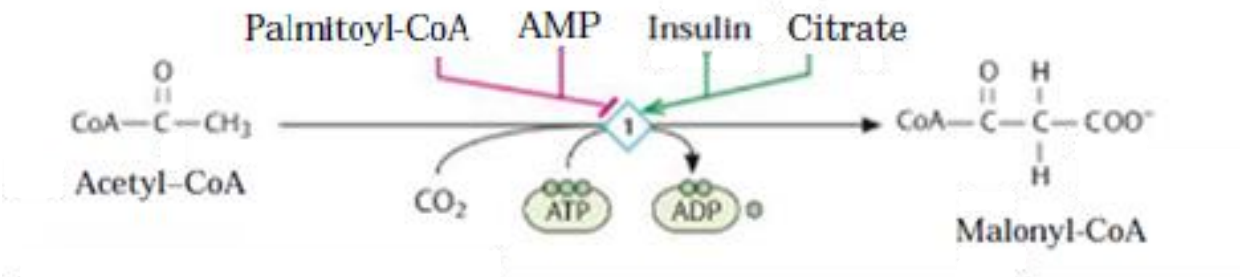
BIOSYNTHESIS OF FATTY ACIDS



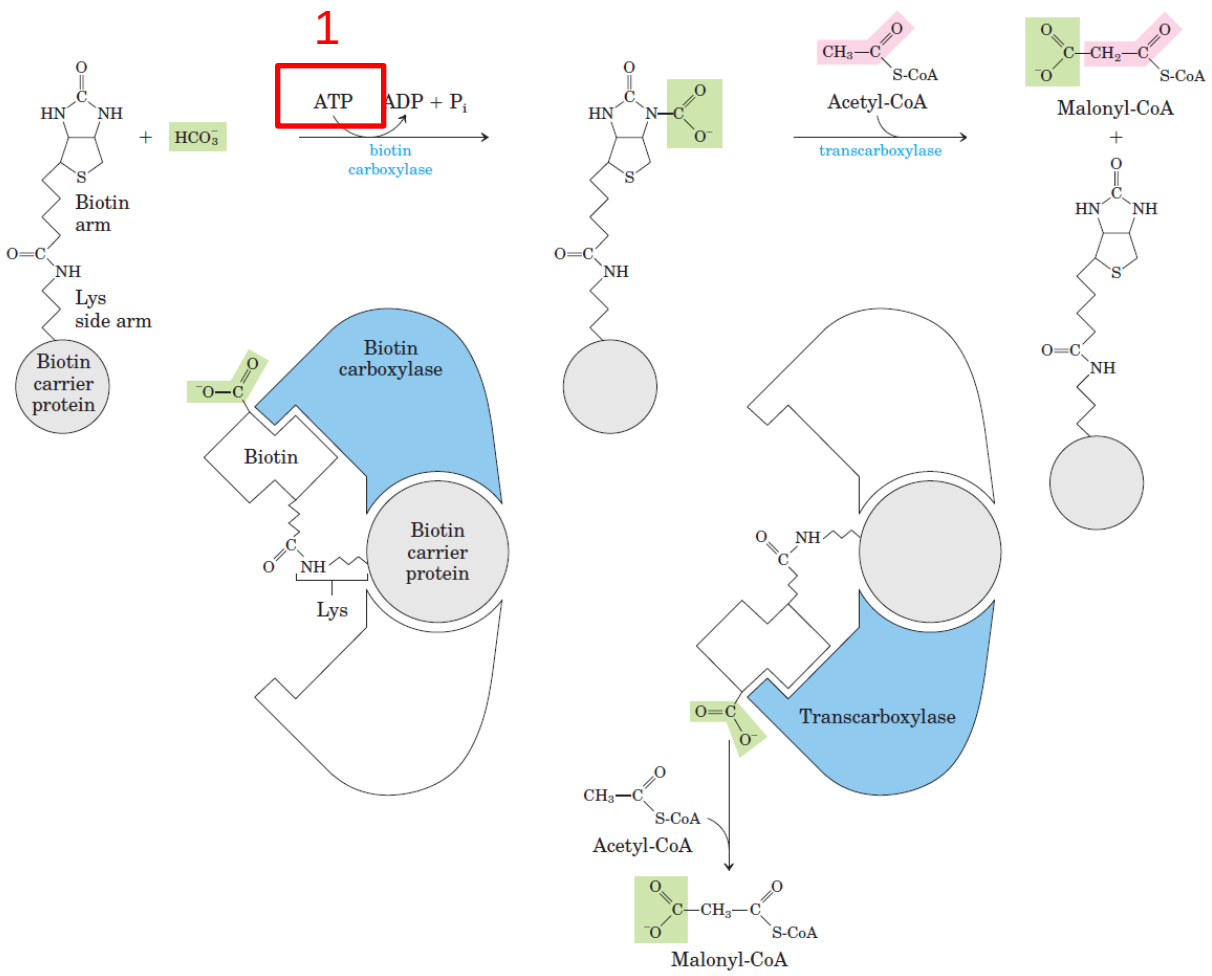
1 = acetyl-CoA carboxylase

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.

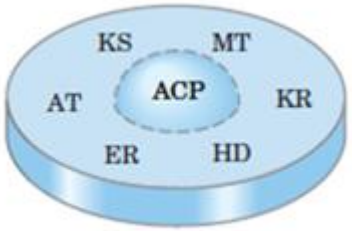
Biosynthesis requires the participation of a three-carbon intermediate, **malonyl-CoA,** that is not involved in fatty acid breakdown.

The acetyl-CoA carboxylase reaction

Acetyl-CoA carboxylase has three functional regions: biotin carrier protein (gray); biotin carboxylase, which activates CO2 by attaching it to a nitrogen in the biotin ring in an ATP-dependent reaction; and transcarboxylase, which transfers activated CO_2 (shaded green) from biotin to acetyl-CoA, producing malonyl-CoA. The long, flexible biotin arm carries the activated CO_2 from the biotin carboxylase region to the transcarboxylase active site. The active enzyme in each step is shaded blue.

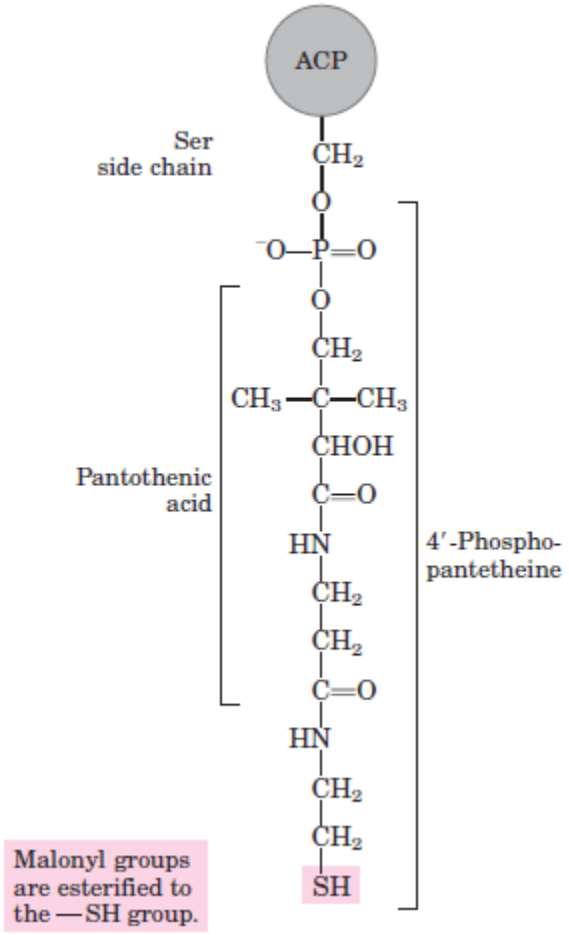


Fatty acid synthase in vertebrates



The vertebrate enzyme is a single large polypeptide.

Component	*Function*
Acyl carrier protein (ACP)	Carries acyl groups in thioester linkage
Acetyl-CoA–ACP transacetylase (AT)	Transfers acyl group from CoA to Cys residue of KS
β-Ketoacyl-ACP synthase (KS)	Condenses acyl and malonyl groups (KS has at least three isozymes)
Malonyl-CoA–ACP transferase (MT)	Transfers malonyl group from CoA to ACP
β-Ketoacyl-ACP reductase (KR)	Reduces β-keto group to β-hydroxyl group
β-Hydroxyacyl-ACP dehydratase (HD)	Removes H_2O from β-hydroxyacyl-ACP, creating double bond
Enoyl-ACP reductase (ER)	Reduces double bond, forming saturated acyl-ACP

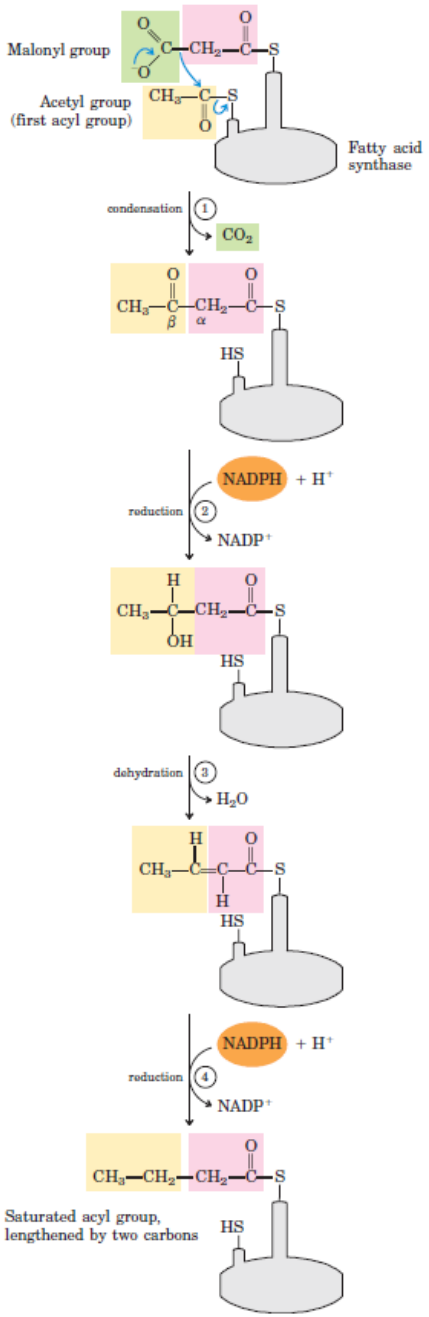


Acyl carrier protein (ACP)

The prosthetic group is 4-phosphopantetheine, which is covalently attached to the hydroxyl group of a Ser residue in ACP.

Phosphopantetheine contains the B vitamin pantothenic acid, also found in the coenzyme A molecule.

Its SH group is the site of entry of malonyl groups during fatty acid synthesis.



Addition of two carbons to a growing fatty acyl chain: a four-step sequence. Each malonyl group and acetyl (or longer acyl) group is activated by a thioester that links it to fatty acid synthase, a multienzyme complex.

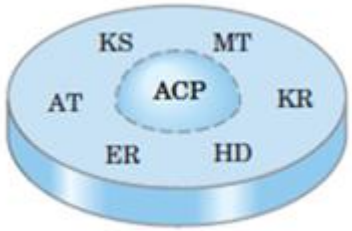
1 Condensation of an activated acyl group (an acetyl group from acetyl-CoA is the first acyl group) and two carbons derived from malonyl-CoA, with elimination of CO_2 from the malonyl group, extends the acyl chain by two carbons. Decarboxylation facilitates condensation.

The β-keto product of this condensation is then reduced in three more steps nearly identical to the reactions of oxidation, but in the reverse sequence:

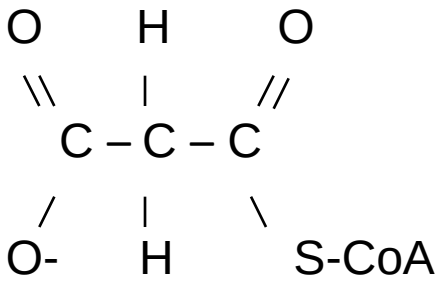
2 the β-keto group is reduced to an alcohol,

3 elimination of H_2O creates a double bond,

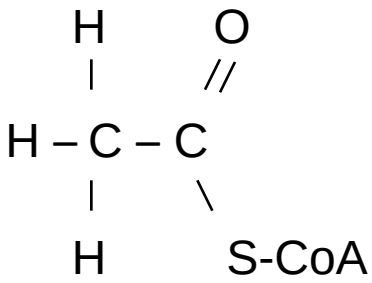
4 the double bond is reduced to form the corresponding saturated fatty acyl group.

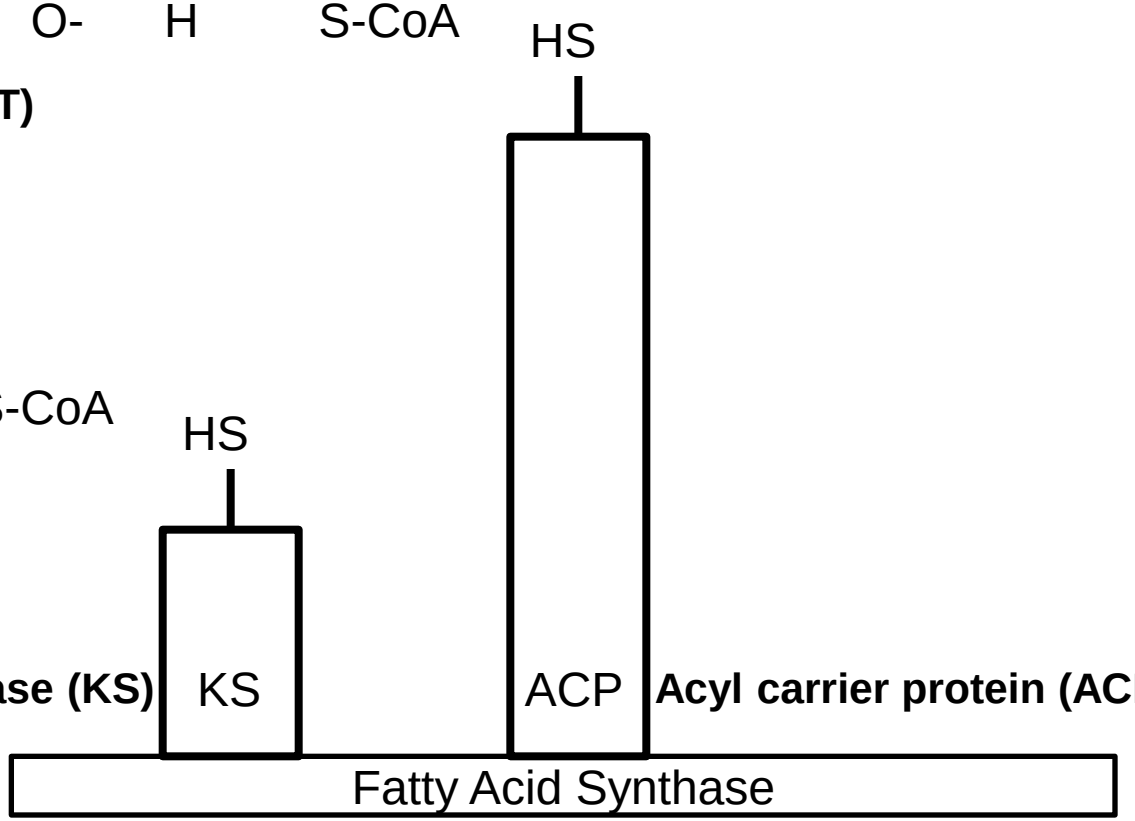


Malonyl-CoA–ACP transferase (MT)



