Lecture 4

BT 203

Biochemistry

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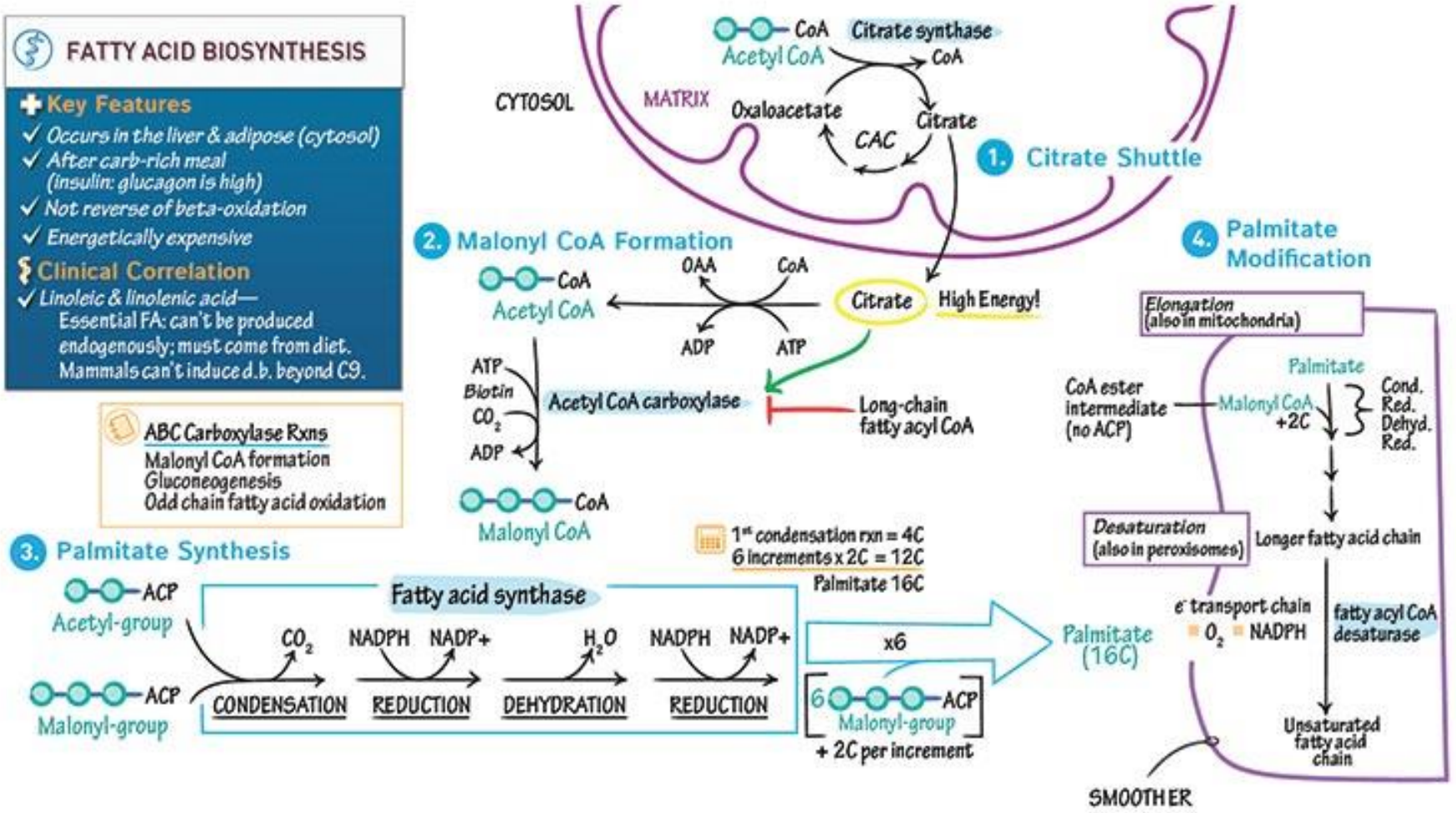
Key Concepts

- How are fatty acids synthesized ?
- What is fatty acid synthesis?
- What is the role of Acetyl-CoA Carboxylase?
- Where does fatty acid synthesis takes place?

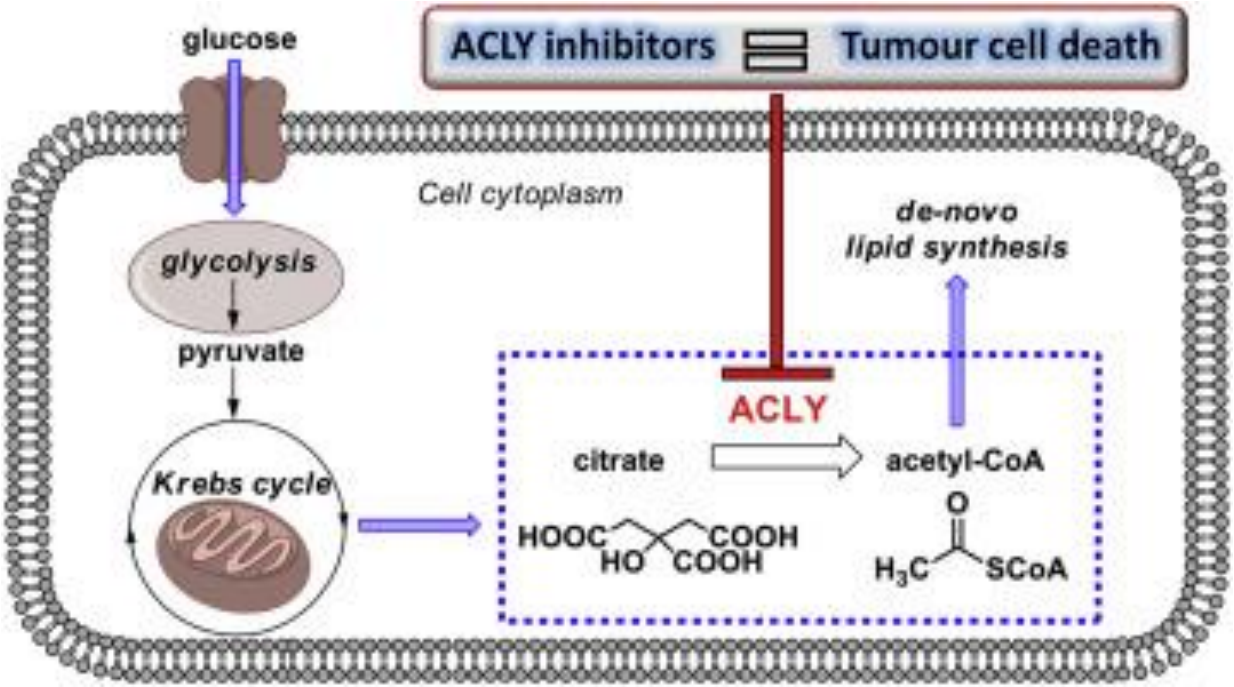
Key Concepts



Citrate shuttle

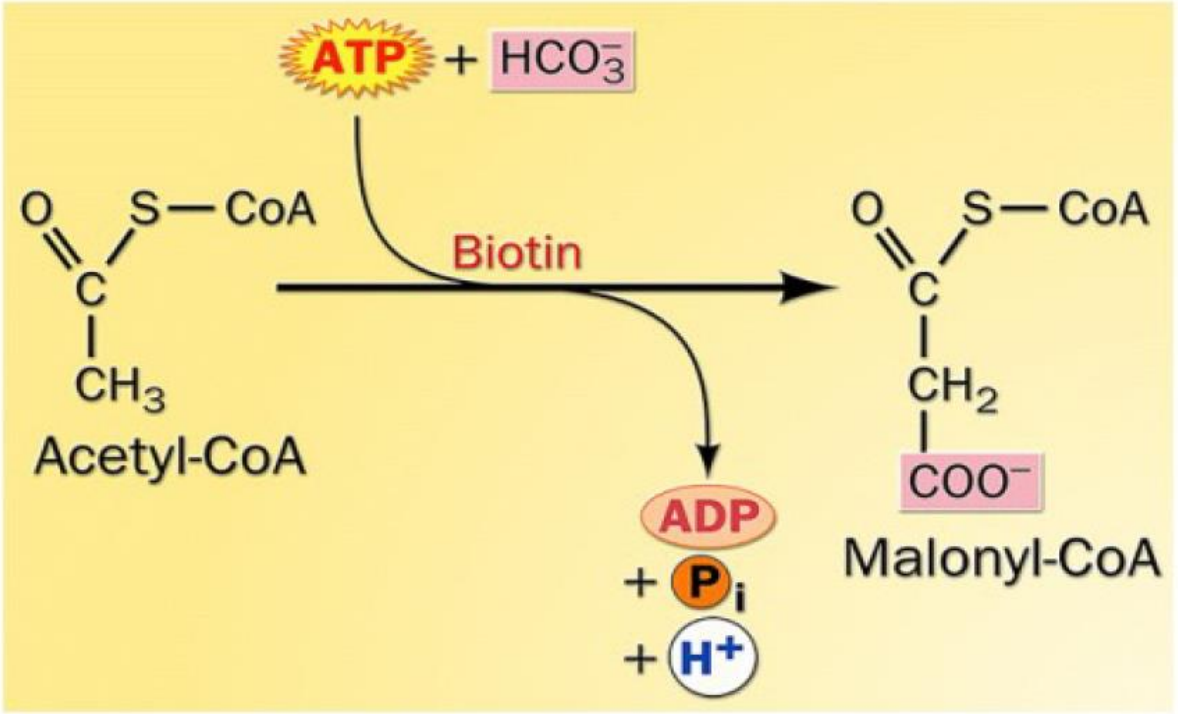
- FAs are synthesized in the cytoplasm from acetyl CoA
- Acetyl CoA generated from pyruvate by the action of PDH and by β-oxidation of fatty acids is in the mitochondria.
- For fatty acid biosynthesis, acetylCoA has to be transported from the mitochondria to the cytoplasm. This is done via a shuttle system called the Citrate Shuttle.
- Acetyl CoA reacts with oxaloacetate to give citrate. A tricarboxylate translocase transports citrate from mitochondria to cytosol.
- In the cytosol, citrate is cleaved back to oxaloacetate and acetylCoA. This reaction is catalyzed by ATP-citrate lyase and requires the hydrolysis of one molecule of ATP.

