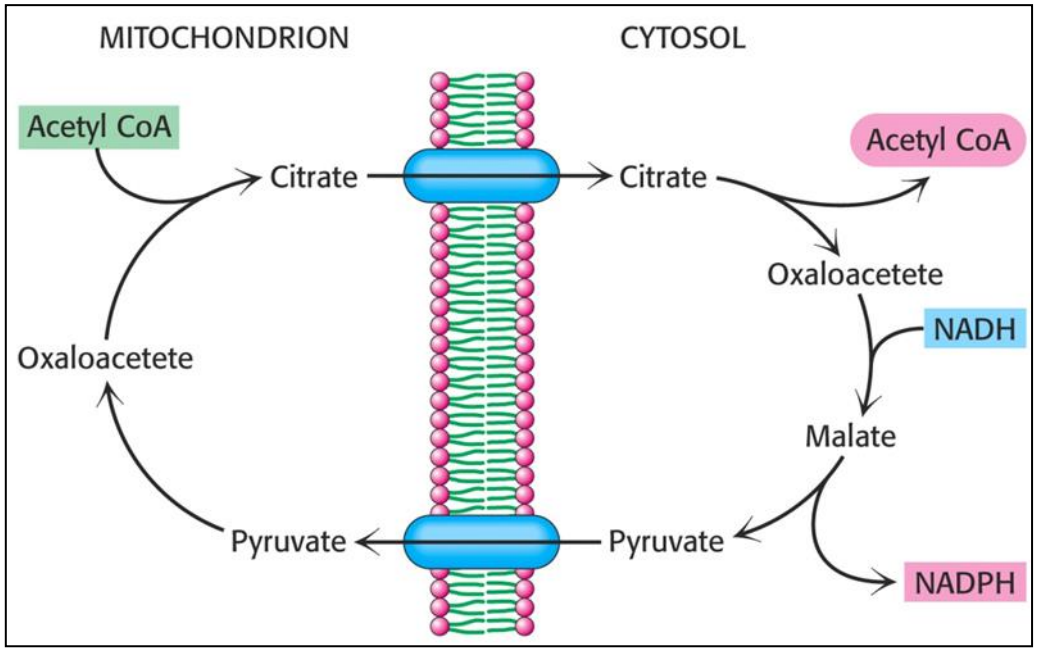
The barrier to acetyl CoA is bypassed by citrate, which carries acetyl groups across the inner mitochondrial membrane. Citrate is formed in the mitochondrial matrix by the condensation of acetyl CoA with oxaloacetate. When present at high levels, citrate is transported to the cytosol, where it is cleaved by ATP-citrate lyase.

$$\text{Citrate} + \text{ATP} + \text{CoA} + \text{H}_2\text{O} \rightleftharpoons \text{acetyl CoA} + \text{ADP} + \text{P}_\text{i} + \text{oxaloacetate}$$

Thus, acetyl CoA and oxaloacetate are transferred from mitochondria to the cytosol at the expense of the hydrolysis of a molecule of ATP.

Sources of NADPH for Fatty Acid Synthesis

Oxaloacetate formed in the transfer of acetyl groups to the cytosol must now be returned to the mitochondria. The inner mitochondrial membrane is impermeable to oxaloacetate. Hence, a series of bypass reactions occurs.



First, the oxaloacetate is reduced to malate catalysed by a *malate dehydrogenase* in the cytosol.

Second, malate is oxidatively decarboxylated by an *$NADP^+$-linked malate enzyme* (also called *malic enzyme*) to form pyruvate.

Cont……

Sources of NADPH for Fatty Acid Synthesis

The pyruvate formed in this reaction readily enters mitochondria, where it is carboxylated to oxaloacetate by pyruvate carboxylase.

Thus, one molecule of NADPH is generated for each molecule of acetyl CoA that is transferred from mitochondria to the cytosol.

Hence, eight molecules of NADPH are formed when eight molecules of acetyl CoA are transferred to the cytosol for the synthesis of palmitate.