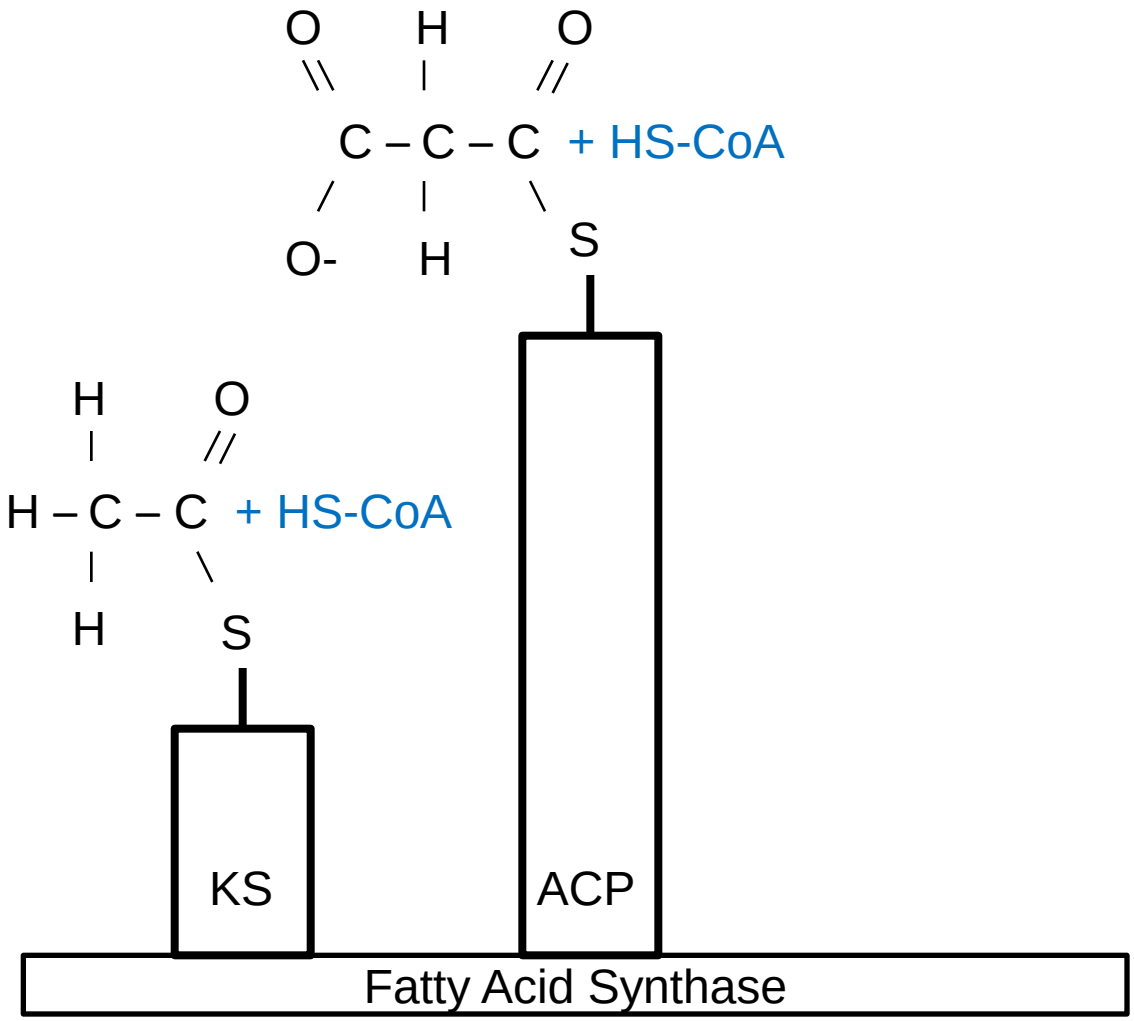
Acetyl-CoA–ACP transacetylase (AT)



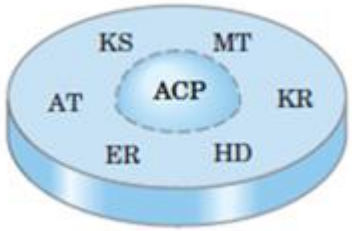


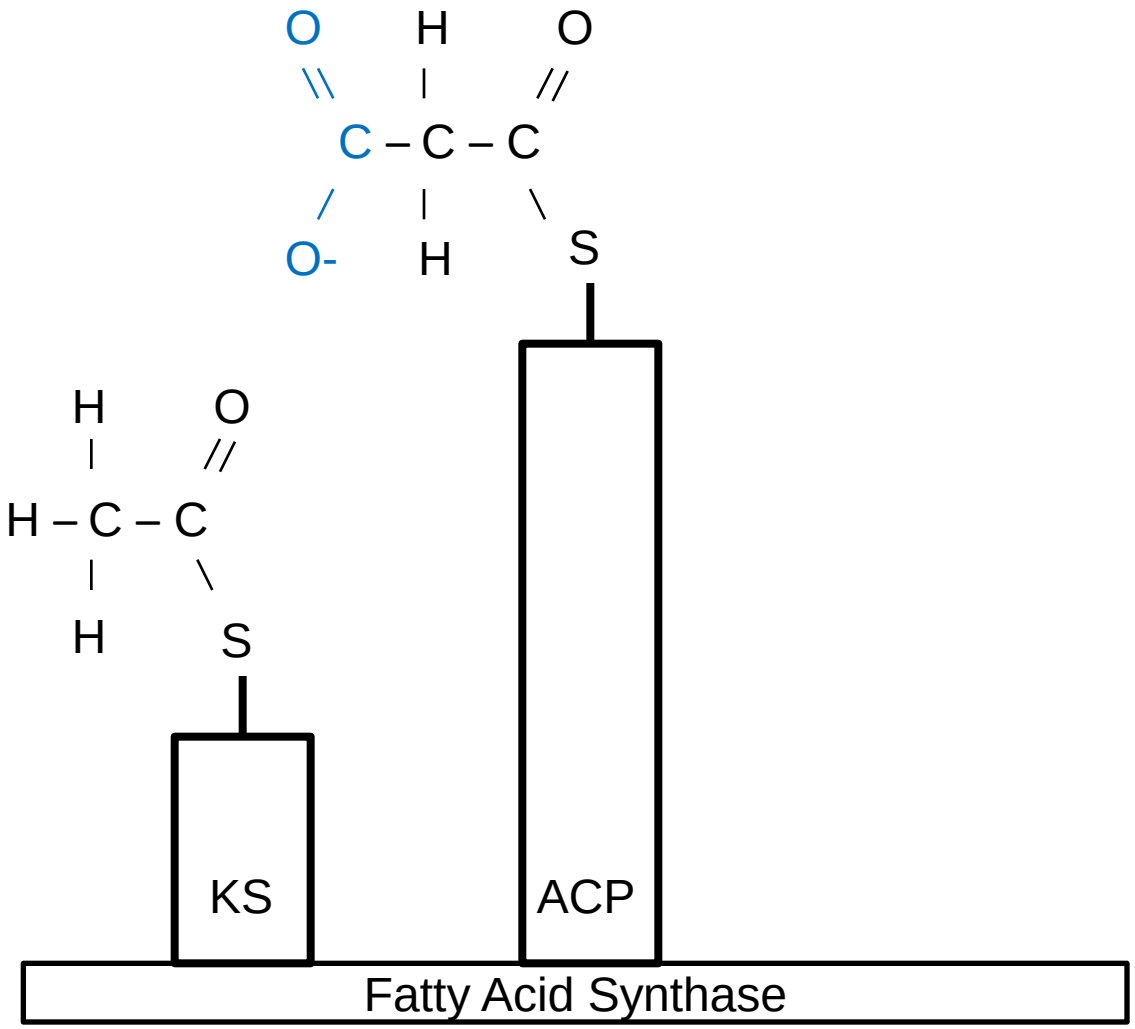
β-ketoacyl-ACP synthase (KS)

Acyl carrier protein (ACP)


O H O
C – C – C + HS-CoA
O- H S
H O
H – C – C + HS-CoA
H S
KS
ACP
Fatty Acid Synthase

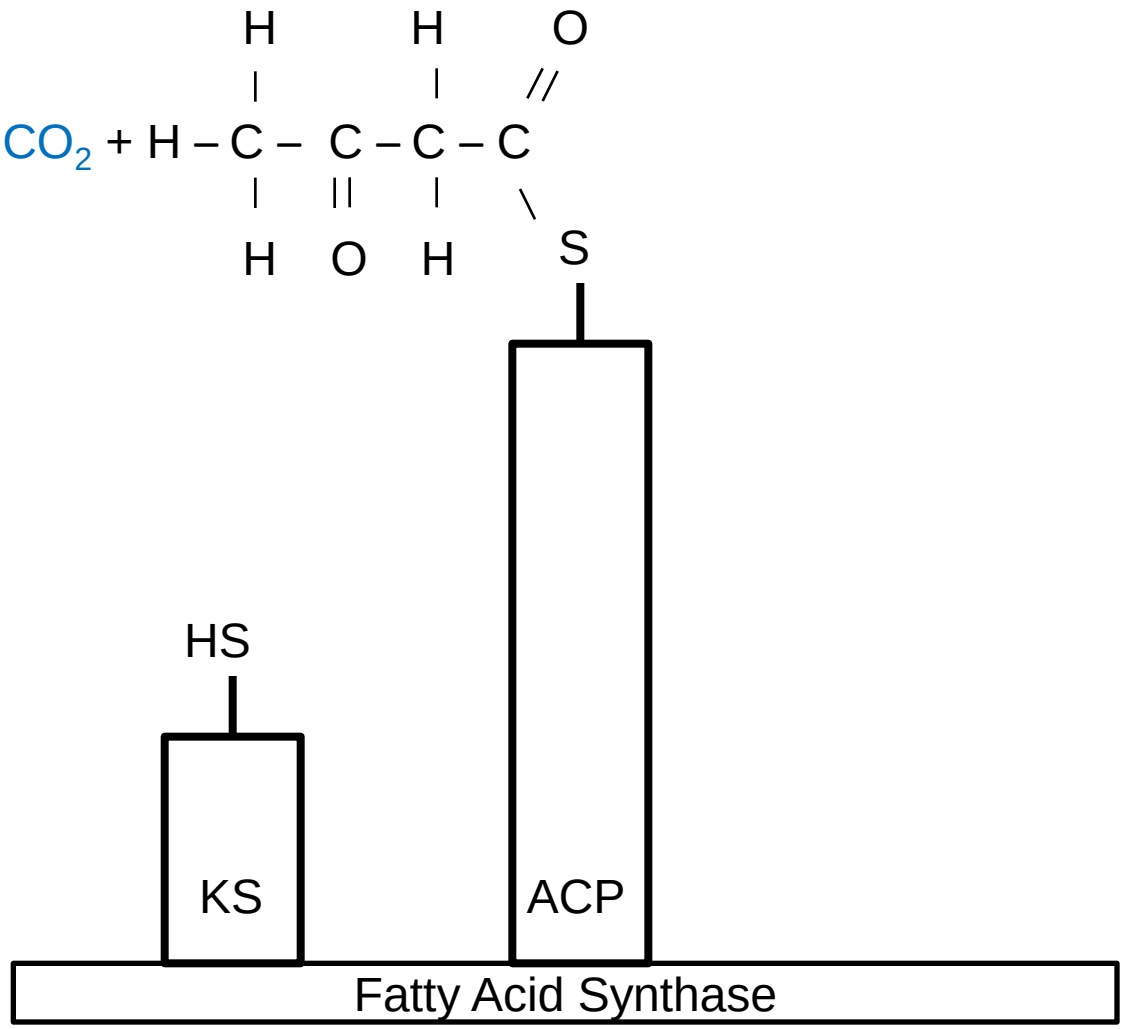
Step 1 Condensation



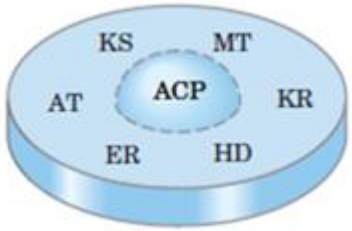


β-ketoacyl-ACP synthase (KS)

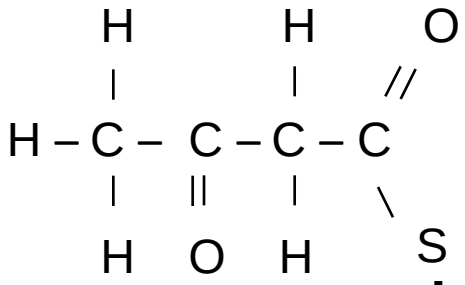
Step 1 Condensation

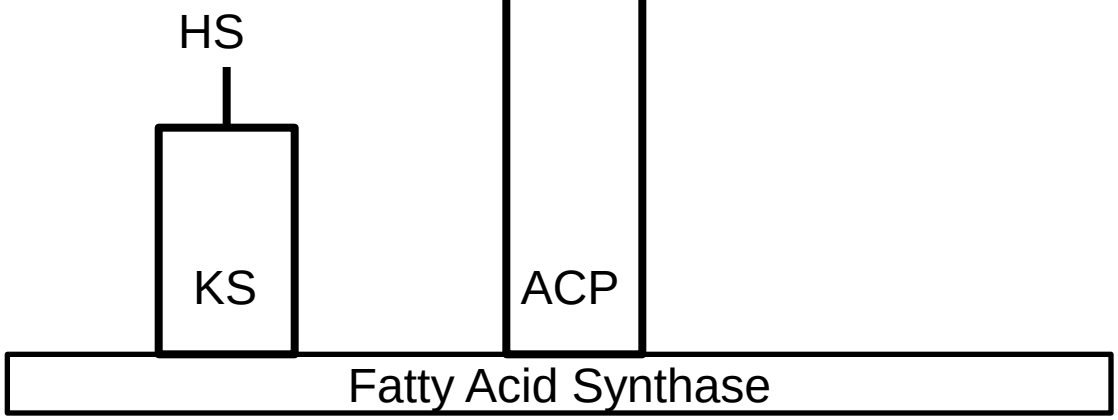


Step 2 Reduction of the Carbonyl Group



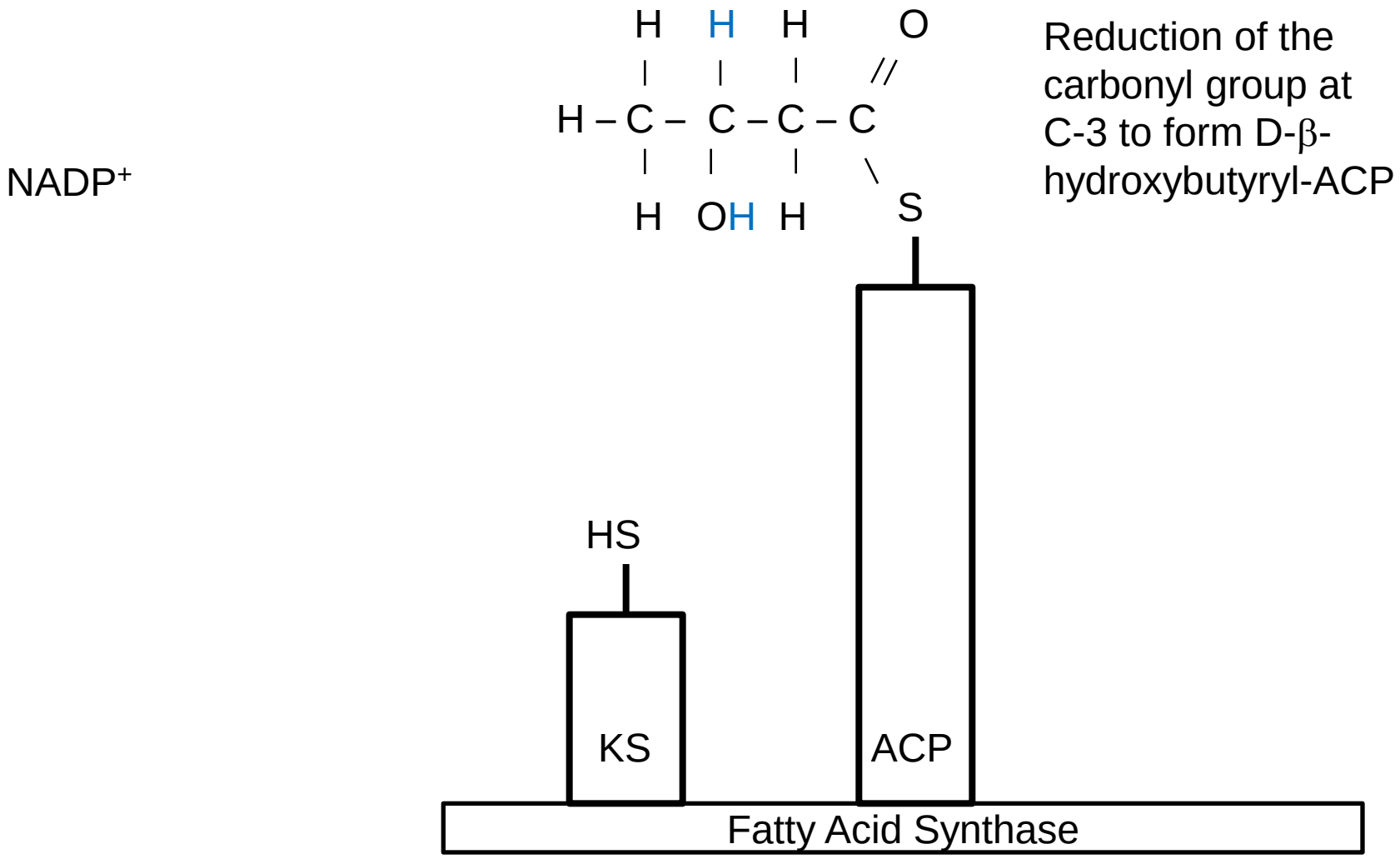
NADPH + H^+





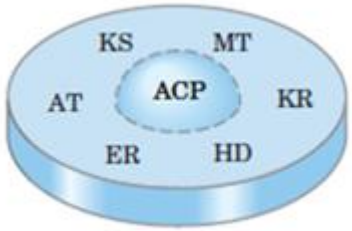
β-ketoacyl-ACP reductase (KR)