Fatty acid synthesis



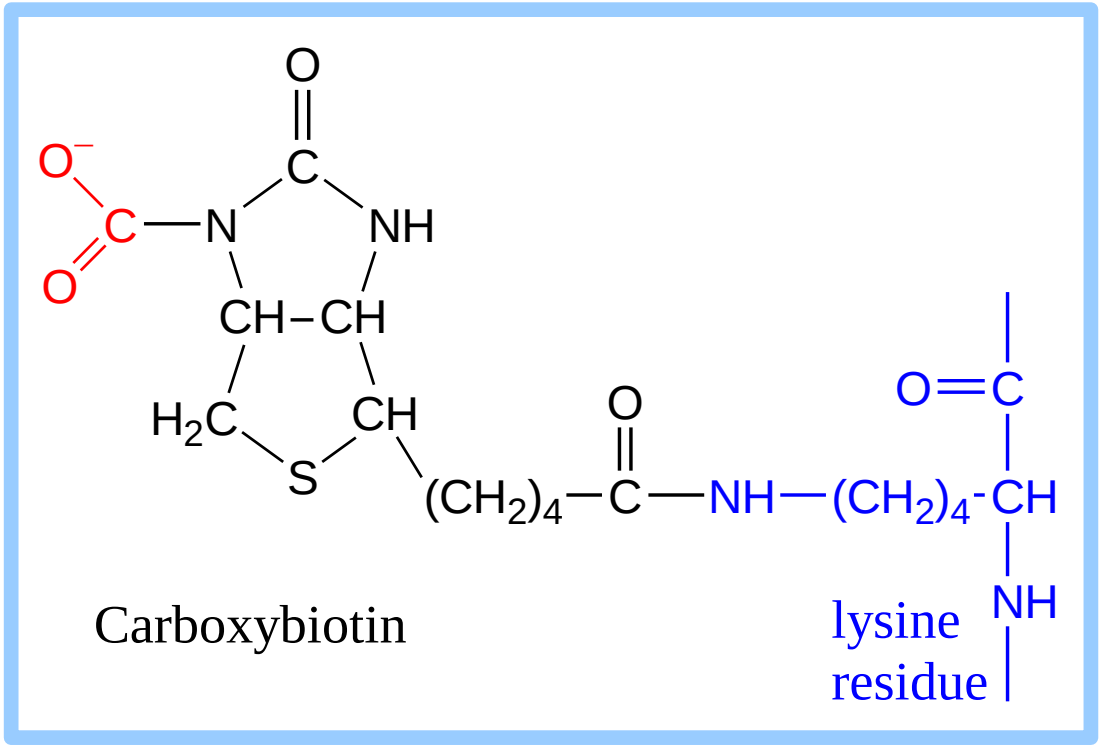
Fatty acid synthesis

Reaction catalyzed by Acetyl CoA Carboxylase



Activation of acetate : Acetyl-CoA to malonyl CoA

What is biotin?



Biotin is linked to the enzyme by an amide bond between the terminal carboxyl of the biotin side chain and the

e-amino group of a **lysine** residue.

The combined biotin and lysine side chains act as a **long flexible arm** and it allows the biotin ring to translocate between the 2 active sites.

Acetyl-CoA Carboxylase

Acetyl-CoA Carboxylase, which converts acetyl-CoA to malonyl-CoA, is the **committed step** of the fatty acid synthesis pathway.

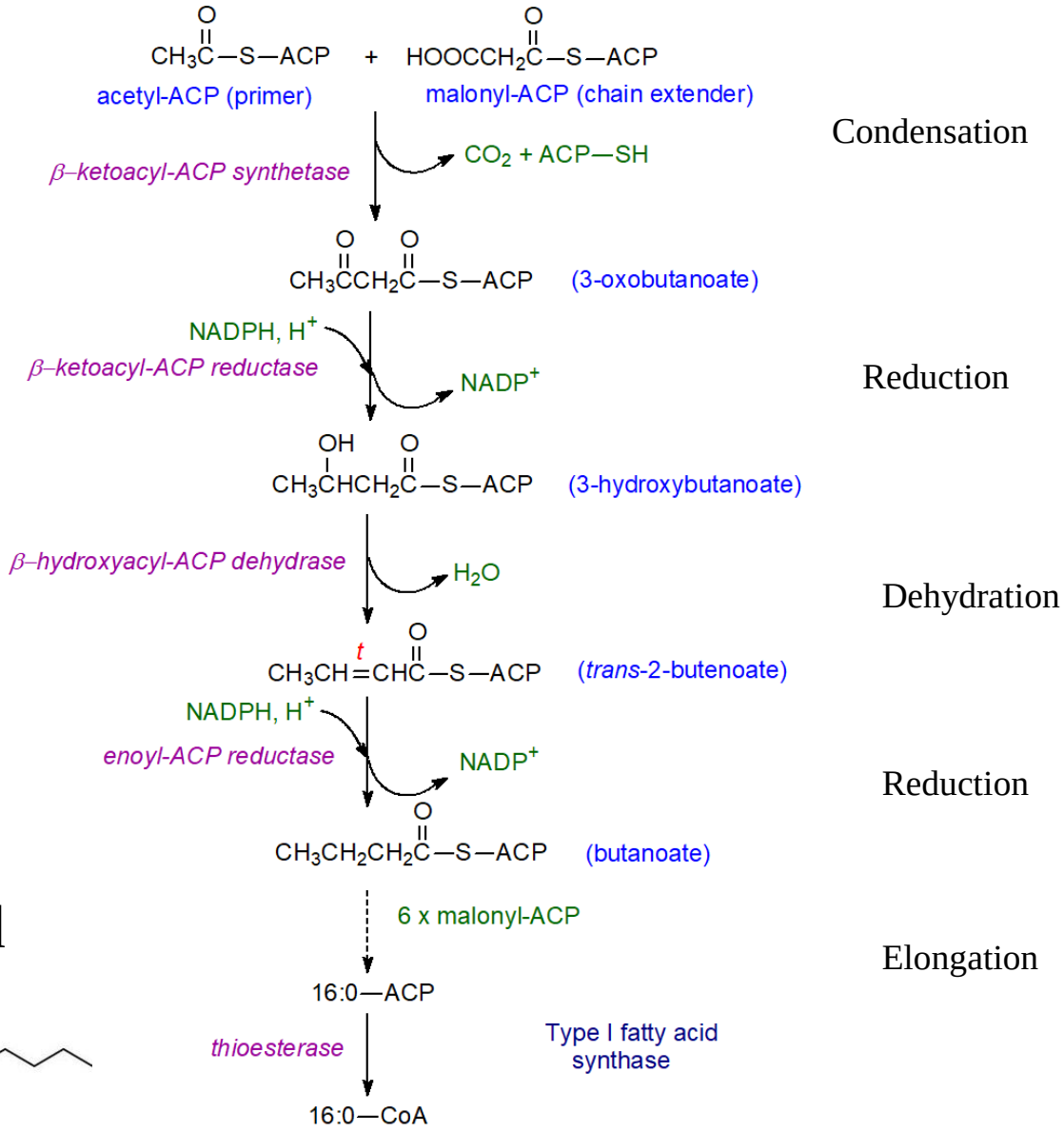
The mammalian enzyme is required to be **regulated**, and it is achieved by

- Phosphorylation

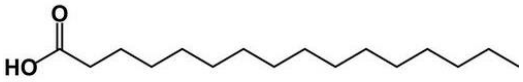
  and

- Allosteric control by local metabolites.