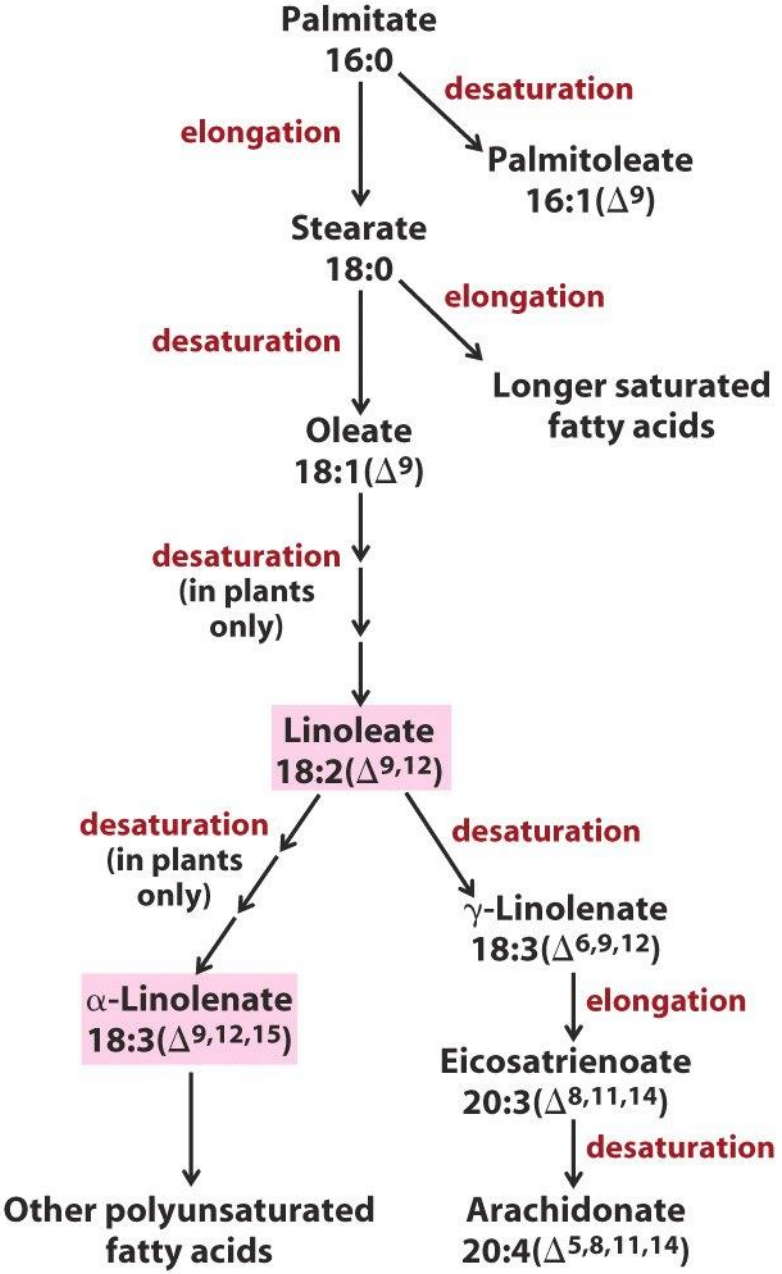
The additional six molecules of NADPH required for this process come from the pentose phosphate pathway.

Palmitic acid modifications

Palmitate is the starting point for other fatty acids that use a set of related reactions to generate the modified chains and head groups of the lipid classes.

Cell makes a pool of palmitic acid that it can elongate and/or desaturate in the ER.

Elongation is similar to synthesis of palmitate because it uses malonyl-CoA as an intermediate.



Control of fatty acid synthesis

When an organism has more than enough metabolic fuel to meet its energy needs, the excess is converted to fatty acids and stored as triglycerides.