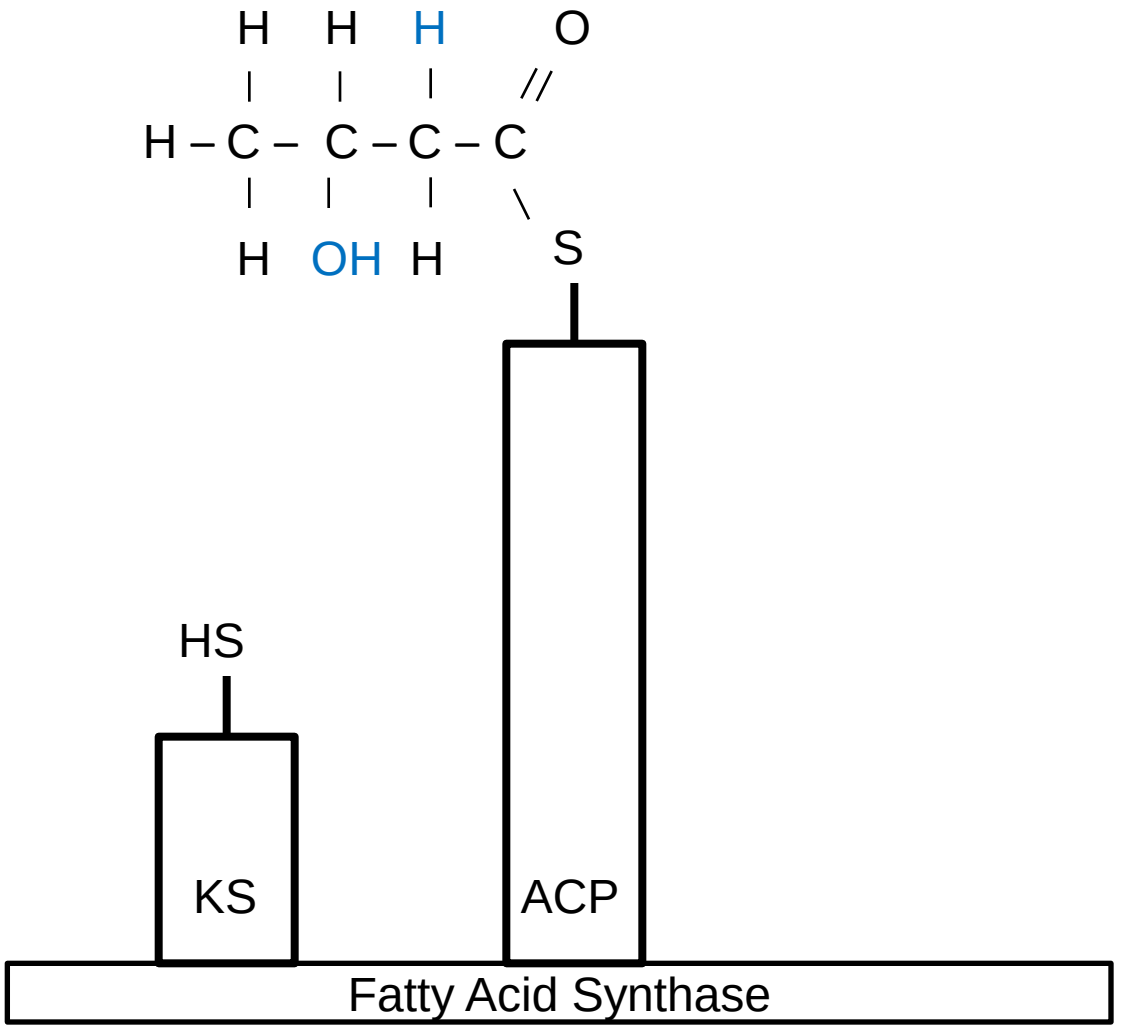
Step 2 Reduction of the Carbonyl Group



Reduction of the carbonyl group at C-3 to form D-β-hydroxybutyryl-ACP

Step 3 Dehydration

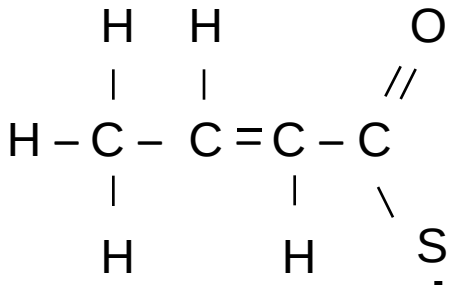




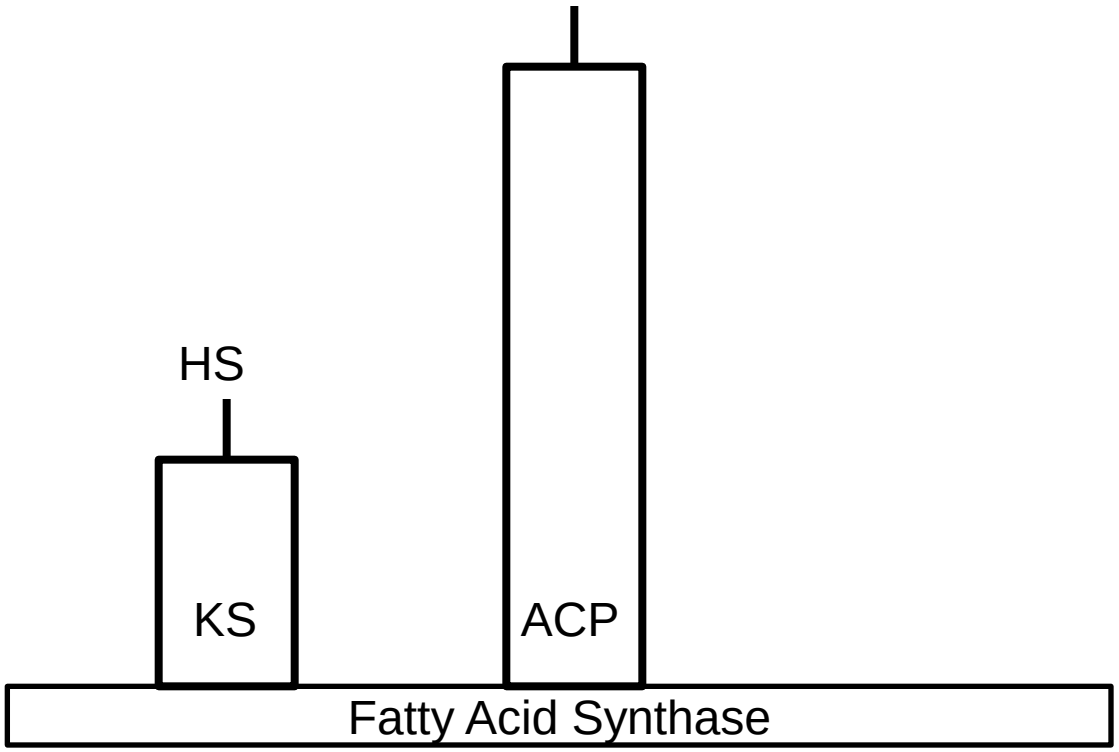
β-hydroxyacyl-ACP dehydratase (HD)

Step 3 Dehydration

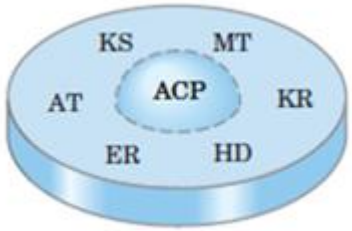
H_2O



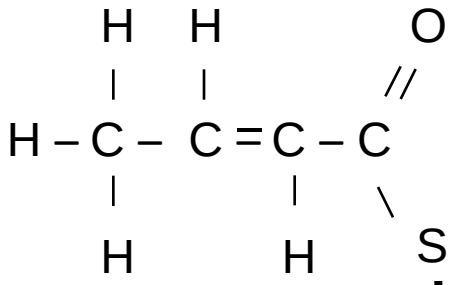
Water is removed from C-2 and C-3 of to yield trans-Δ_2-butenoyl-ACP

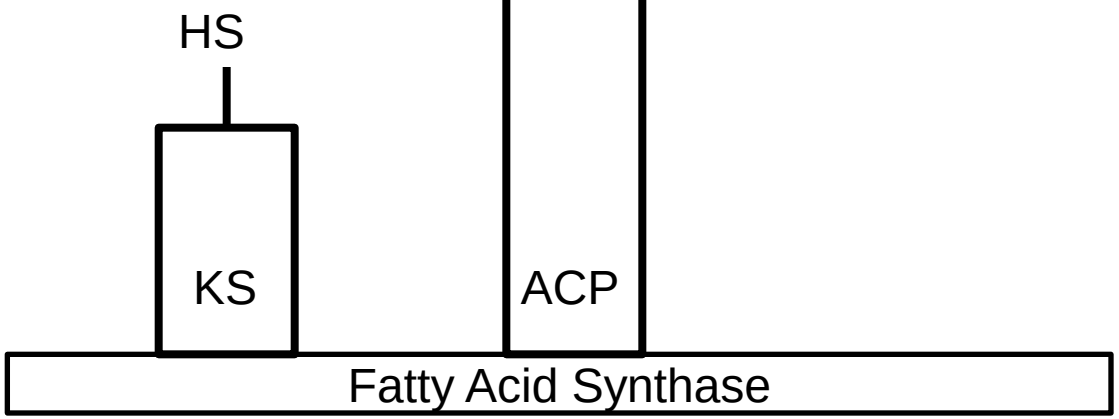


Step 4 Reduction of the Double Bond



NADPH + H^+

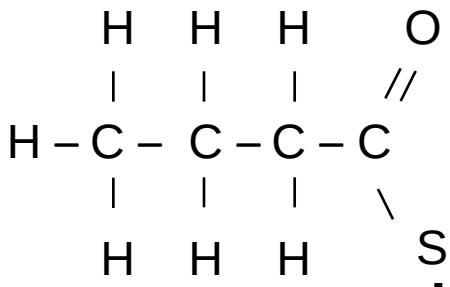




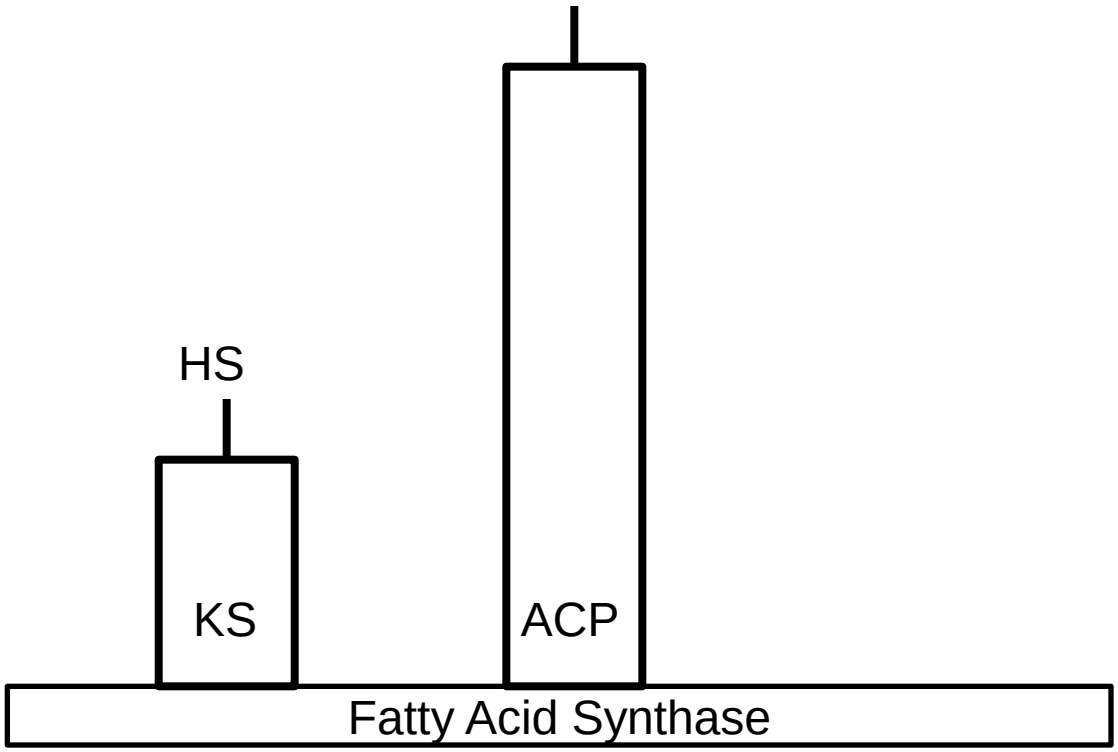
enoyl-ACP reductase (ER)