Key Concepts



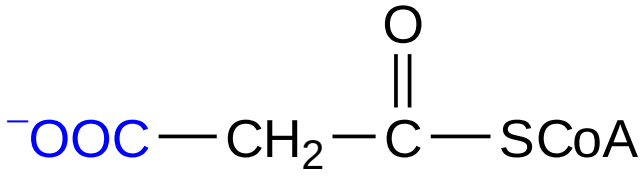
Palmitic acid



Fatty acid precursor

The input to fatty acid synthesis is **acetyl-CoA**, a two carbon compound, which is carboxylated to three carbon compound **malonyl-CoA**.

acetyl-CoA



malonyl-CoA

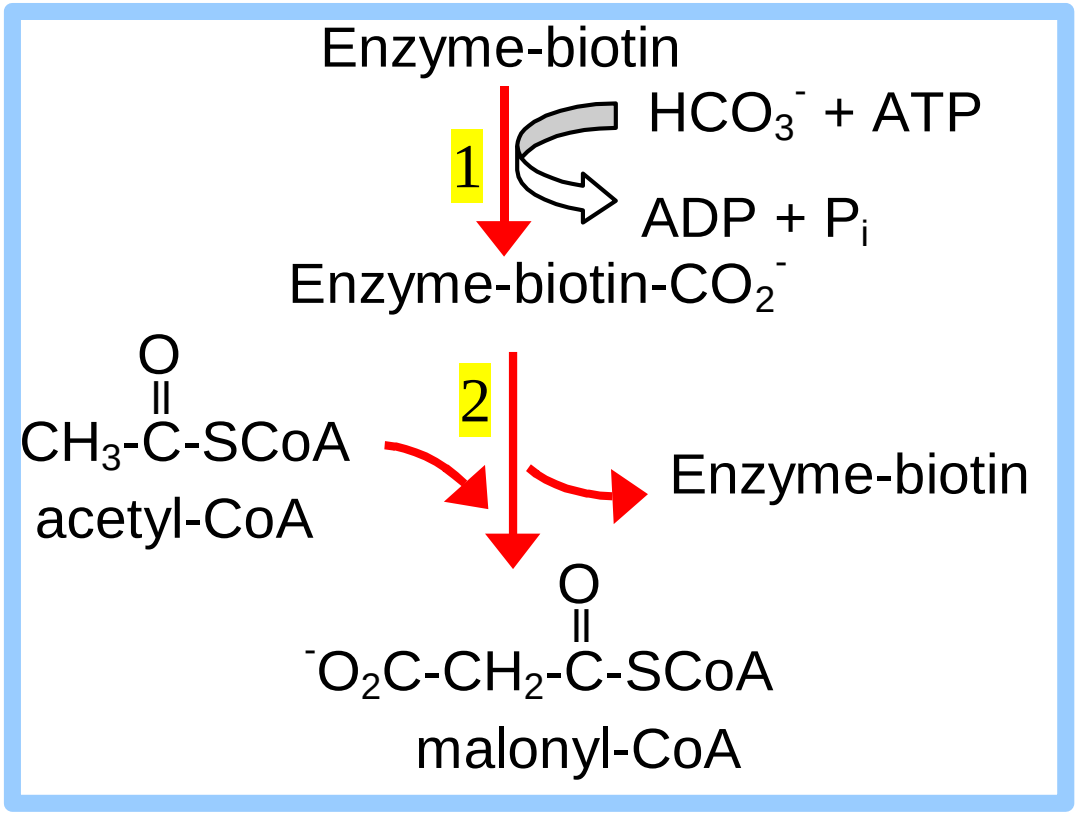
ATP-dependent carboxylation provides energy input.

The CO_2 is lost later during condensation with the growing fatty acid.

The spontaneous decarboxylation drives the condensation reaction.

Fatty acid precursor

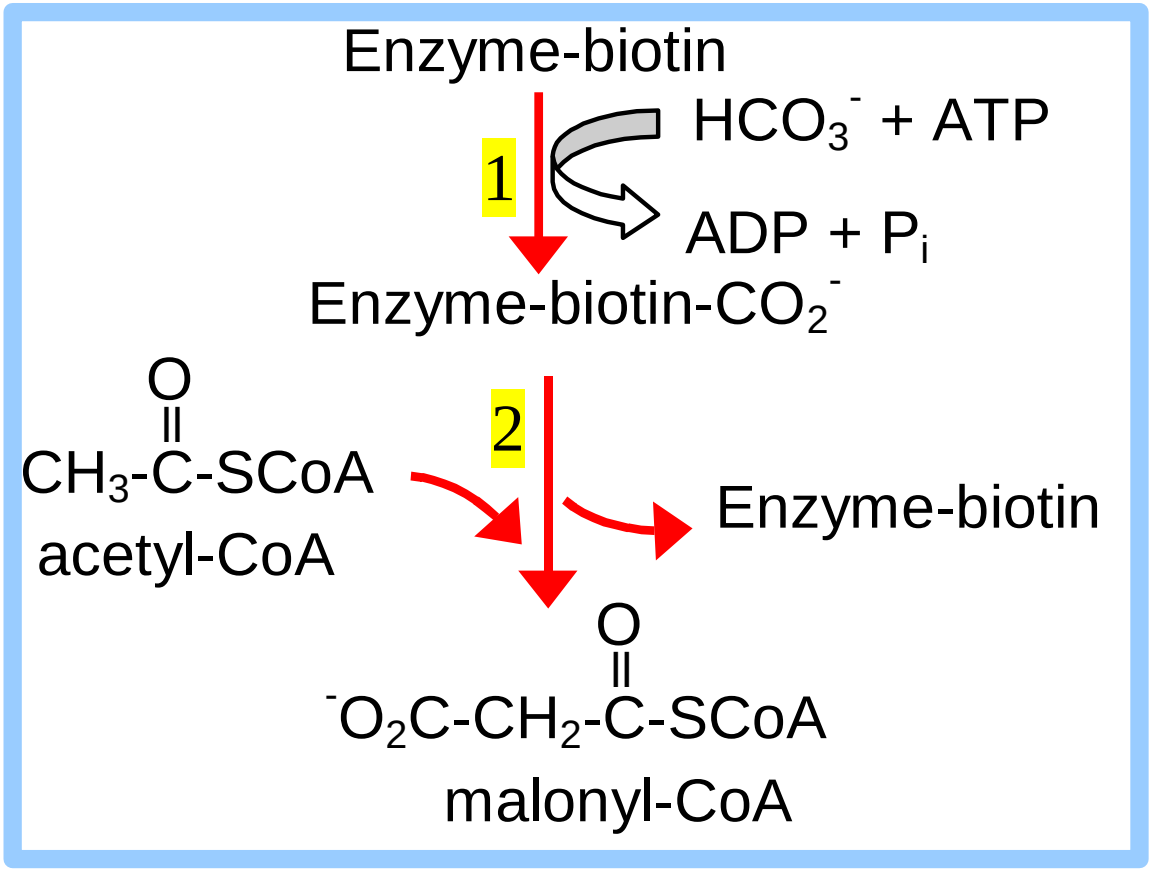
Acetyl-CoA Carboxylase catalyzes the **2-step** reaction by which acetyl-CoA is carboxylated to form **malonyl-CoA**.



As with other carboxylation reactions, the enzyme prosthetic group is **biotin**.

ATP-dependent carboxylation of the biotin, carried out at one active site , is followed by transfer of the carboxyl group to acetyl-CoA at a second active site 2 .

Fatty acid precursor



The overall reaction, which is **spontaneous**, is summarized as: **HCO_3^- + ATP + Acetyl-CoA → ADP + P_i + Malonyl-CoA**

Fatty acid synthesis

Fatty acid synthesis from acetyl-CoA & malonyl-CoA occurs by a series of reactions that are:

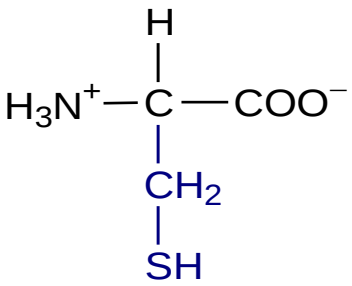
- in **bacteria** catalyzed by 6 different enzymes plus a separate acyl carrier protein (ACP)
- in **mammals** catalyzed by individual domains of a very large polypeptide that includes an ACP domain.

  Evolution of the mammalian Fatty Acid Synthase apparently has involved **gene fusion**.

NADPH serves as **electron donor** in the two reactions involving substrate reduction.