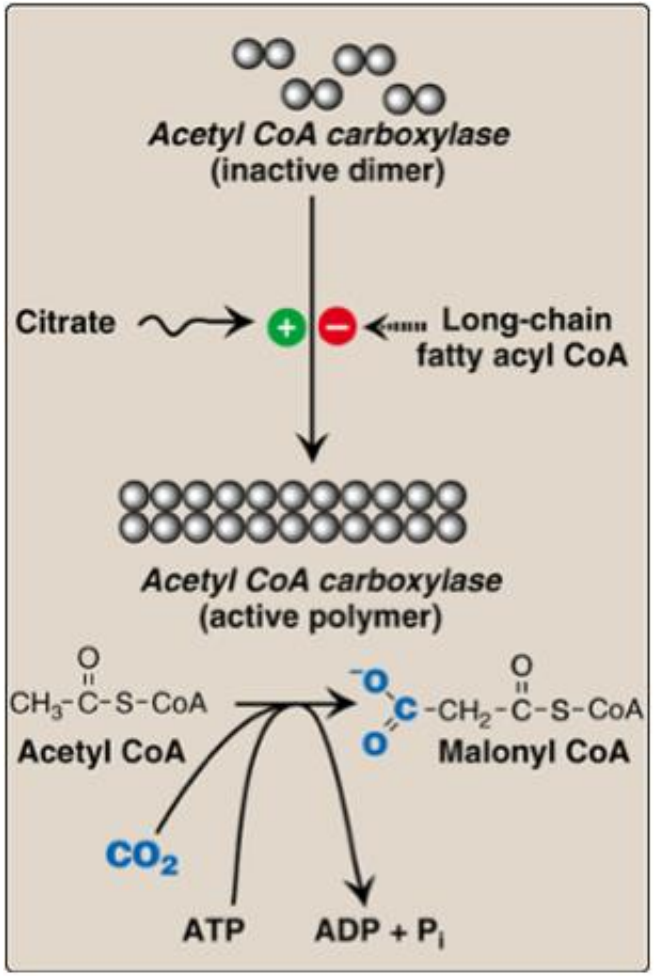
One of the key regulatory step that control fatty acid synthesis is at the level of malonyl CoA. The synthesis of malonyl CoA is catalysed by *Acetyl-CoA Carboxylase* which is a rate limiting step in the biosynthesis of fatty acids.

Regulation of Acetyl-CoA Carboxylase

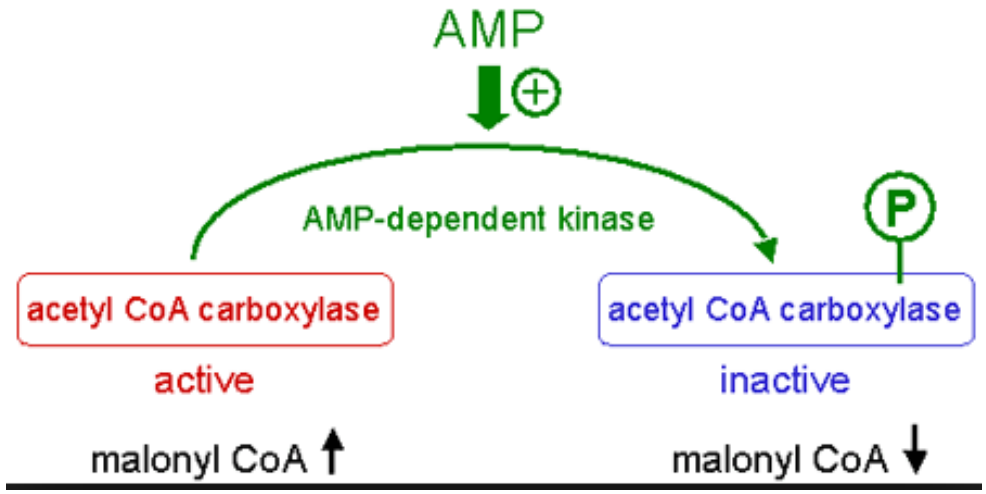
The mammalian enzyme is regulated by
A) Allosteric control by local metabolites
B) Phosphorylation
C) Conformational change
    1. active conformation is multimeric filamentous structure
    2. inactive conformation is non- filamentous form