Step 4 Reduction of the Double Bond

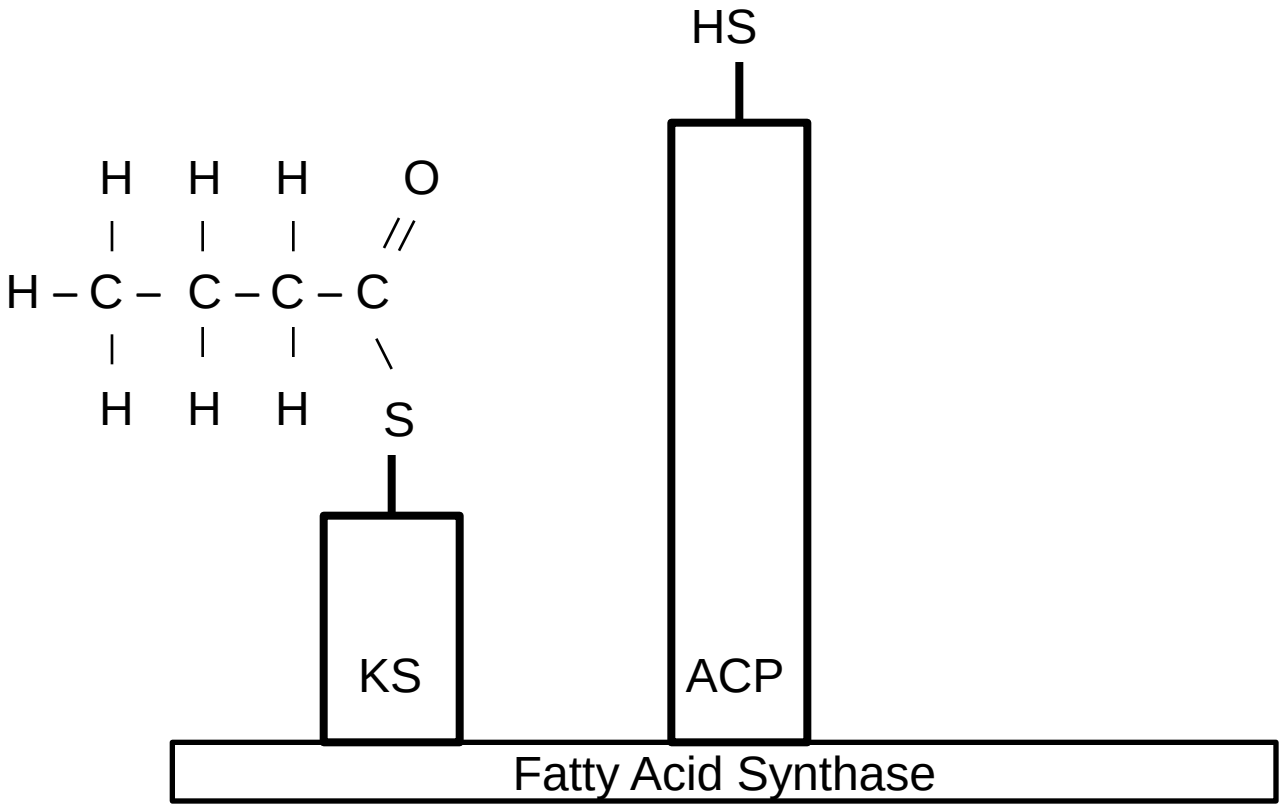
NADP$^+$



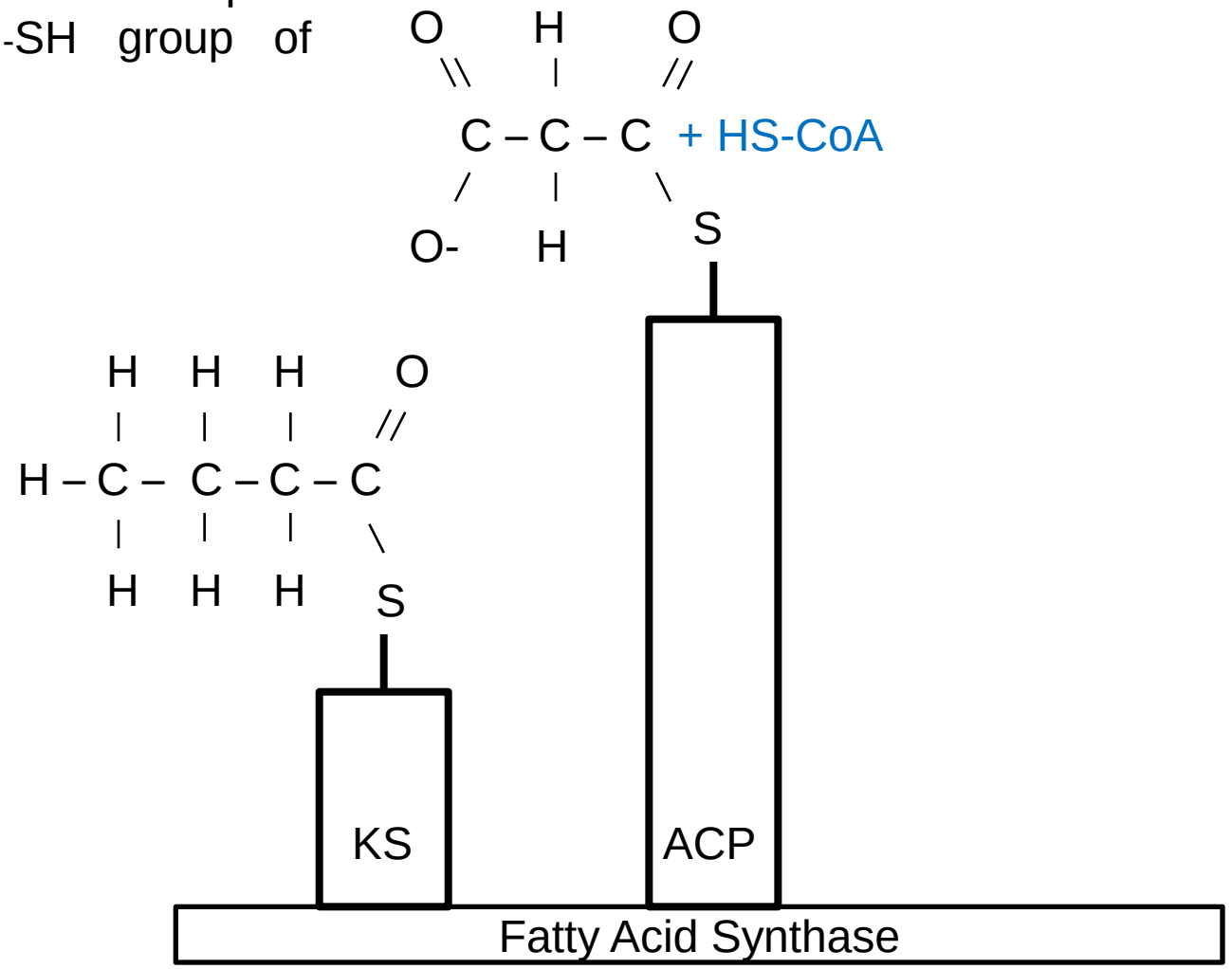
The double bond of *trans*-Δ_2-butenoyl-ACP is reduced (saturated) to form **butyryl-ACP**

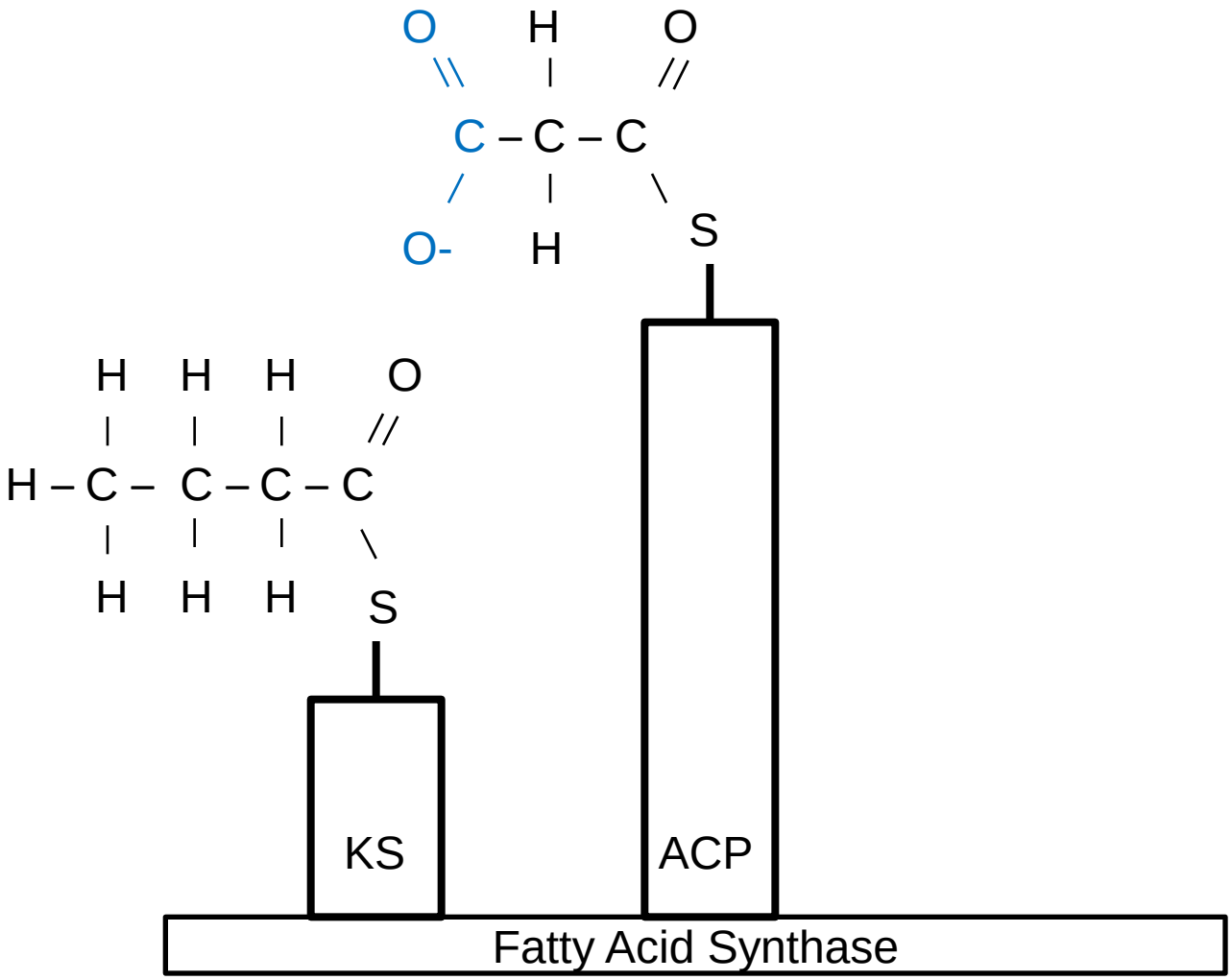


The butyryl group is now transferred from the phosphopantetheine -SH group of ACP to the Cys –SH group of β-ketoacyl-ACP synthase, which initially bore the acetyl group.

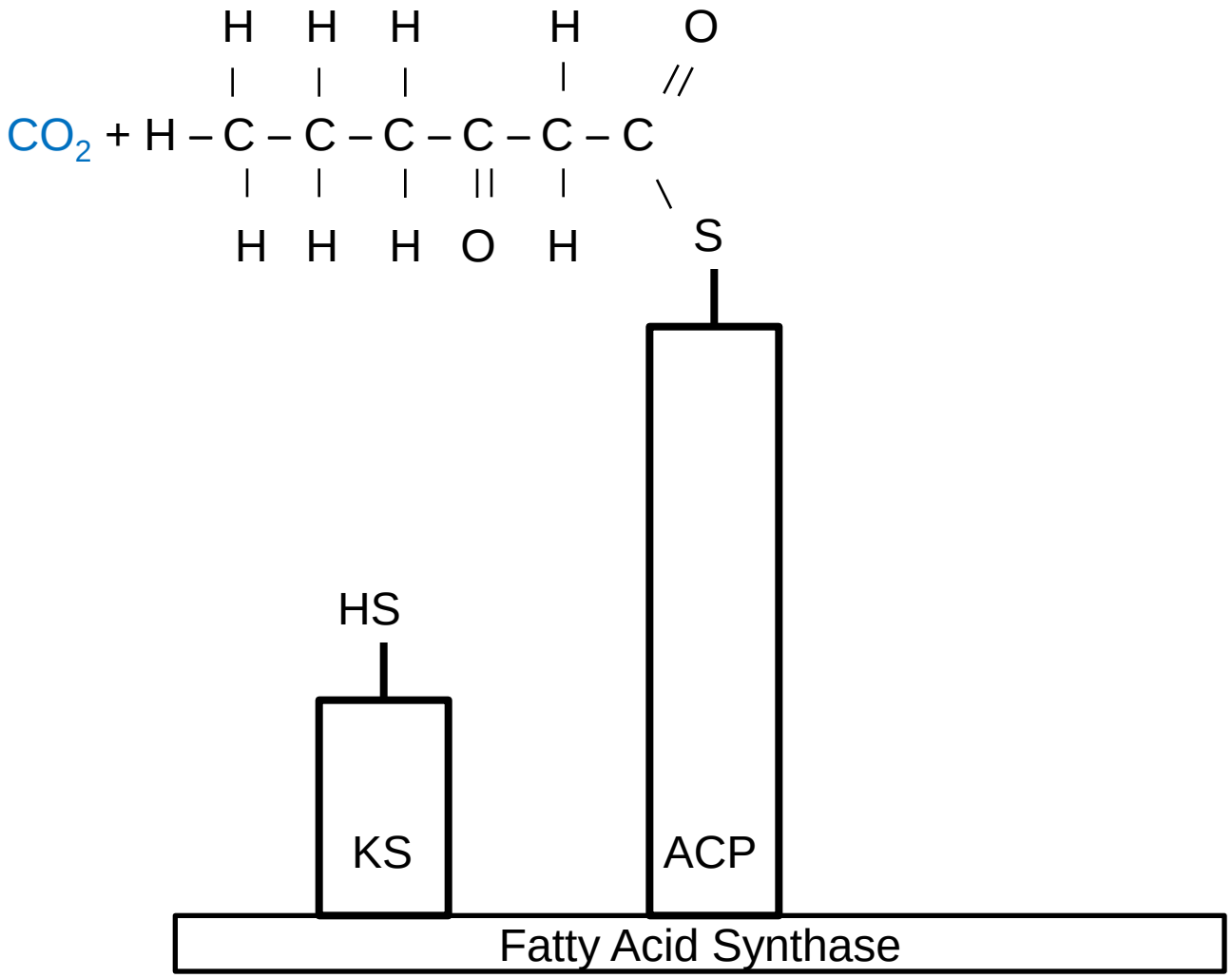


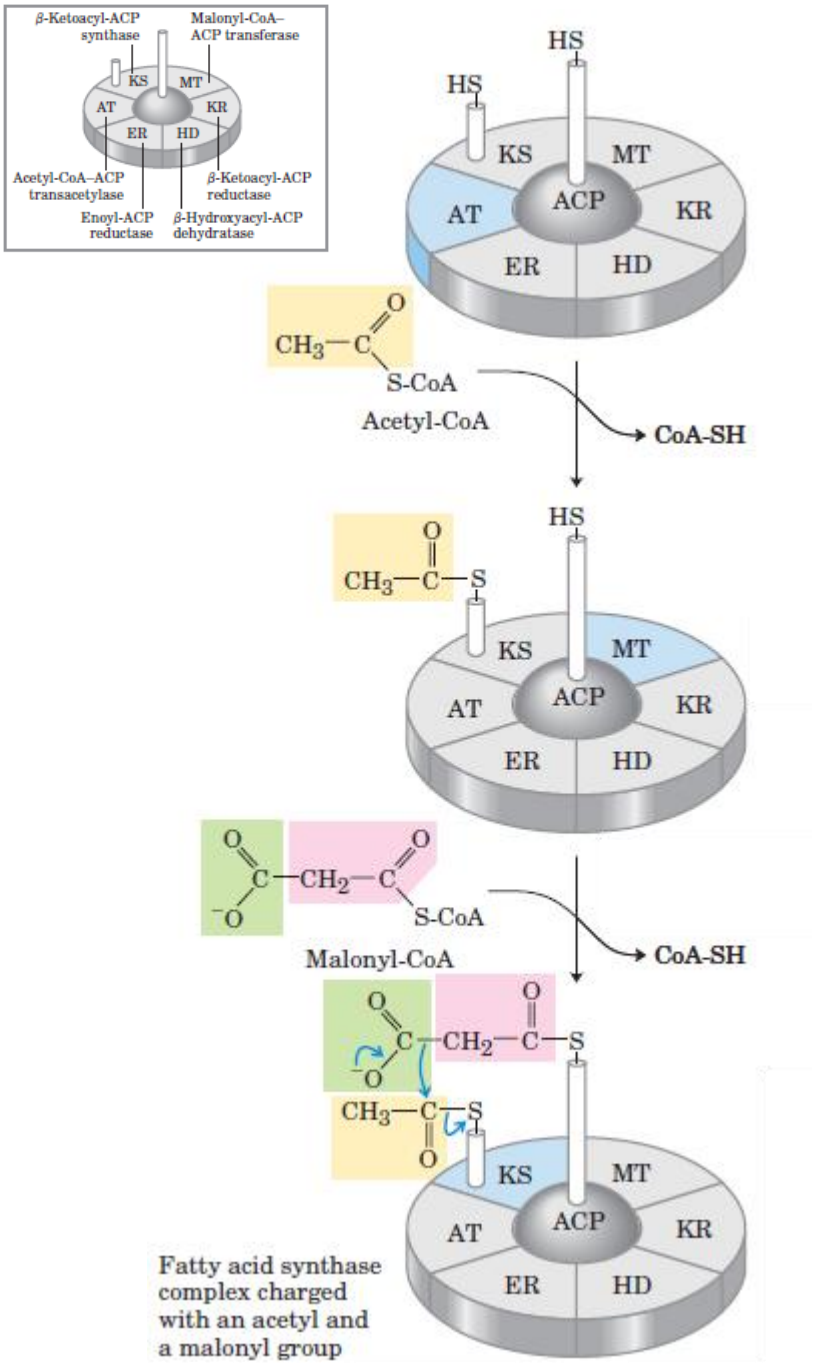
To start the next cycle of four reactions that lengthens the chain by two more carbons, another malonyl group is linked to the now unoccupied phosphopantetheine -SH group of ACP




O
H
O
C – C – C
O-
H
S
H H H O
H – C – C – C – C
H H H
S
KS
ACP
Fatty Acid Synthase

Step 1 Condensation [2^ elongation cycle]



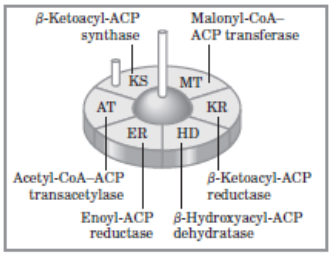


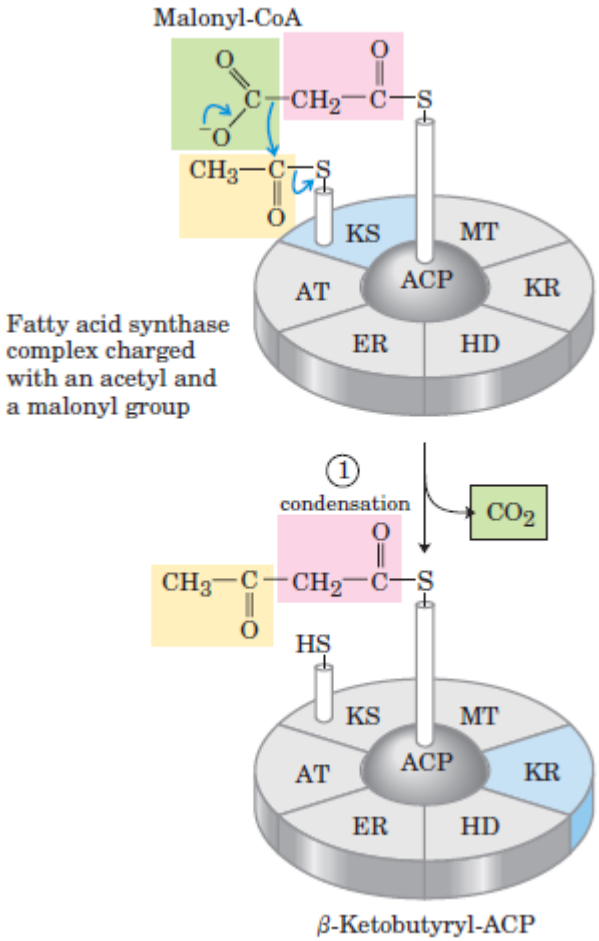
Before the condensation reactions that build up the fatty acid chain can begin, the two thiol groups on the enzyme complex must be charged with the correct acyl groups.