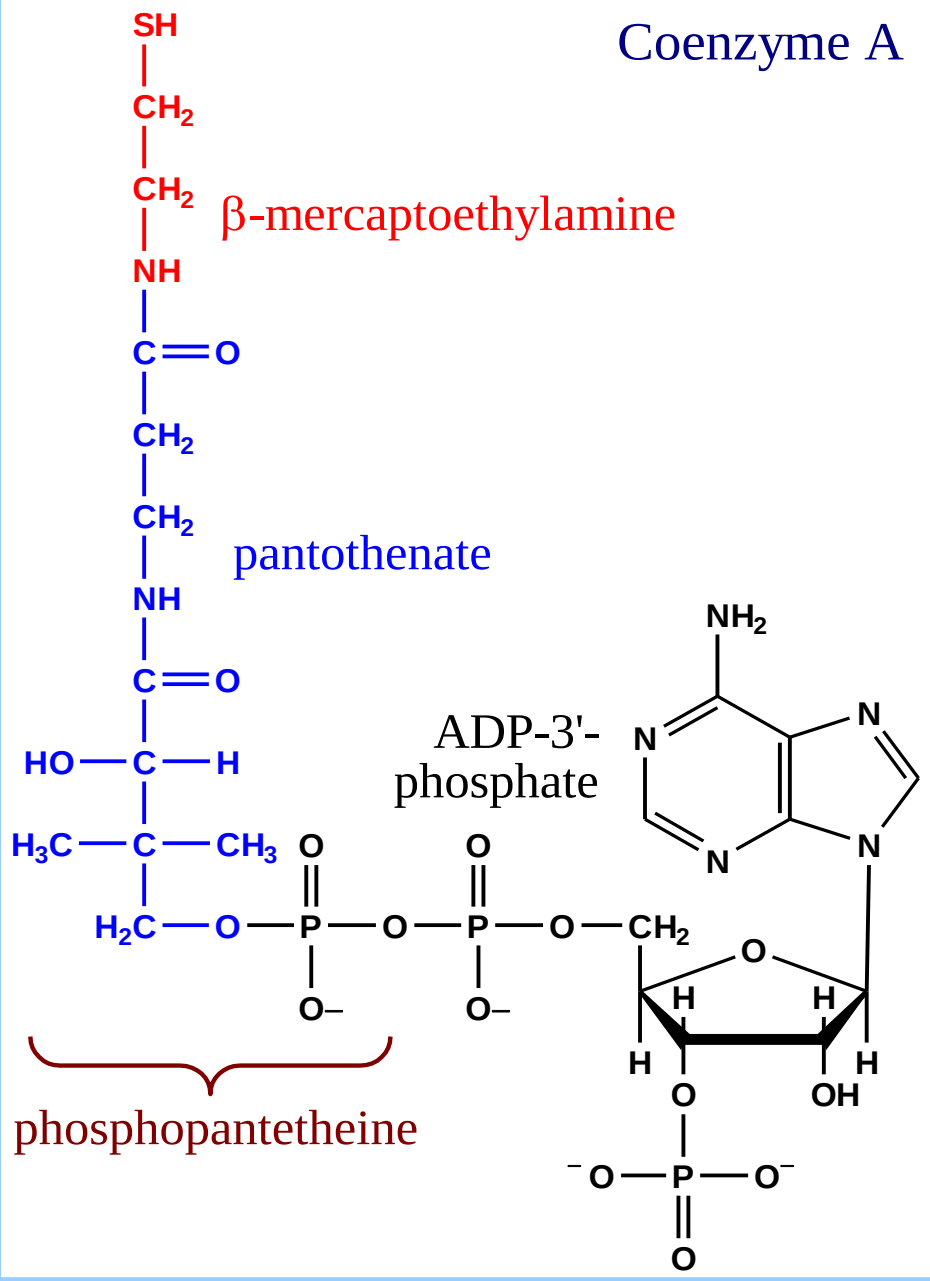
The NADPH is produced mainly by the Pentose Phosphate Pathway.

Fatty acid synthesis



Fatty Acid Synthase prosthetic groups:

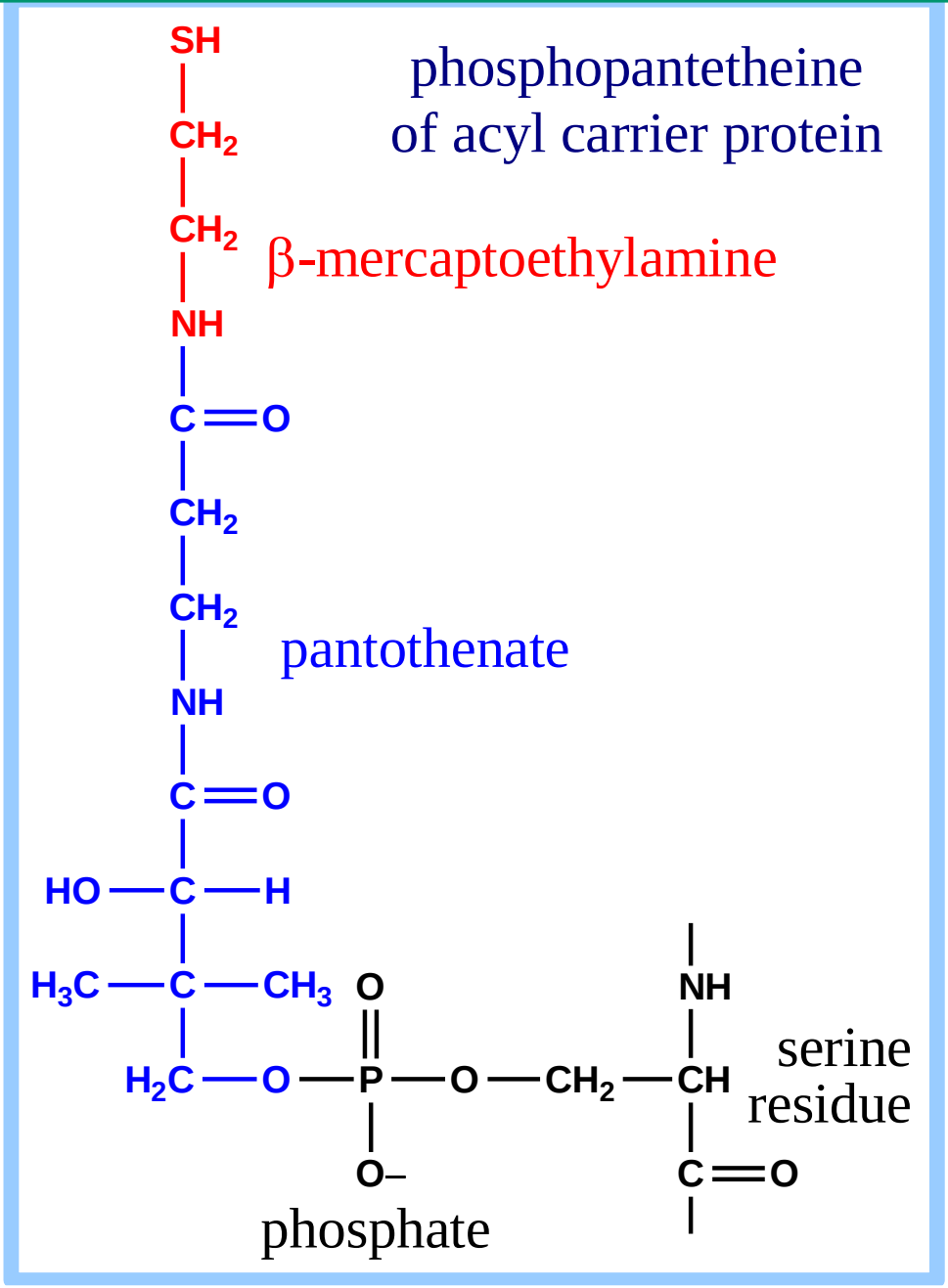
- the **thiol** of the side-chain of a **cysteine** residue of Condensing Enzyme domain.
- the **thiol** of **phosphopantetheine**, equivalent in structure to part of coenzyme A.



Fatty acid synthesis

Phosphopantetheine (Pant) is covalently linked via a phosphate ester to a serine OH of the **acyl carrier protein** domain of Fatty Acid Synthase.

The **long flexible arm** of phosphopantetheine helps its thiol to move from one active site to another within the complex.