Regulation of Acetyl-CoA Carboxylase

Regulation by
Conformational change

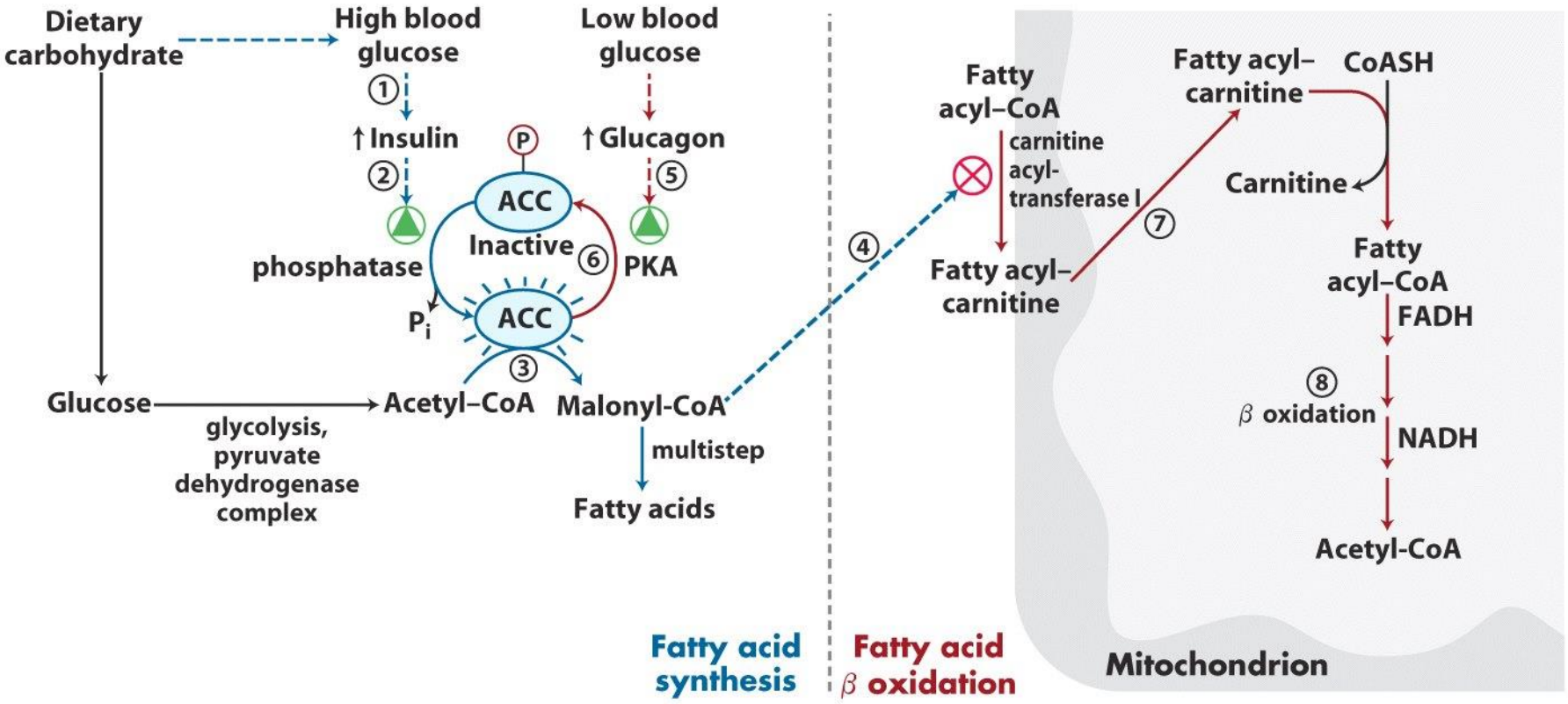


Regulation by
Allosteric control and
Phosphorylation



glucagon,
epinephrine
trigger phosphorylation/
inactivation

Nutritional state regulates fatty acid synthesis



During well fed state, the FA synthesis is elevated due to high level of glucose and acetyl-CoA which is also supported by insulin that activates ***Acetyl-CoA Carboxylase (ACC)*** by dephosphorylation .

In low fed state, the blood glucose is low, that activates PKA which inhibits ***Acetyl-CoA Carboxylase*** by phosphorylation *that in turn reduces fatty acid synthesis.*

Differences between Fatty acid synthesis and degradation (beta oxidation)

Fatty acid (FA) synthesis is not a reversal of the beta oxidation of FA (degradative pathway). Some important differences between the pathways are:

- Synthesis takes place in the *cytosol*, however degradation, takes place primarily in the mitochondrial matrix.
- Intermediates in FA synthesis are covalently linked to the sulfhydryl groups of ACP, whereas intermediates in FA breakdown are linked to Coenzyme A.
- The enzymes of FA synthesis in higher organisms are joined in a *single polypeptide chain* called *fatty acid synthase*. In contrast, the degradative enzymes do not seem to be associated.
- The growing FA chain is elongated by the *sequential addition of two-carbon units* derived from acetyl CoA. The activated donor of two carbon units in the elongation step is *malonyl ACP*.
- The reductant in FA synthesis is NADPH, whereas the oxidants in FA degradation are NAD^+ and FAD.
- Elongation stops at C_{16} (palmitic acid)

Importance of Fatty acid synthesis

It is a critical anabolic pathway in most organisms. In addition to being the major component of membranes, fatty acids are important energy storage molecules, and fatty acyl derivatives possess a variety of physiological functions, including post-translational modification of numerous proteins. Main functions of fatty acids are:

Energy – high per gram (37 kJ/gram fat)

Transportable form of energy – blood lipids (e.g. triacylglycerol in lipoproteins)

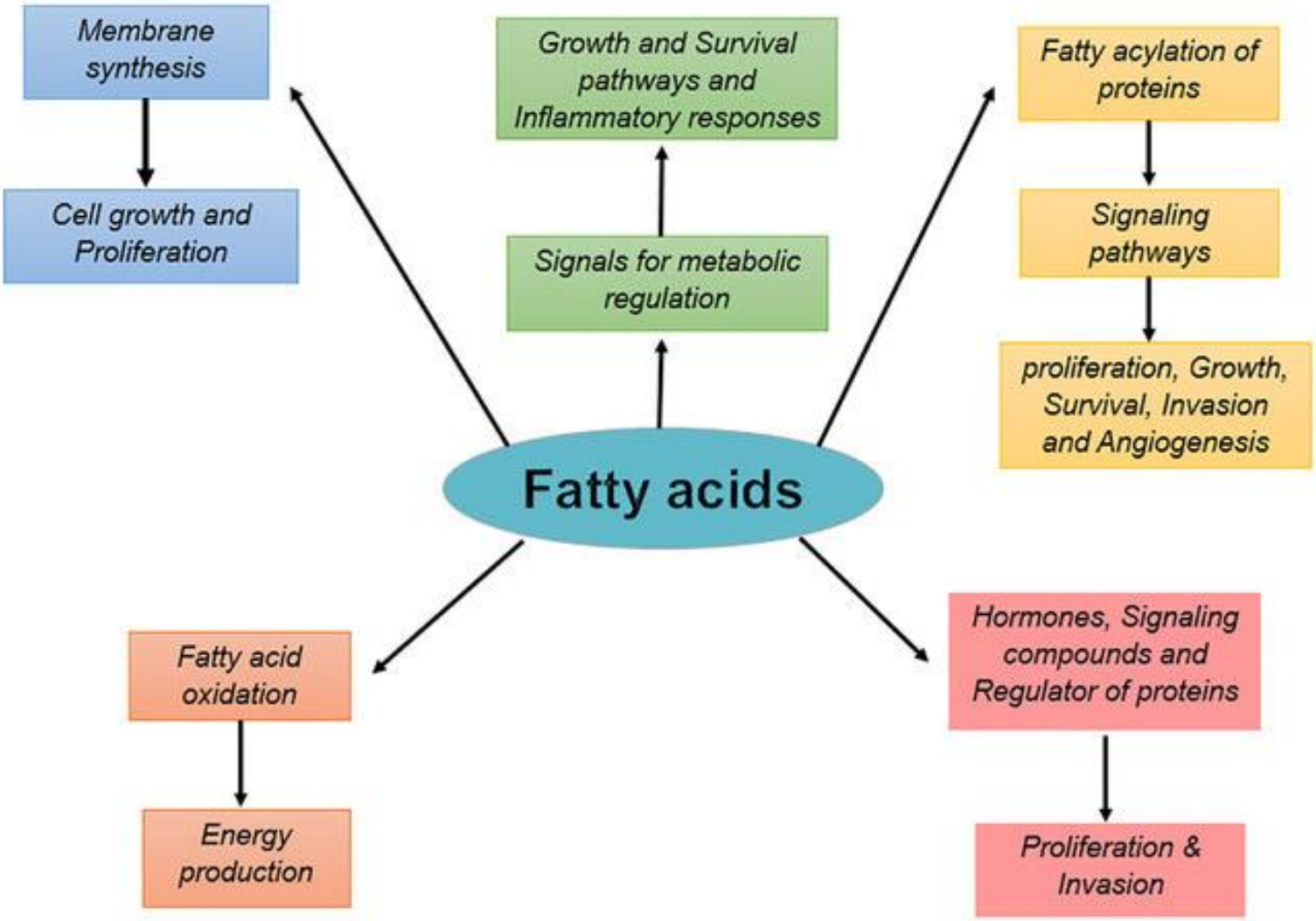
Storage of energy, e.g. in adipose tissue and skeletal muscle

Component of cell membranes (phospholipids)

Insulation - thermal etc

Signals – eicosanoids, gene regulation (transcription)

Importance of Fatty acids in animals



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