First, the acetyl group of acetyl-CoA is transferred to the Cys -SH group of the β-ketoacyl-ACP synthase. This reaction is catalyzed by **acetyl-CoA–ACP transacetylase** (AT)

The second reaction, transfer of the malonyl group from malonyl-CoA to the -SH group of ACP, is catalyzed by **malonyl-CoA–ACP transferase** (MT), also part of the complex.

In the charged synthase complex, the acetyl and malonyl groups are very close to each other and are activated for the chain-lengthening process.

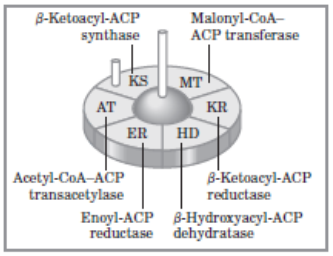




Step 1 Condensation

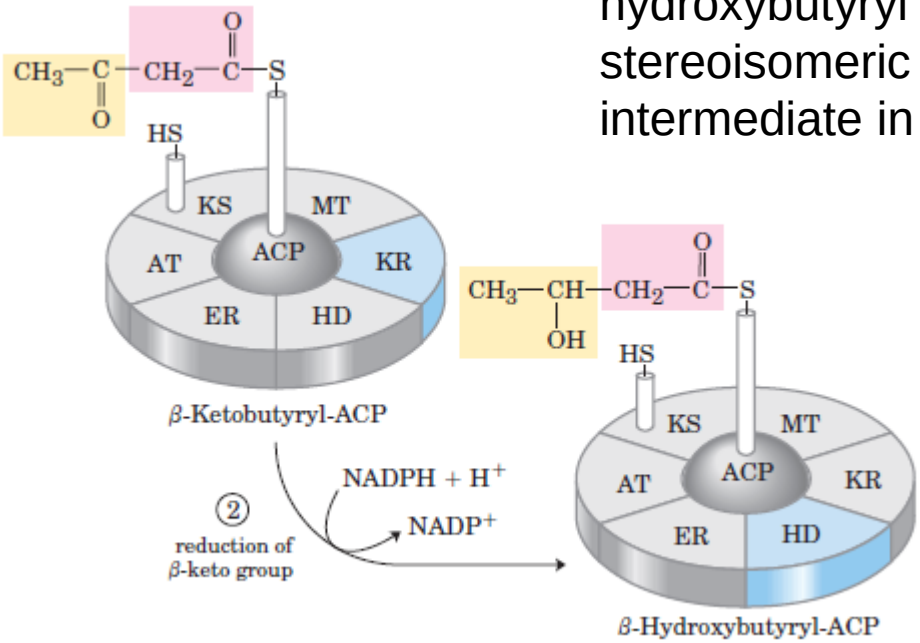
The first reaction in the formation of a fatty acid chain is condensation of the activated acetyl and malonyl groups to form **acetoacetyl-ACP,** an acetoacetyl group bound to ACP through the phosphopantetheine -SH group; simultaneously, a molecule of CO_2 is produced.

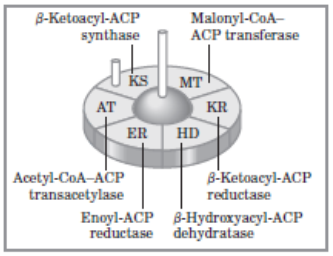
In this reaction, catalyzed by **β-ketoacyl-ACP synthase** (KS), the acetyl group is transferred from the Cys -SH group of the enzyme to the malonyl group on the -SH of ACP, becoming the methyl-terminal two-carbon unit of the new acetoacetyl group.

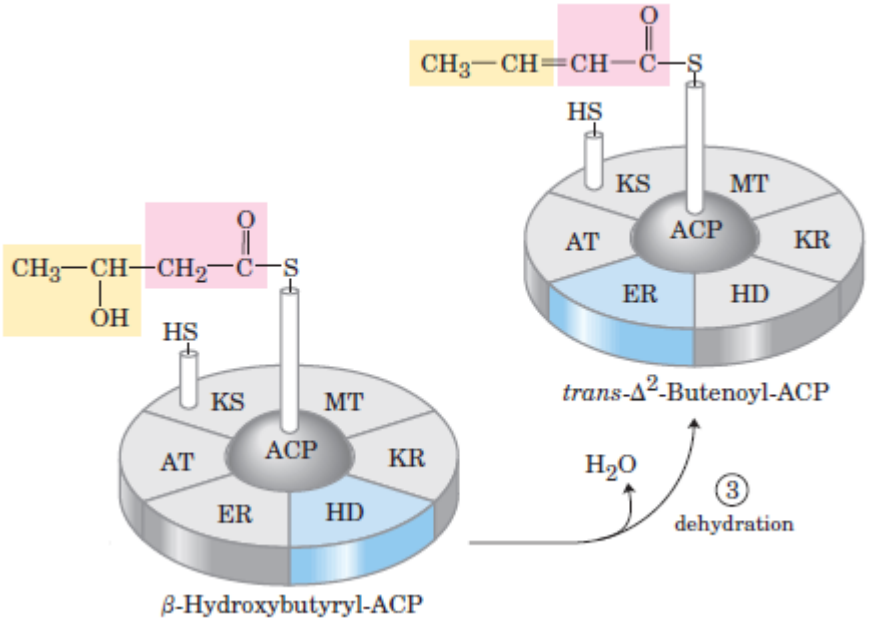


Step 2 Reduction of the Carbonyl Group

The acetoacetyl-ACP formed in the condensation step now undergoes reduction of the carbonyl group at C-3 to form D-β-hydroxybutyryl-ACP. This reaction is catalyzed by **β-ketoacyl-ACP reductase** (KR) and the electron donor is NADPH. Notice that the D-β-hydroxybutyryl group does not have the same stereoisomeric form as the L-β- hydroxyacyl intermediate in fatty acid oxidation.



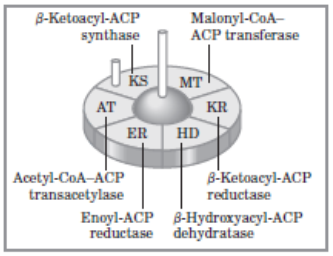


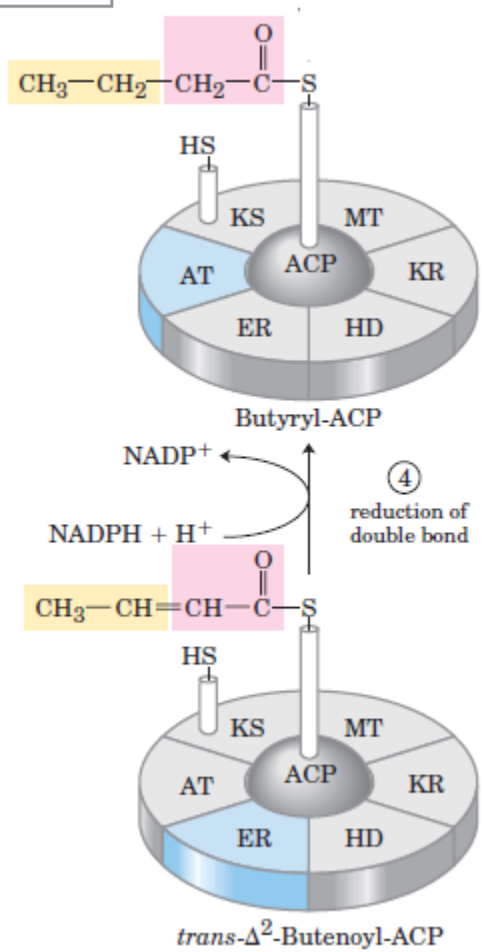


Step 3 Dehydration

The elements of water are now removed
from C-2 and C-3 of D-β-hydroxybutyryl-ACP to yield a double bond in the product, **trans-Δ_2- butenoyl-ACP.**

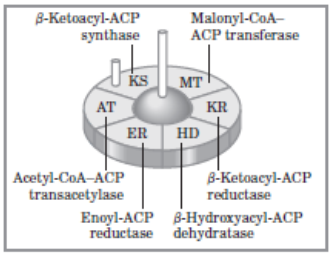
The enzyme that catalyzes this dehydration is the **β-hydroxyacyl-ACP dehydratase** (HD).

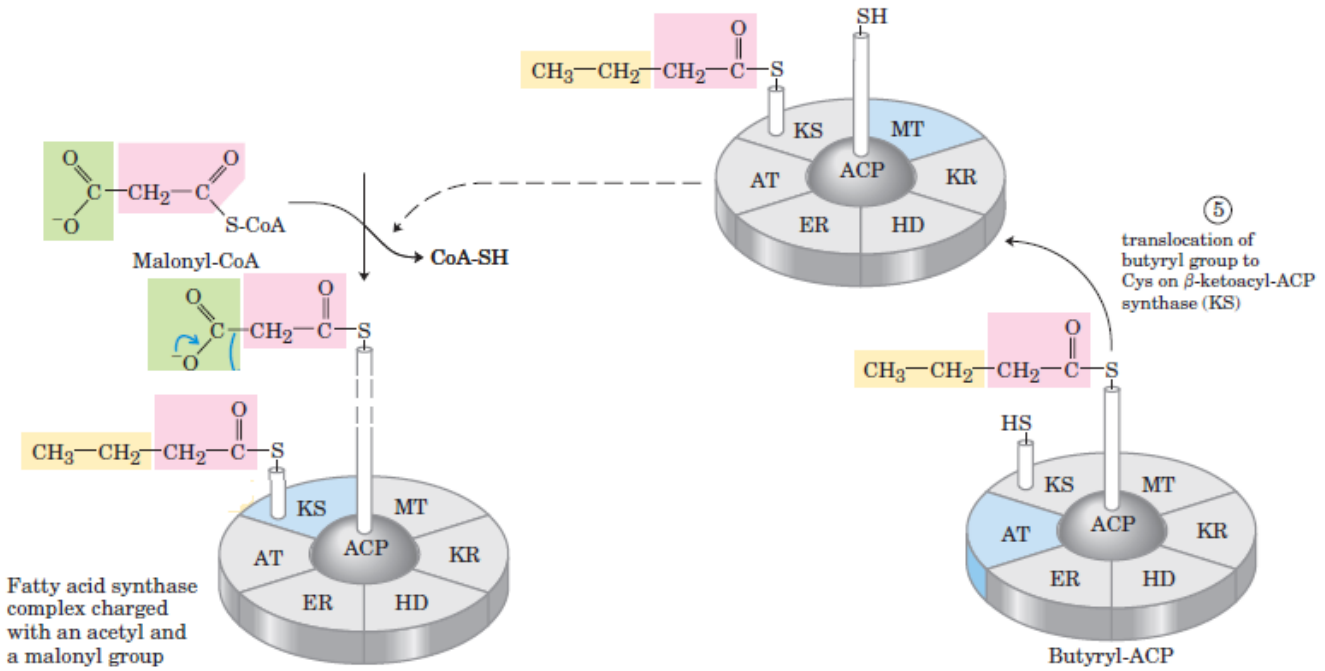




Step 4 Reduction of the Double Bond

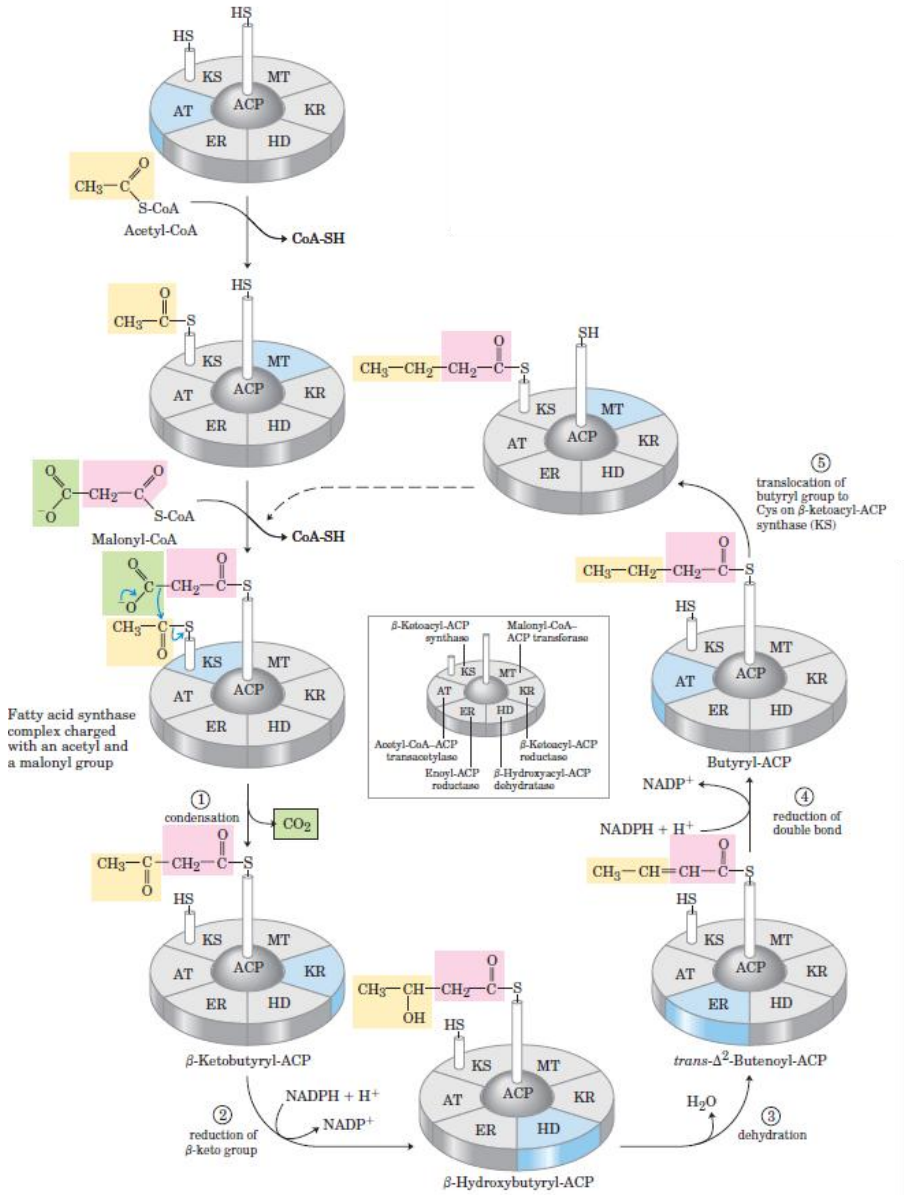
Finally, the double bond of *trans*-Δ_2-butenoyl-ACP is reduced (saturated) to form **butyryl-ACP** by the action of **enoyl-ACP reductase** (ER);
again, NADPH is the electron donor.





To start the next cycle of four reactions that lengthens the chain by two more carbons, another malonyl group is linked to the now unoccupied phosphopantetheine -SH group of ACP

The butyryl group is now transferred from the phosphopantetheine -SH group of ACP to the Cys –SH group of β-ketoacyl-ACP synthase, which initially bore the acetyl group.