Fatty acid synthesis

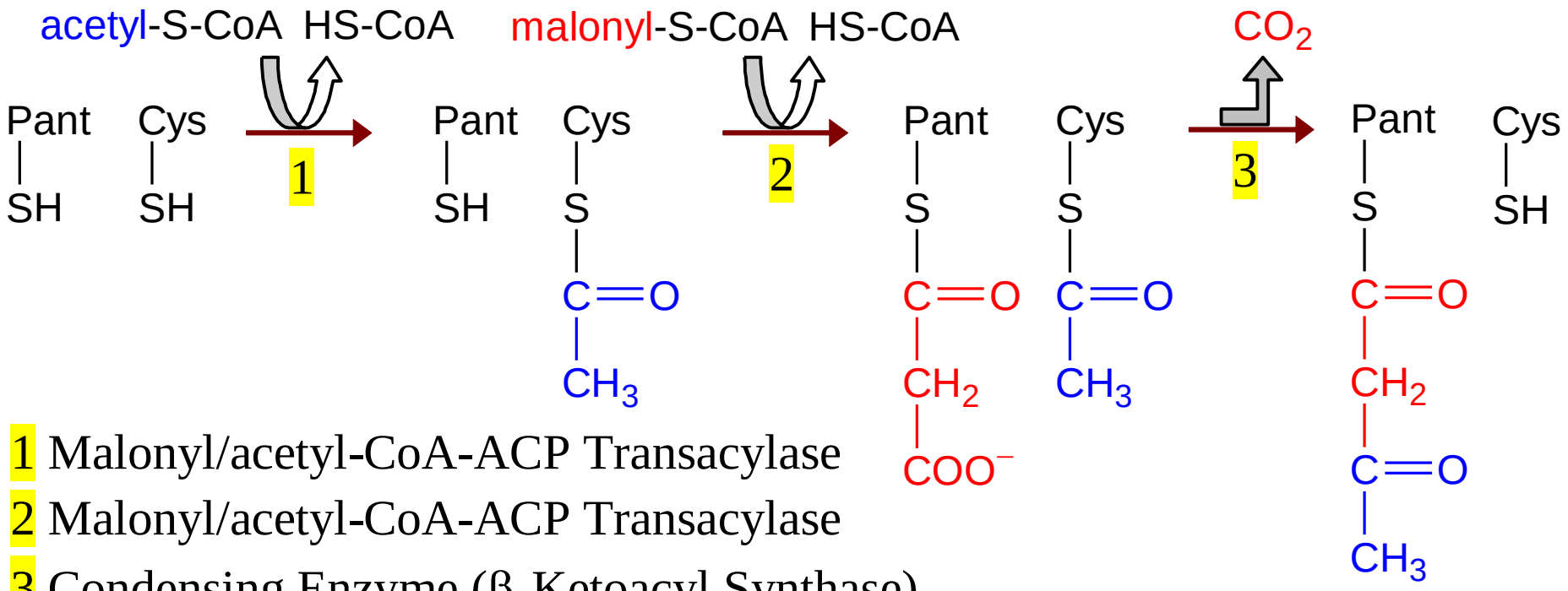
Order of domains in primary structure of mammalian Fatty Acid Synthase

As each of the substrates acetyl-CoA & malonyl-CoA bind to the complex, the initial attacking group is the oxygen of a **serine hydroxyl** group of the Malonyl/acetyl-CoA Transacylase enzyme domain.

Each acetyl or malonyl moiety is transiently in ester linkage to this serine hydroxyl, before being transferred into thioester linkage with the **phosphopantetheine thiol** of the acyl carrier protein (ACP) domain.

Acetate is subsequently transferred to a **cysteine thiol** of the Condensing Enzyme domain.

Fatty acid synthesis



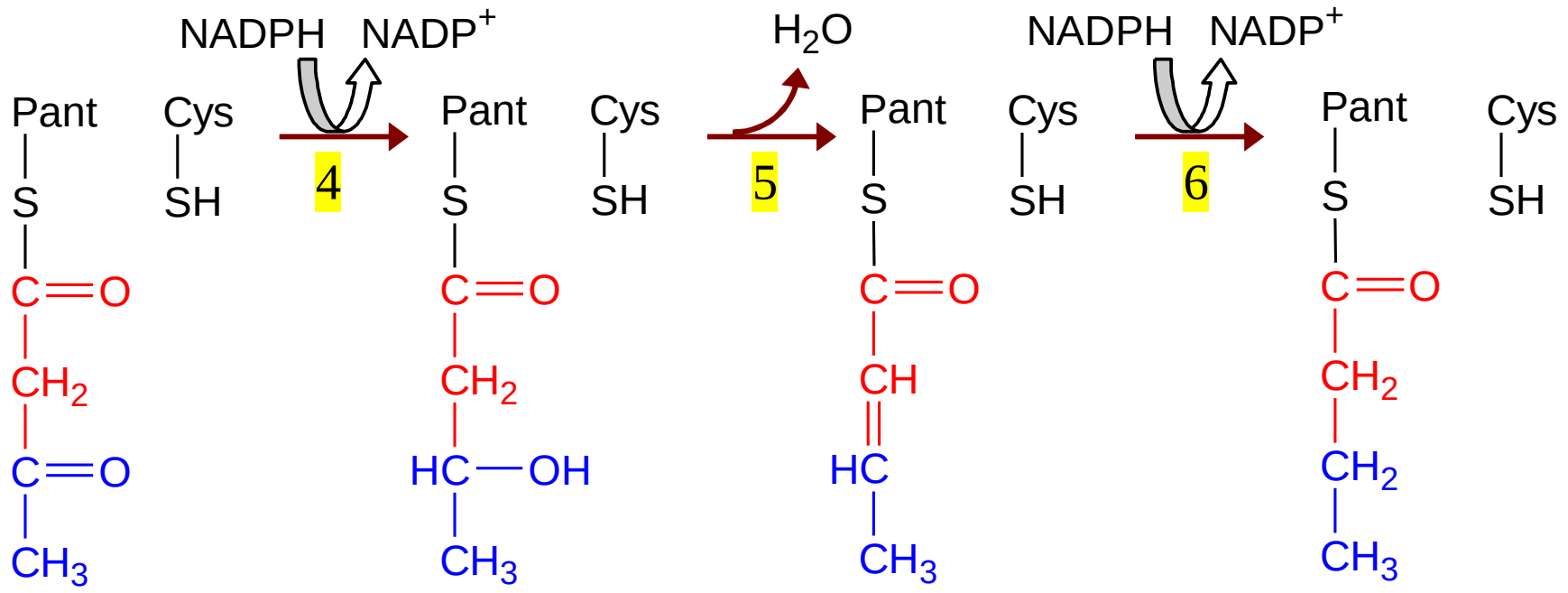
1 Malonyl/acetyl-CoA-ACP Transacylase

2 Malonyl/acetyl-CoA-ACP Transacylase

3 Condensing Enzyme (β-Ketoacyl Synthase)

The **condensation** reaction (step 3) involves decarboxylation of the malonyl moiety, followed by attack of the resultant carbanion on the carbonyl carbon of the acetyl (or acyl) moiety.

Fatty acid synthesis



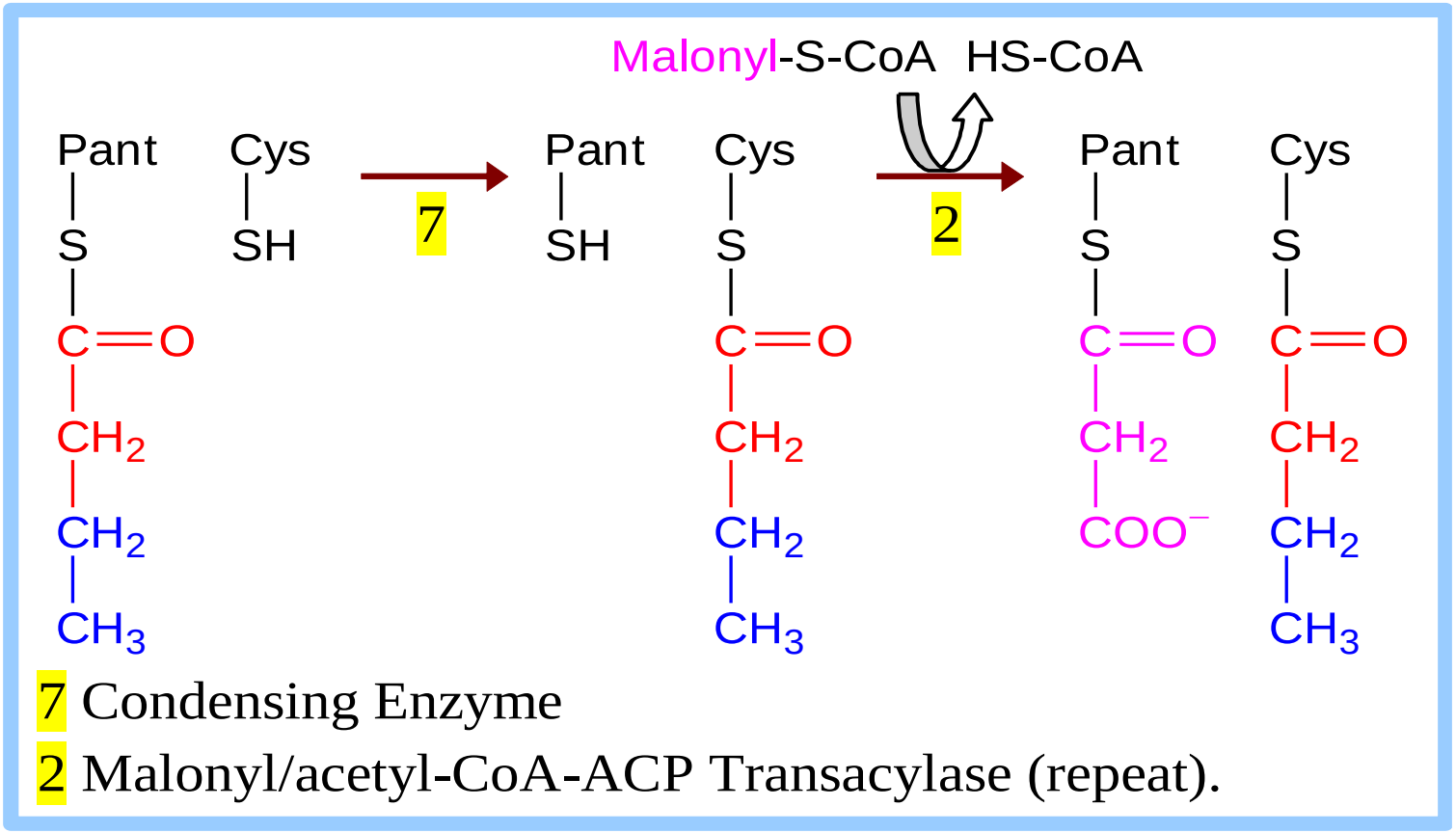
4 β-Ketoacyl-ACP Reductase

5 β-Hydroxyacyl-ACP Dehydratase

6 Enoyl-ACP Reductase

4. The β-ketone is **reduced** to an alcohol by e^- transfer from NADPH.
5. **Dehydration** yields a trans double bond.
6. **Reduction** by NADPH yields a saturated chain.