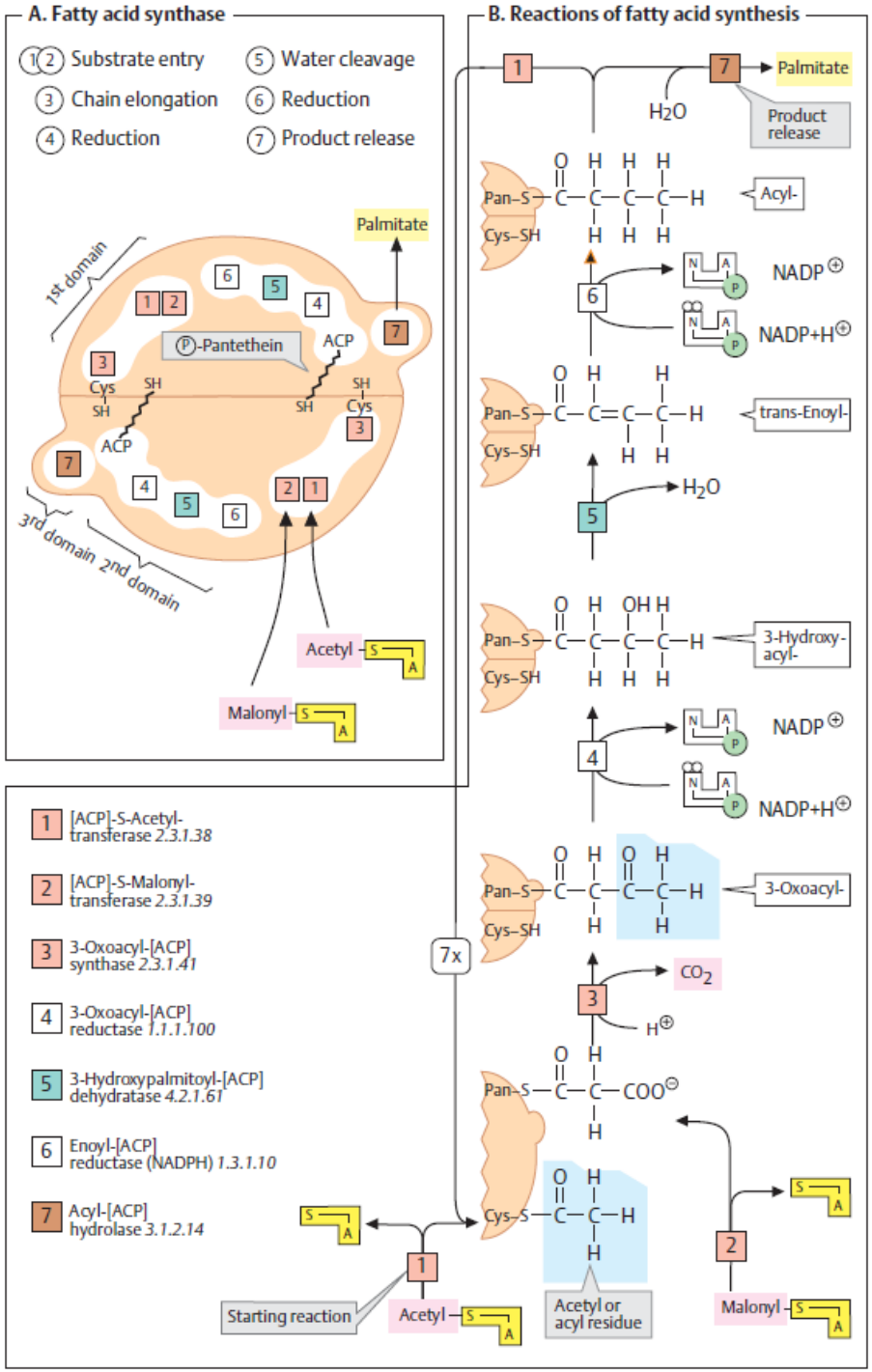


Sequence of events during synthesis of a fatty acid

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.

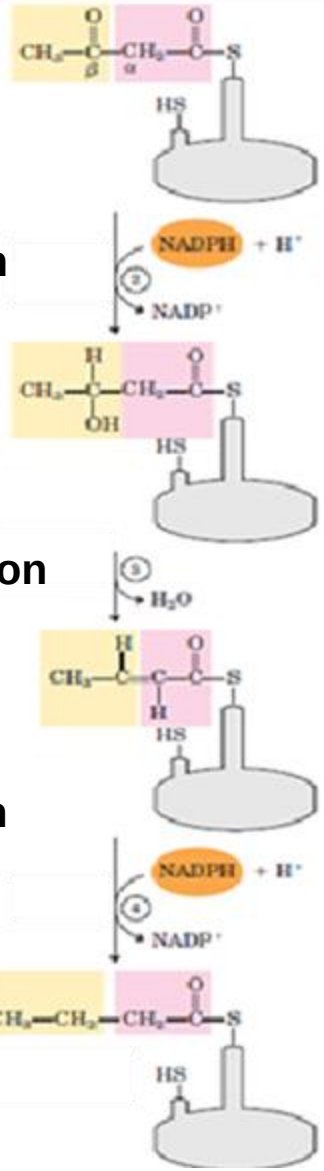
The initial acetyl group is shaded yellow, C-1 and C-2 of malonate are shaded pink, and the carbon released as CO_2 is shaded green. Steps 1 to 4 were described in the previous slides.



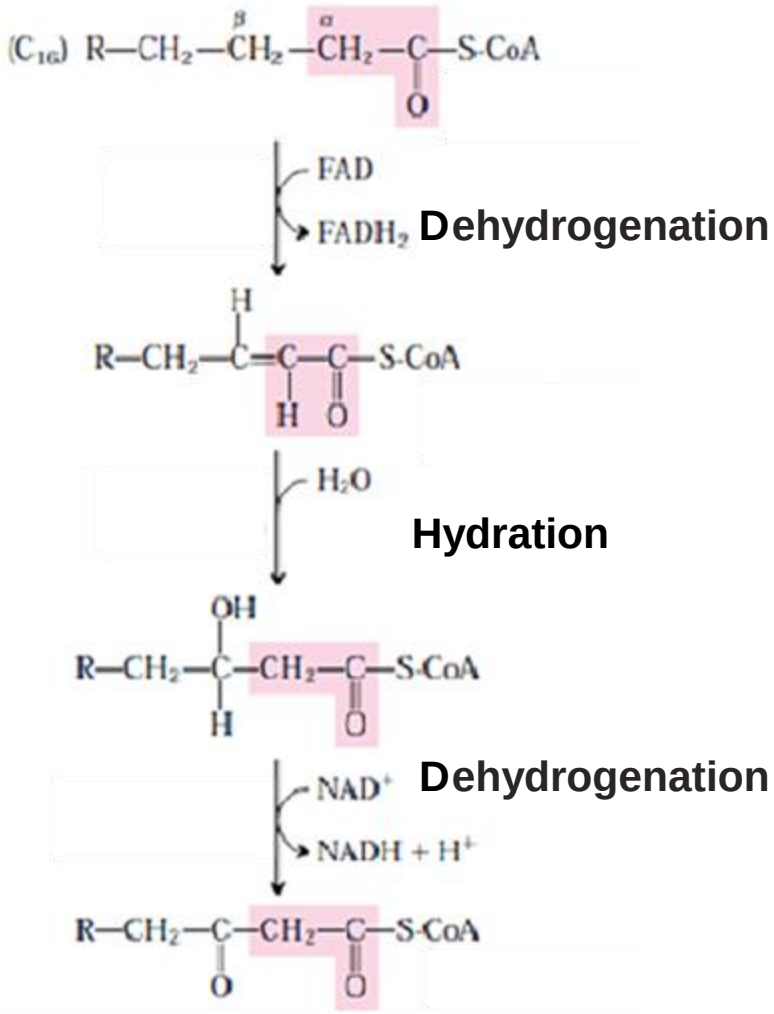
J. Koolman, K.-H. Roehm
Color atlas of biochemistry

Synthesis	vs.	Degradation
Reduction	**vs.**	**Dehydrogenation**
Dehydration	**vs.**	**Hydration**
Reduction	**vs.**	**Dehydrogenation**

Synthesis vs. Degradation



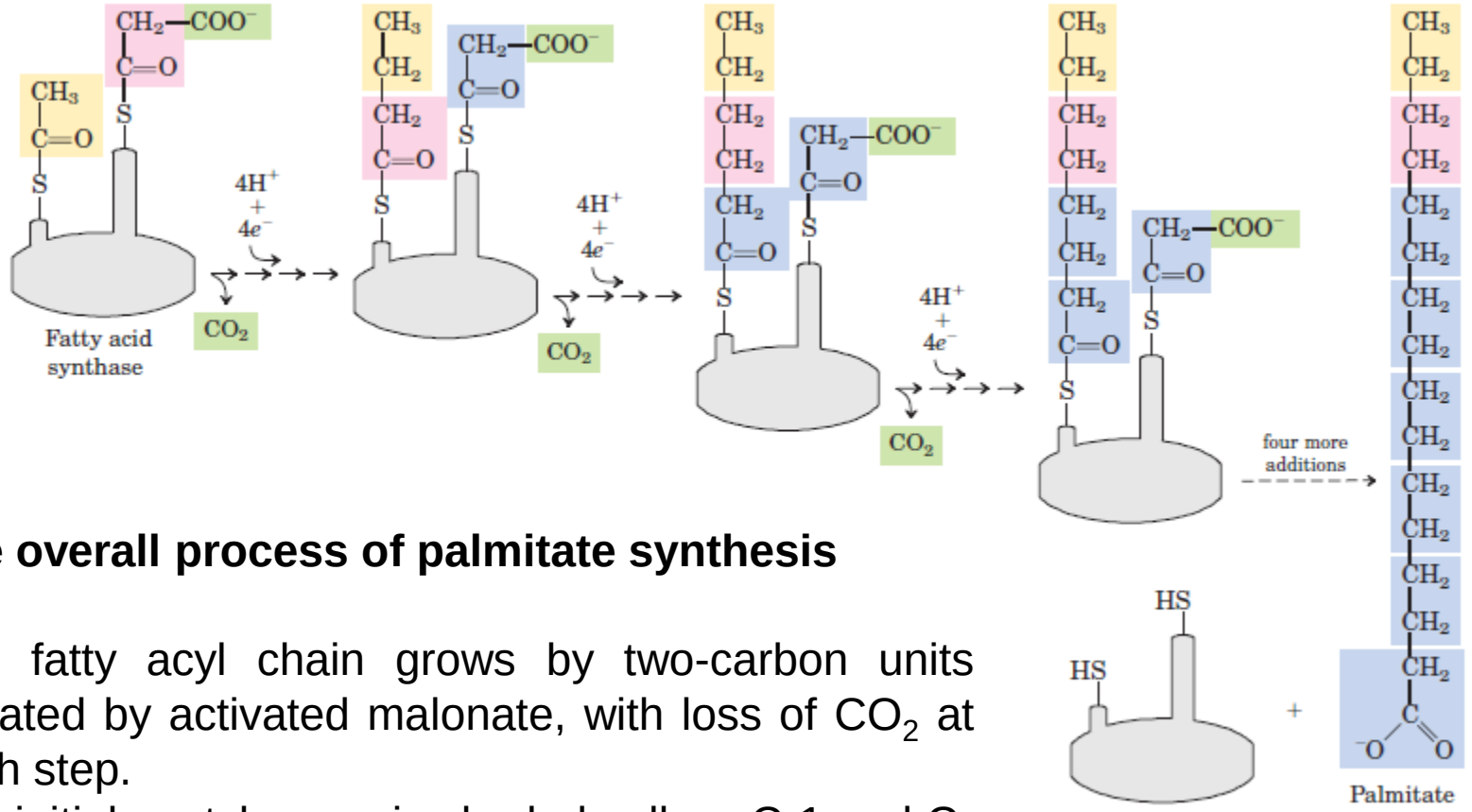
vs.



vs.

vs.

vs.



The overall process of palmitate synthesis

The fatty acyl chain grows by two-carbon units donated by activated malonate, with loss of CO_2 at each step.
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. After each two-carbon addition, reductions convert the growing chain to a saturated fatty acid of four, then six, then eight carbons, and so on. The final product is palmitate (16:0).

Your questions:

Why the final product of biosynthesis of fatty acids is palmitate?
How does its release take place?

Palmitate is the final product of fatty acid biosynthesis since Fatty Acid Synthase (FAS) is designed to stop elongation at 16 carbons. The enzyme has a thioesterase (TE) domain that specifically recognizes and hydrolyzes the 16-carbon saturated fatty acyl chain.

Once a 16-carbon chain is formed and saturated, the TE domain of FAS hydrolyzes the thioester bond between palmitoyl group and the acyl carrier protein (ACP), releasing free palmitate.

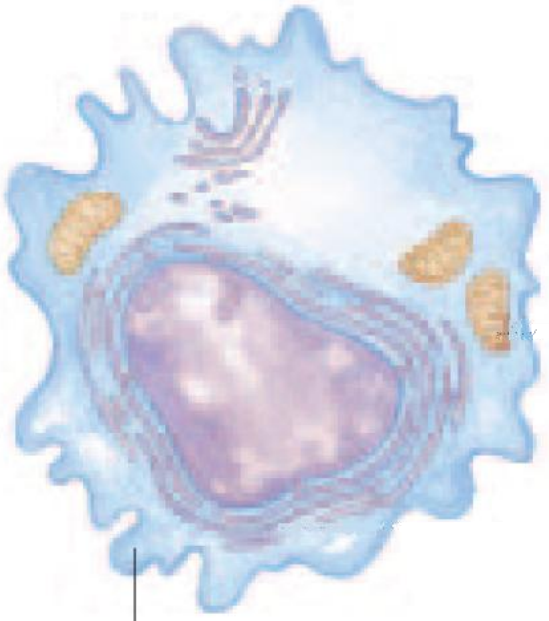
Once released, palmitate can be:
- activated to palmitoyl-CoA (by acyl-CoA synthetase);
- used in lipid synthesis (e.g., triglycerides, phospholipids);
- modified (e.g., elongated, desaturated);
- stored in lipid droplets.

Your question:

Where the synthesis of a fatty acid does take place?

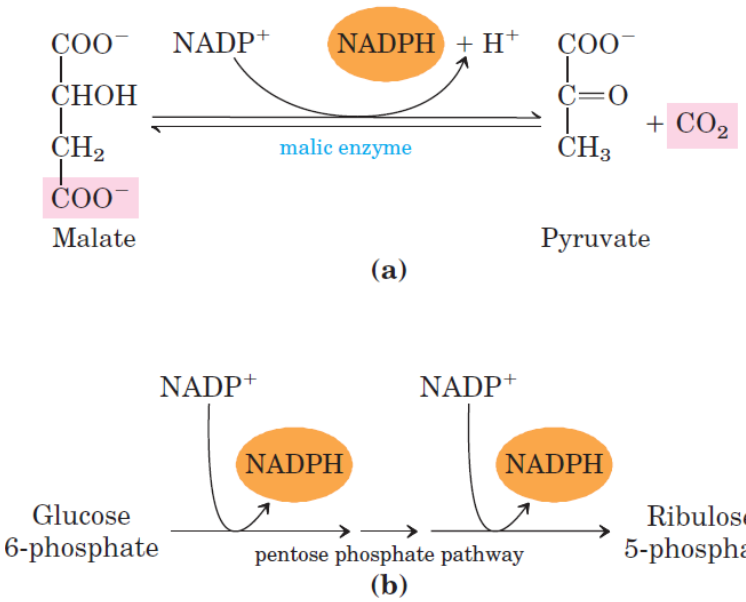
Fatty acid synthesis takes place in the compartment in which NADPH is available for reductive synthesis (i.e., where the [NADPH]/[NADP] ratio is high).