Fatty acid synthesis



7 Condensing Enzyme

2 Malonyl/acetyl-CoA-ACP Transacylase (repeat).

Following **transfer** of the growing fatty acid from phosphopantetheine to the Condensing Enzyme's cysteine sulfhydryl, the **cycle begins again**, with another malonyl-CoA.

Fatty acid synthesis

Product release:

When the fatty acid is 16 carbon atoms long, a **Thioesterase** domain catalyzes hydrolysis of the thioester linking the fatty acid to phosphopantetheine.

The **16-C** saturated fatty acid **palmitate** is the final product of the Fatty Acid Synthase complex.

Fatty acid synthesis

Summary (ignoring H^+ & water):

Write a balanced equation for synthesis of palmitate from acetyl-CoA, listing net inputs and outputs:

8 acetyl-CoA + 14 NADPH + 7 ATP →

palmitate + 14 $NADP^+$ + 8 CoA + 7 ADP + 7 P_i

Summary based on malonate as an input:

acetyl-CoA + 7 malonyl-CoA + 14 NADPH →

palmitate + 7 CO_2 + 14 $NADP^+$ + 8 CoA

Fatty acid synthesis occurs in the **cytosol**. Acetyl-CoA generated in mitochondria is transported to the cytosol via a shuttle mechanism involving **citrate**.

Fatty acid synthesis

Fatty Acid Synthase is **transcriptionally regulated**.

In liver:

- **Insulin**, a hormone produced when blood glucose is high, **stimulates** Fatty Acid Synthase expression.

  Thus excess glucose is stored as fat.

  Transcription factors that mediate the stimulatory effect of insulin include **USFs** (upstream stimulatory factors) and **SREBP-1**.

  SREBPs (sterol response element binding proteins) were first identified for their regulation of cholesterol synthesis.

- Polyunsaturated **fatty acids diminish** transcription of the Fatty Acid Synthase gene in liver cells, by suppressing production of SREBPs.

Fatty acid synthesis

In fat cells:

Expression of SREBP-1 and of Fatty Acid Synthase is **inhibited** by **leptin**, a hormone that has a role in regulating food intake and fat metabolism.

Leptin is produced by fat cells in response to excess fat storage.

Leptin regulates body weight by decreasing food intake, increasing energy expenditure, and inhibiting fatty acid synthesis.

Fatty acid synthesis

Elongation beyond the 16-C length of the palmitate product of Fatty Acid Synthase is mainly catalyzed by enzymes associated with the **endoplasmic reticulum** (ER).

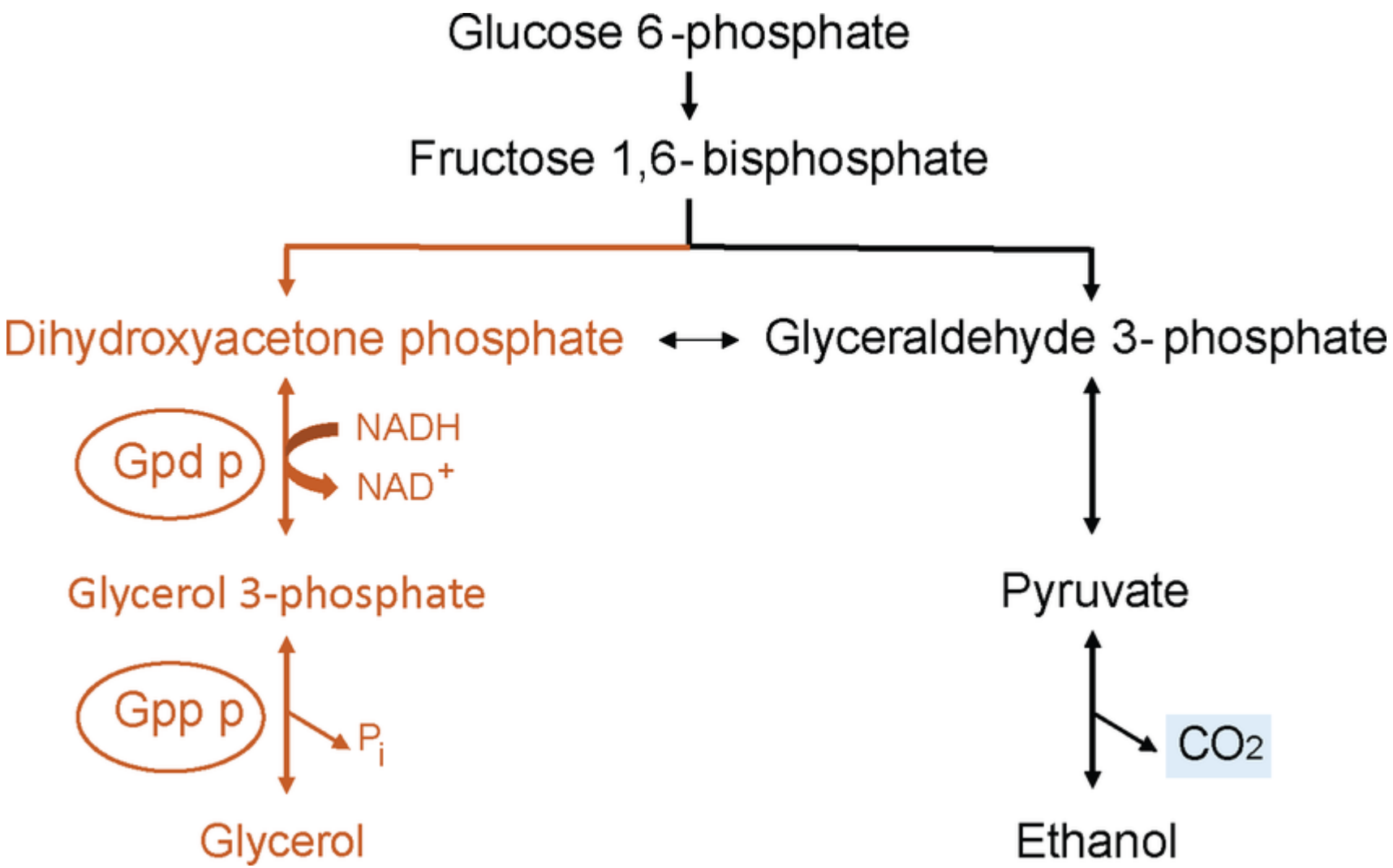
ER enzymes lengthen fatty acids produced by Fatty Acyl Synthase as well as dietary polyunsaturated fatty acids.

Fatty acids esterified to **coenzyme A** serve as substrates.

Malonyl-CoA is the donor of 2-carbon units in a reaction sequence **similar** to that of Fatty Acid Synthase except that individual steps are catalyzed by **separate proteins**.

A family of enzymes designated **Fatty Acid Elongases** or **ELOVL** (elongation of very long chain fatty acid) catalyze the initial condensation step.