Animal cells, yeast cells



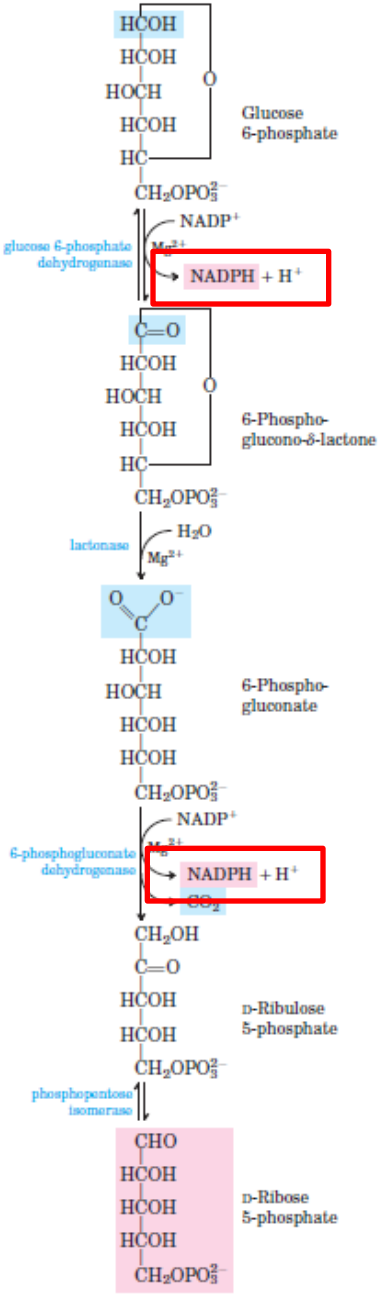
Cytosol

- NADPH production (pentose phosphate pathway; malic enzyme)
- [NADPH]/[$NADP^+$] high
- Fatty acid synthesis



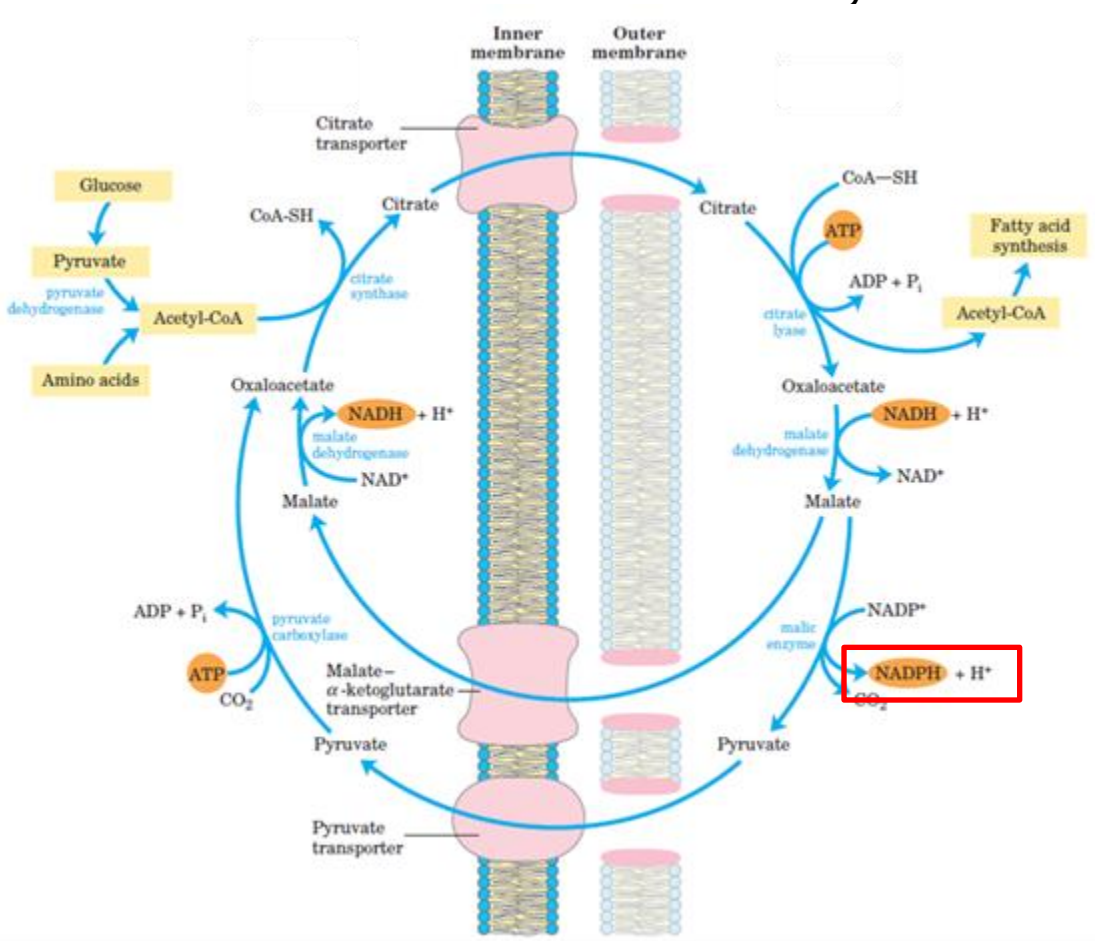
Two routes to NADPH, catalyzed by:
(a) malic enzyme;
(b) the pentose phosphate pathway.

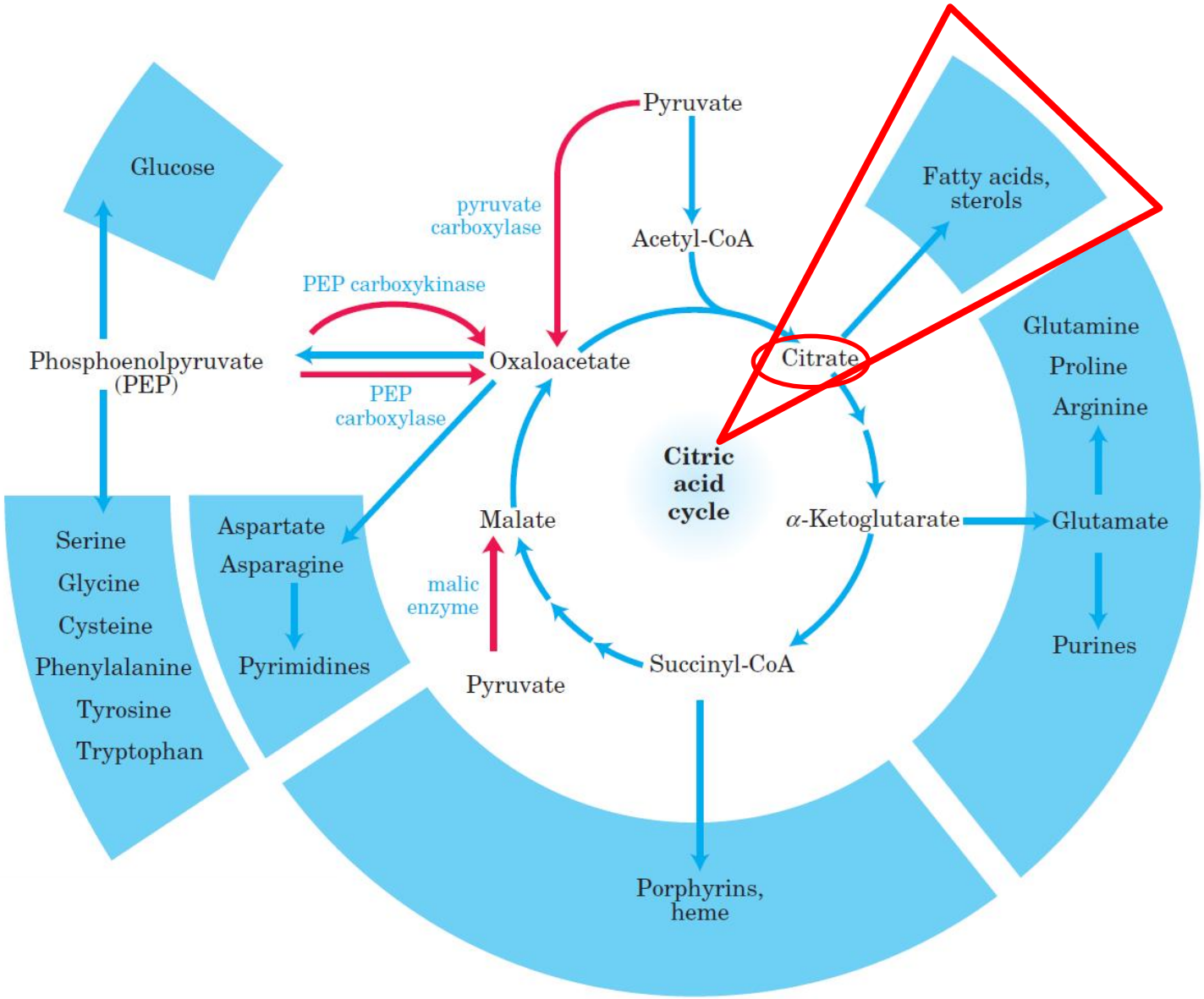
The **Pentose Phosphate Pathway** is a metabolic pathway, parallel to glycolysis, that generates NADPH and pentose, namely ribose 5-phosphate precursor for the synthesis of nucleotides. The first reaction of the pentose phosphate pathway is the oxidation of glucose 6-phosphate (G6P) by **glucose 6-phosphate dehydrogenase (G6PD)** that **is the rate-limiting enzyme of the pentose phosphate pathway**. Thus, regulation of G6PD has downstream consequences for the activity of the rest of the pentose phosphate pathway. **Insulin induces increase in the glucose 6-phosphate dehydrogenase activity, assumed to be due to *de novo* enzyme biosynthesis** involving new RNA production.



Shuttle for transfer of acetyl groups from mitochondria to the cytosol

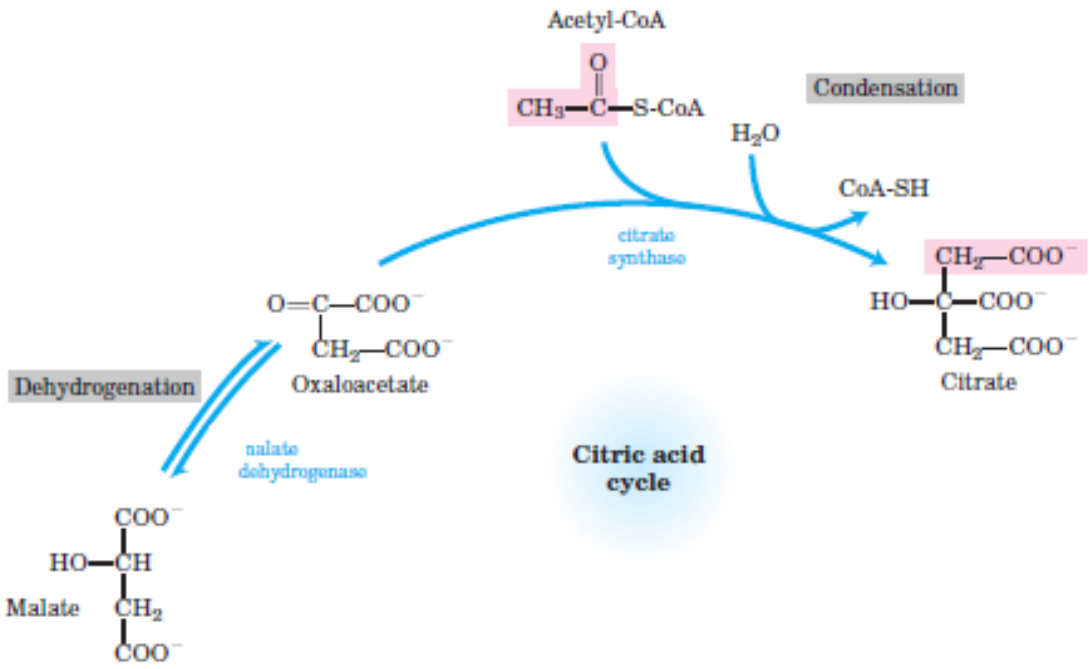
The mitochondrial outer membrane is freely permeable to all these compounds. Pyruvate derived from amino acid catabolism in the mitochondrial matrix, or from glucose by glycolysis in the cytosol, is converted to acetyl-CoA in the matrix. Acetyl groups pass out of the mitochondrion as citrate; in the cytosol they are delivered as acetyl-CoA for fatty acid synthesis. Oxaloacetate is reduced to malate, which returns to the mitochondrial matrix and is converted to oxaloacetate. An alternative fate for cytosolic malate is oxidation by malic enzyme to generate cytosolic NADPH; the pyruvate produced returns to the mitochondrial matrix.



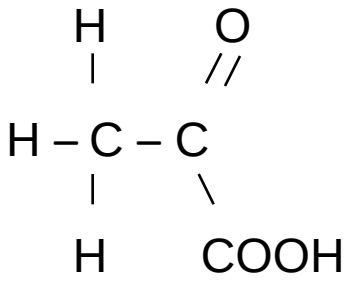


Intermediates of the citric acid cycle as precursors in many biosynthetic pathways

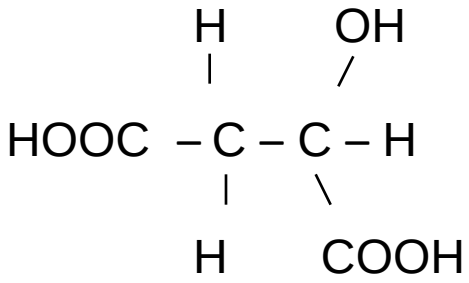
Mitochondrial matrix



Acetyl groups pass out of the mitochondrion as citrate

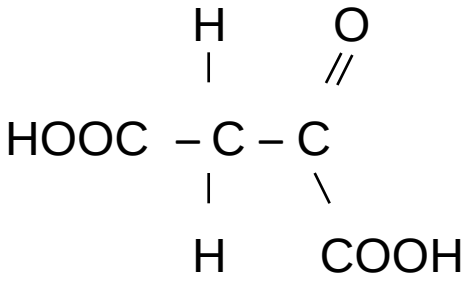


Pyruvate

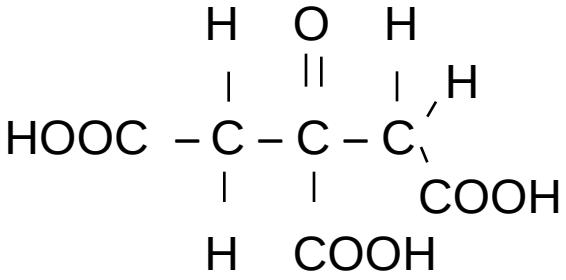


Malate

Oxaloacetate



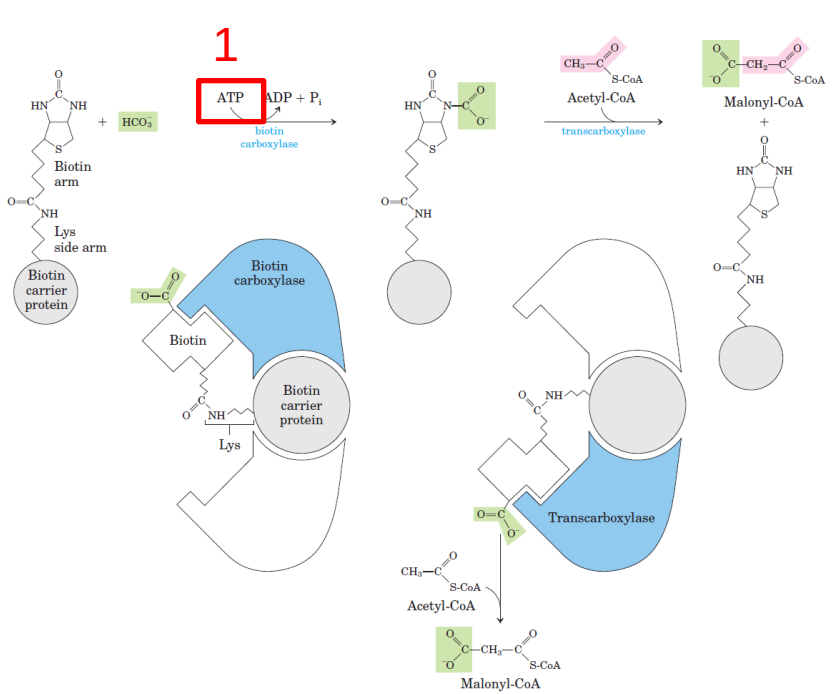
Citrate

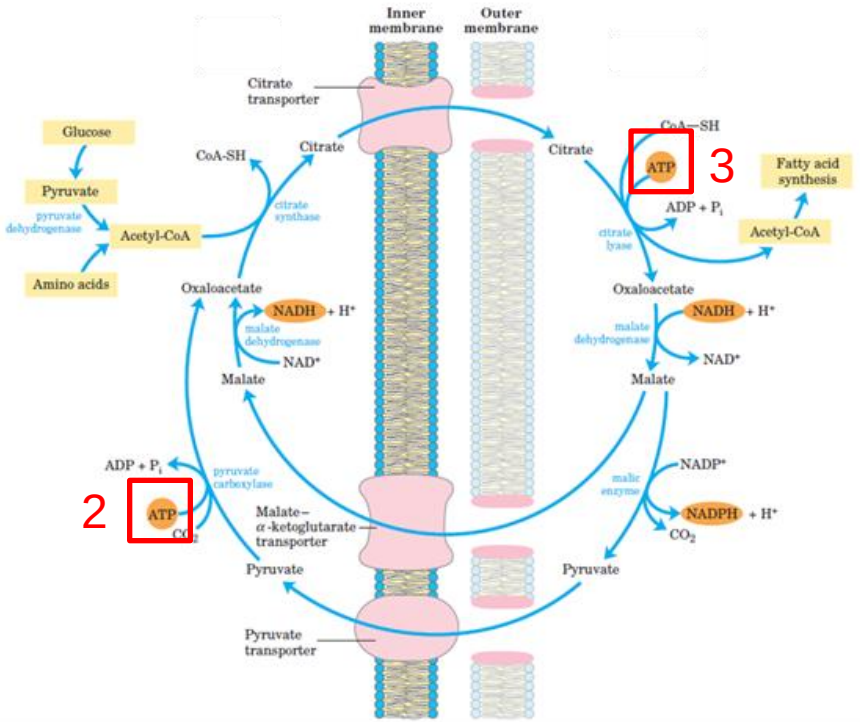


Your question:

What is the energy expenditure for the synthesis of a fatty acid?