Glycerol synthesis



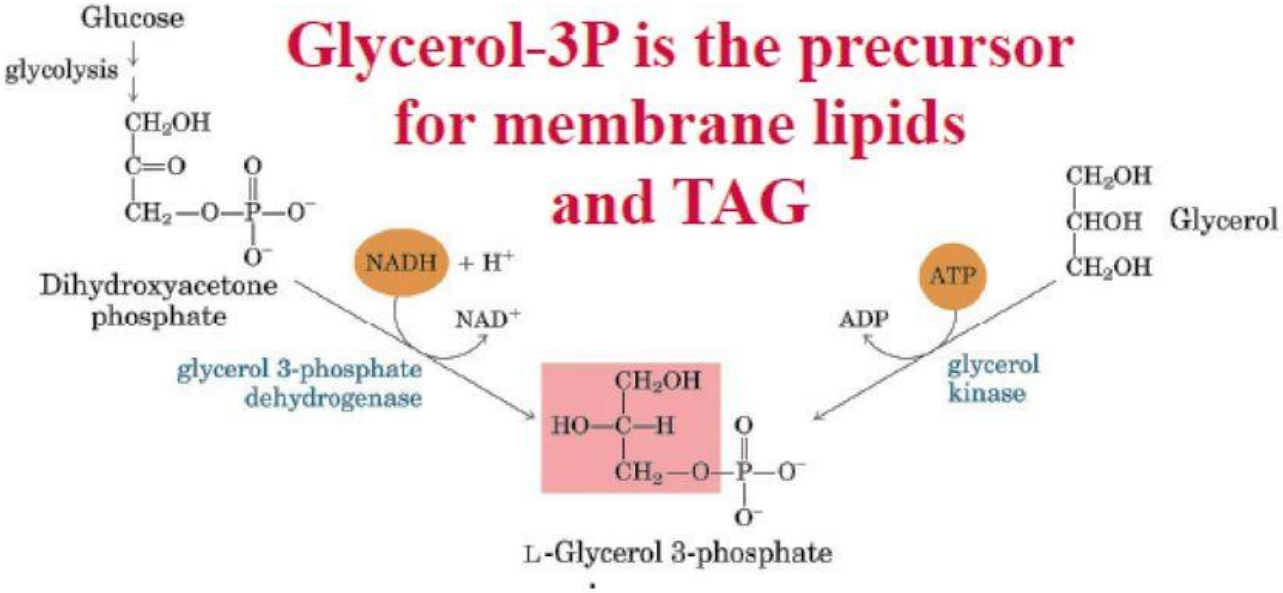
Glycerol synthesis

Biosynthesis of glycerol

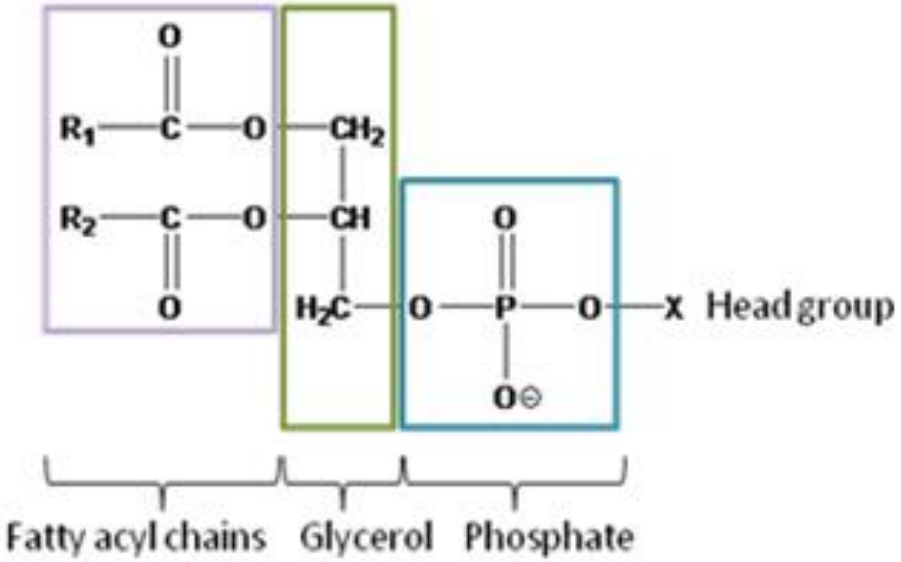
- Glucose is oxidized via glycolysis to **dihydroxy acetone phosphate**

  ↓

  reduced to **glycerol-3 phosphate** by the enzyme glycerol-3 phosphate dehydrogenase.



Glycerol synthesis

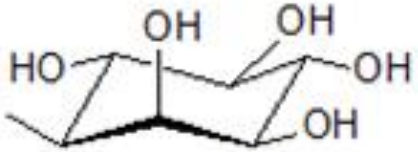


X = —H
phosphatidic acid

—CH_2-CH_2-$\overset{+}{N}(CH_3)_3$
phosphatidylcholine

—CH_2-CH_2-$\overset{+}{N}H_3$
phosphatidylethanolamine

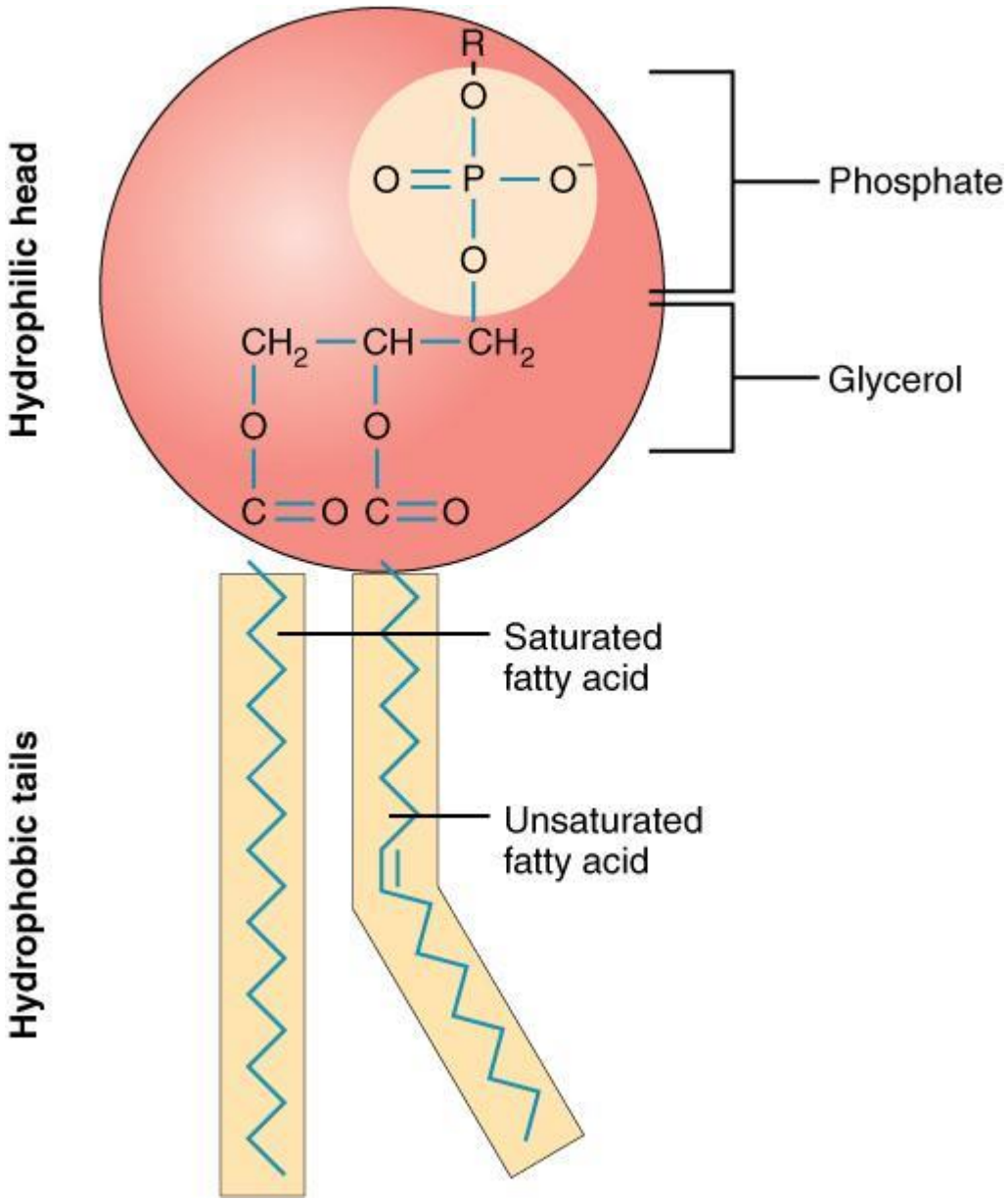
X = —CH_2-$\underset{COO^-}{CH}$-$\overset{+}{N}H_3$
phosphatidylserine