The energy required to make fatty acid synthesis favorable is provided by the 1 ATP molecule used to synthesize malonyl-CoA from acetyl-CoA and HCO_3.
The acetate export cycle from mitochondria consumes 2 molecules of ATP (by citrate lyase and pyruvate carboxylase) for each molecule of acetyl-CoA that enters the synthesis of fatty acids.

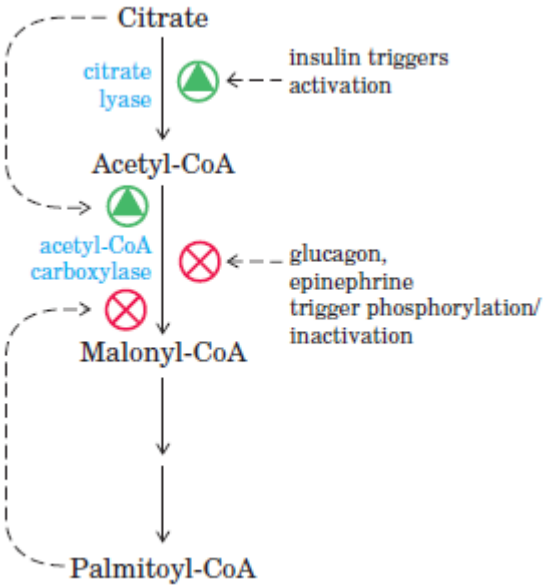


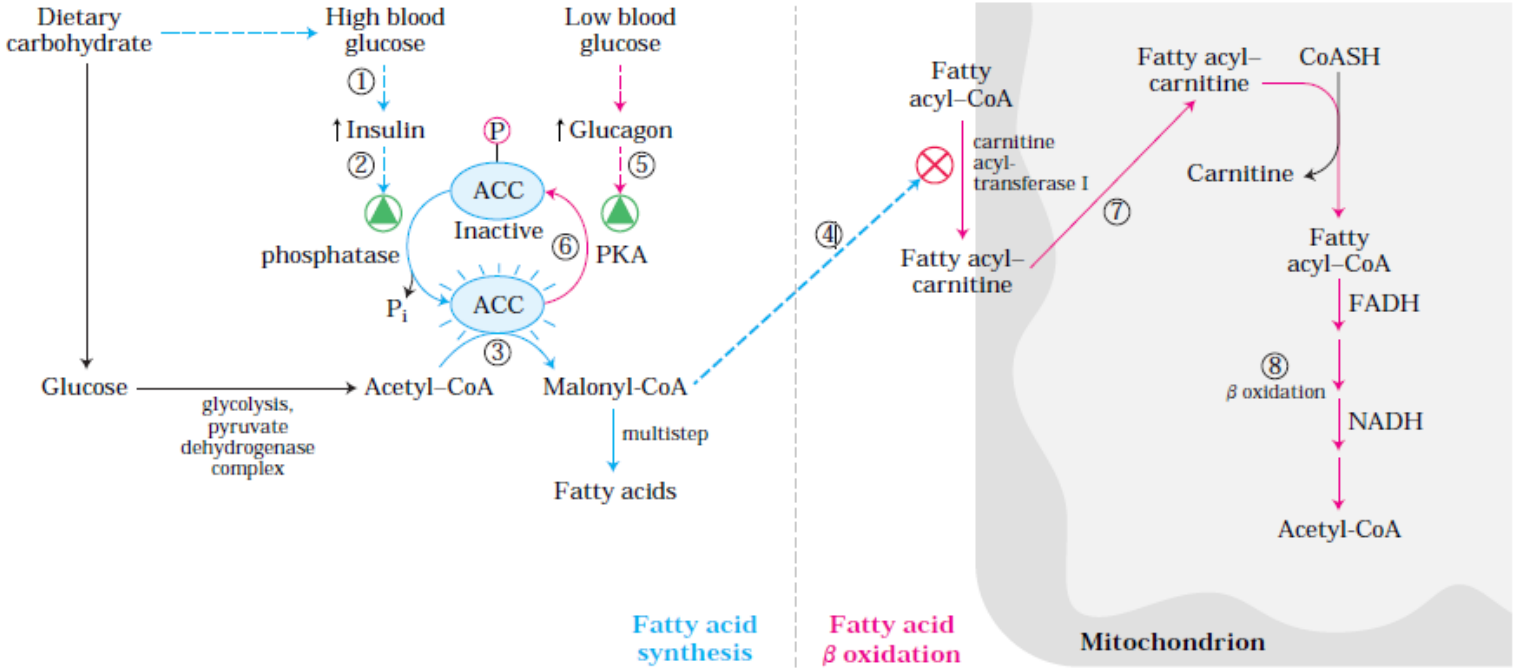


Then **a total of 3 molecules of ATP are consumed for each two-carbon block included in a fatty acid growing chain**.

Regulation of fatty acid synthesis

In the cells of vertebrates, both allosteric regulation and hormone-dependent covalent modification influence the flow of precursors into malonyl-CoA.





Malonyl-CoA, the first intermediate in the cytosolic biosynthesis of long-chain fatty acids from acetyl-CoA increases in concentration whenever an animal is well supplied with carbohydrate; excess glucose that cannot be oxidized or stored as glycogen is converted in the cytosol into fatty acids for storage as triacylglycerol.

The **inhibition of carnitine acyltransferase I by malonyl-CoA ensures that the oxidation of fatty acids is inhibited** whenever the liver is amply supplied with glucose as fuel and is actively making triacylglycerols from excess glucose.

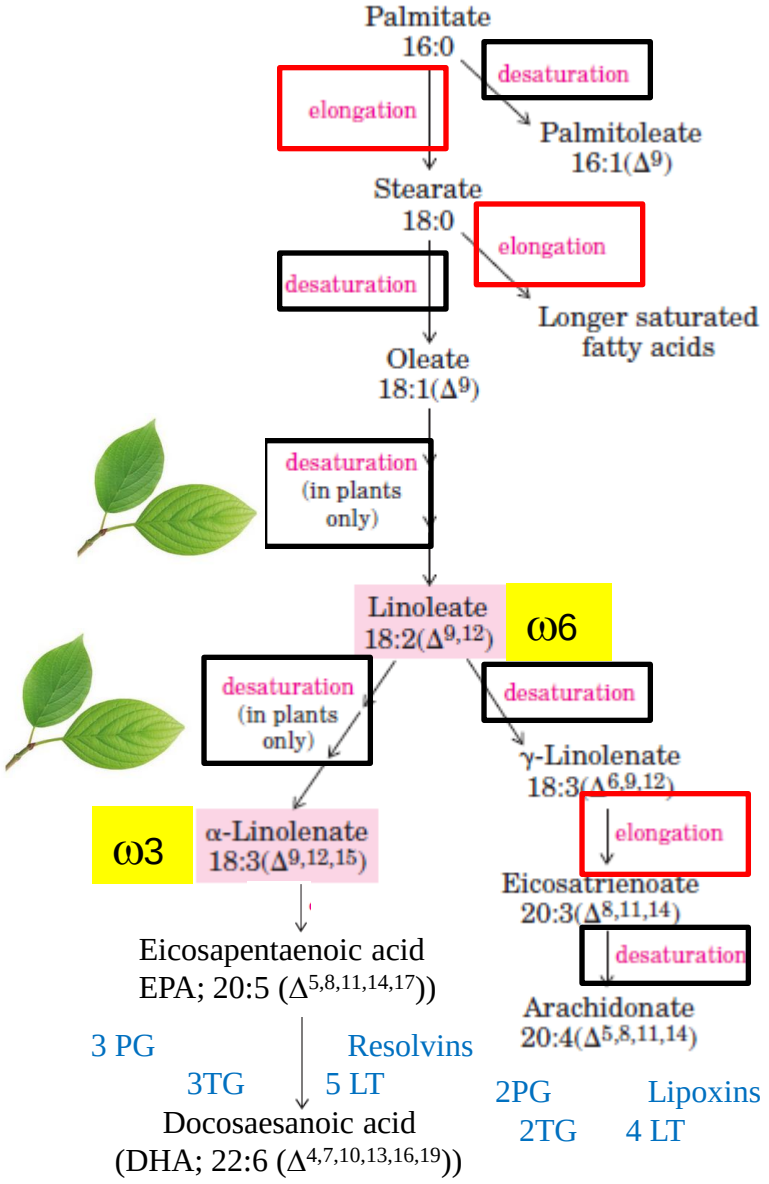
Routes of synthesis of other fatty acids

Palmitate is the precursor of stearate and longer-chain saturated fatty acids, as well as the monounsaturated acids palmitoleate and oleate.

Mammals cannot convert oleate to linoleate or α-linolenate (shaded pink), which are therefore required in the diet as essential fatty acids.

Conversion of linoleate to other polyunsaturated fatty acids and eicosanoids is outlined.

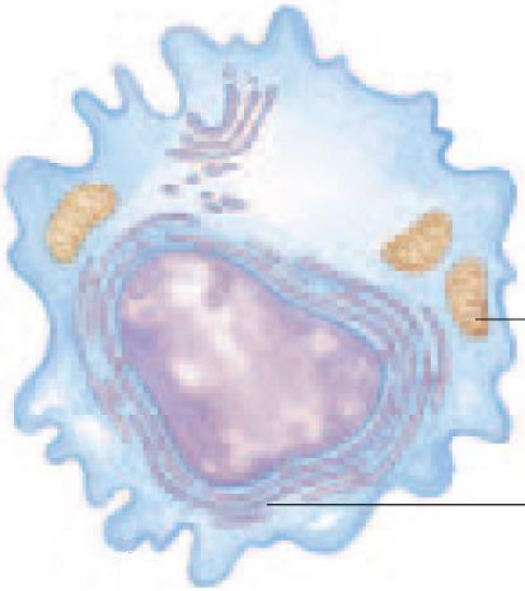
Unsaturated fatty acids are symbolized by indicating the number of carbons and the number and position of the double bonds.



Your question:

Where elongation and desaturation of a fatty acid does take place?

Animal cells, yeast cells



Mitochondria
- Fatty acid elongation

Endoplasmic reticulum
- Fatty acid elongation
- Fatty acid desaturation

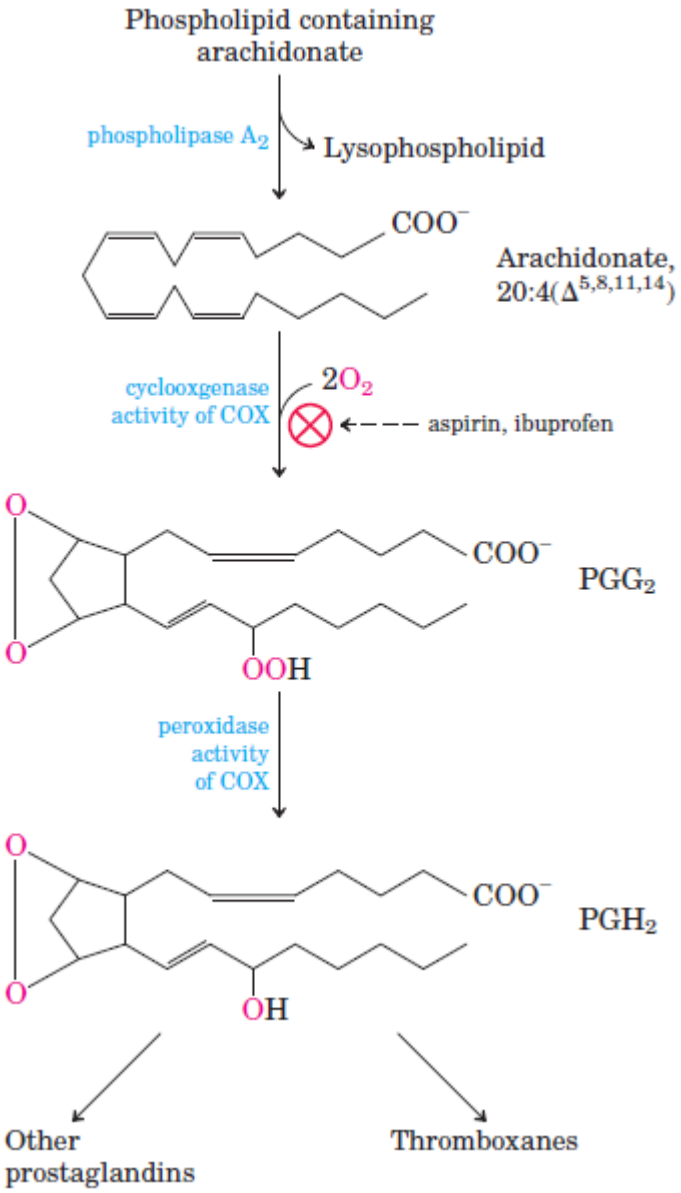
Your question:

Which enzymes elongate fatty acids in the ER?

The enzymes responsible for elongating fatty acids in the ER belong to a group of enzymes known as the elongation of very long-chain fatty acids (ELOVL) enzymes.

The ELOVL enzyme family includes ELOVL1 through ELOVL7, each with substrate specificity for different chain lengths and saturation levels.

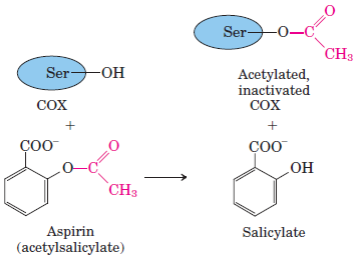
These enzymes catalyze the elongation cycle, namely the condensation of and acyl-CoA with malonyl-CoA followed by reduction, dehydration, and a second reduction - analogous to fatty acid synthesis in the cytoplasm, but distinct in location and substrate.



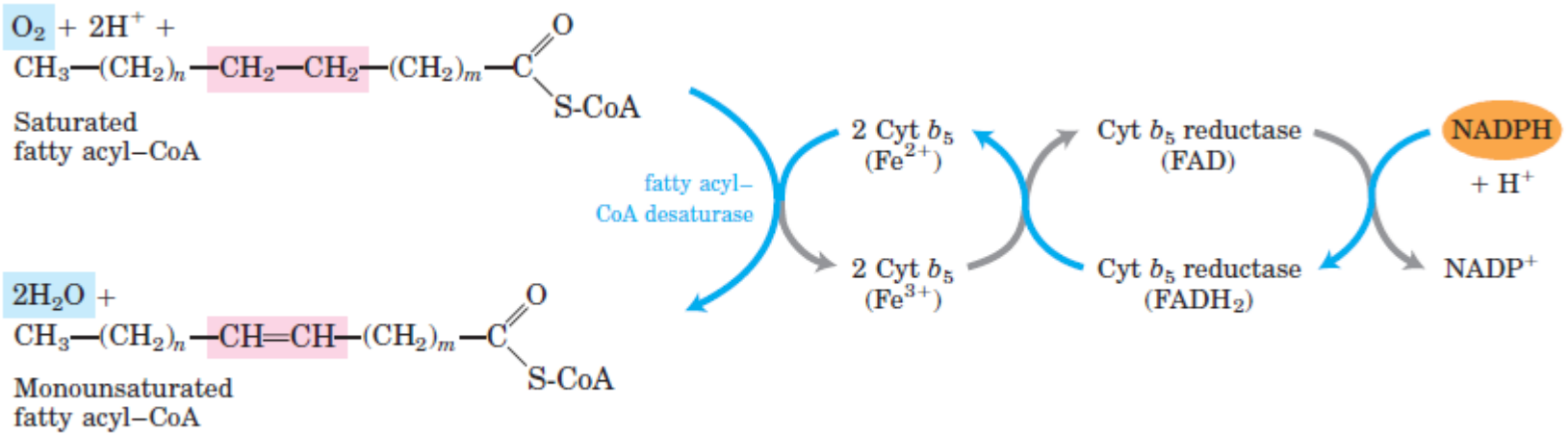
The "cyclic" pathway from arachidonate to prostaglandins and thromboxanes

After arachidonate is released from phospholipids by the action of phospholipase A_2, the cyclooxygenase and peroxidase activities of COX (also called prostaglandin H_2 synthase) catalyze the production of PGH_2, the precursor of other prostaglandins and thromboxanes.

Aspirin inhibits the first reaction by acetylating an essential Ser residue on the enzyme.



Ibuprofen and naproxen inhibit the same step, probably by mimicking the structure of the substrate or an intermediate in the reaction.



Electron transfer in the desaturation of fatty acids in vertebrates

Blue arrows show the path of electrons as two substrates - a fatty acyl–CoA and NADPH - undergo oxidation by molecular oxygen.
These reactions take place on the lumenal face of the smooth ER.

The “linear” pathway from arachidonate to leukotrienes