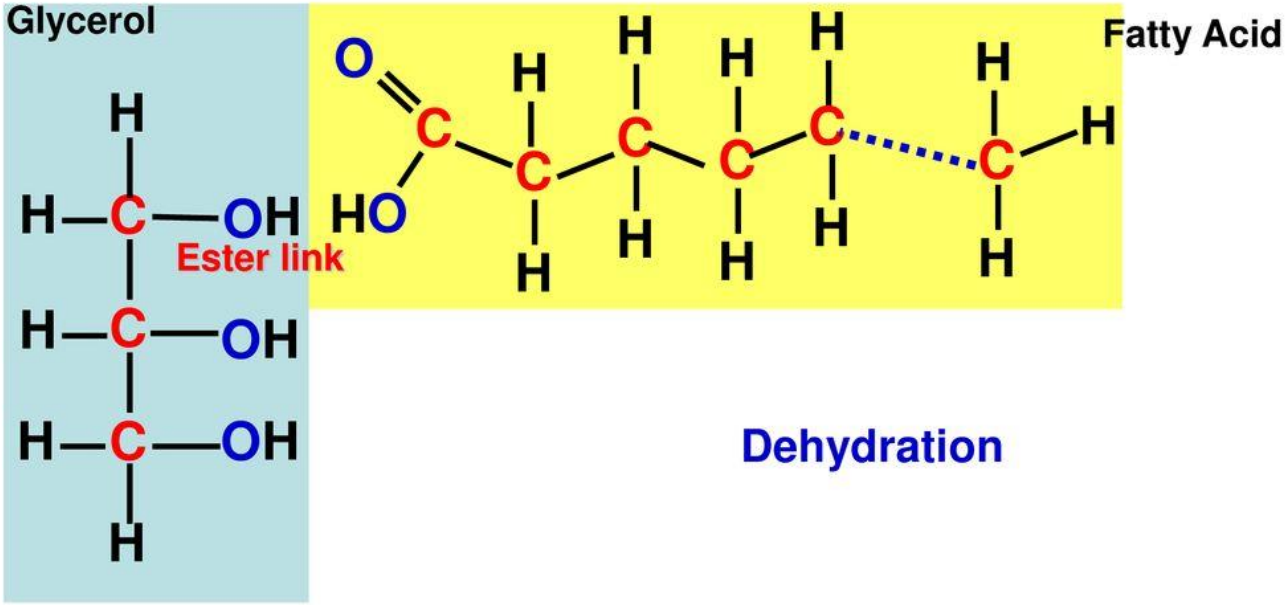
phosphatidylinositol

—CH_2-$\underset{OH}{CH}$-CH_2OH
phosphatidylglycerol

Phospholipid synthesis



Phospholipid synthesis



A fat is composed of <u>three fatty acids</u> linked with glycerol.

Fats are classified into Saturated and Un-saturated fats

Glycerol consists of a three C skeleton with an OH group attached to each.

A fatty acid consists of a carboxyl group (C=O) attached to a long carbon skeleton, often 16 to 18 carbons long.

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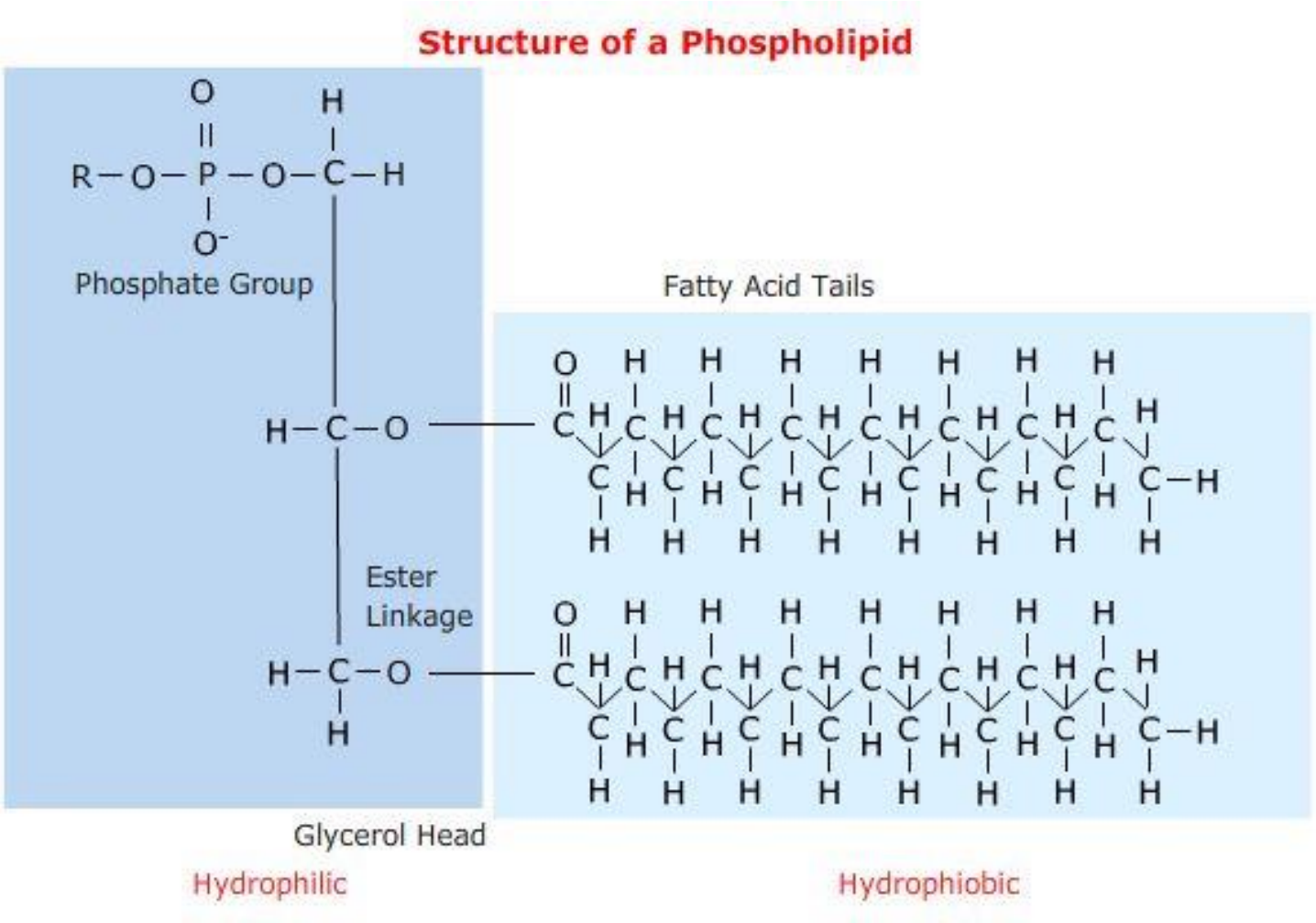
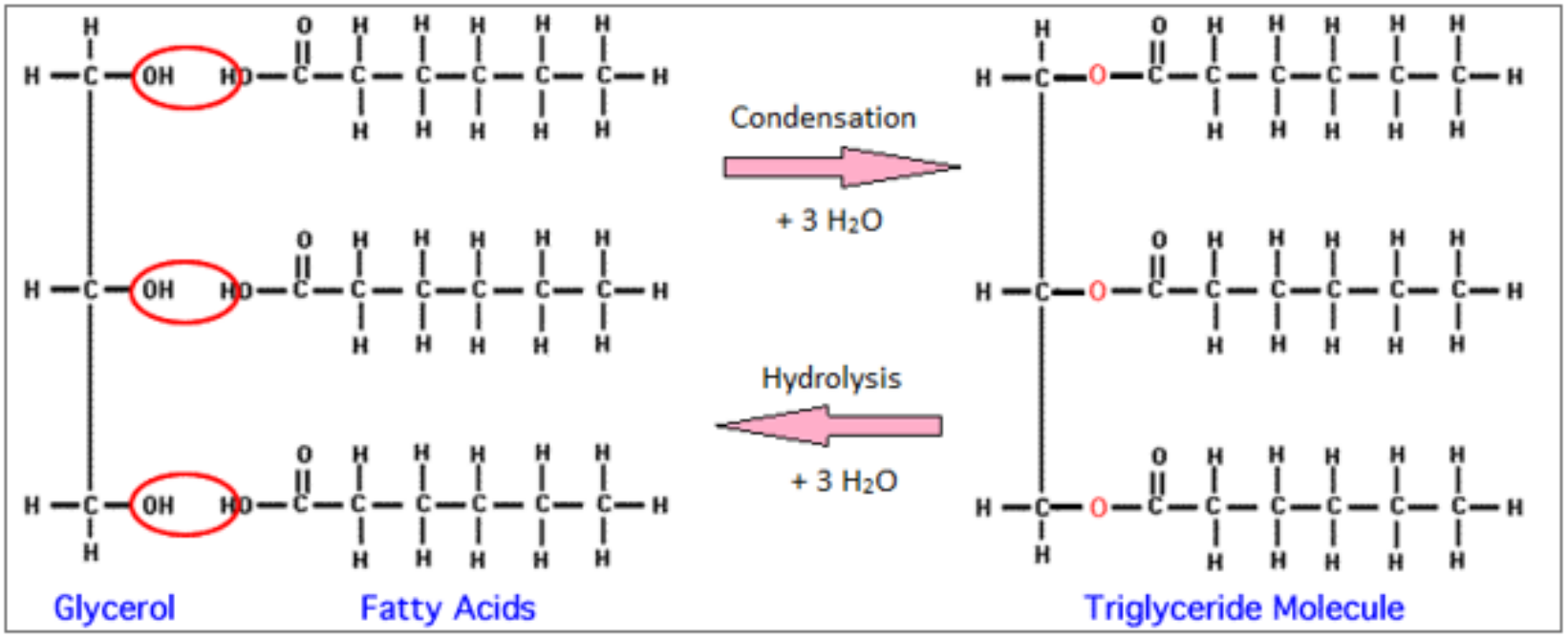
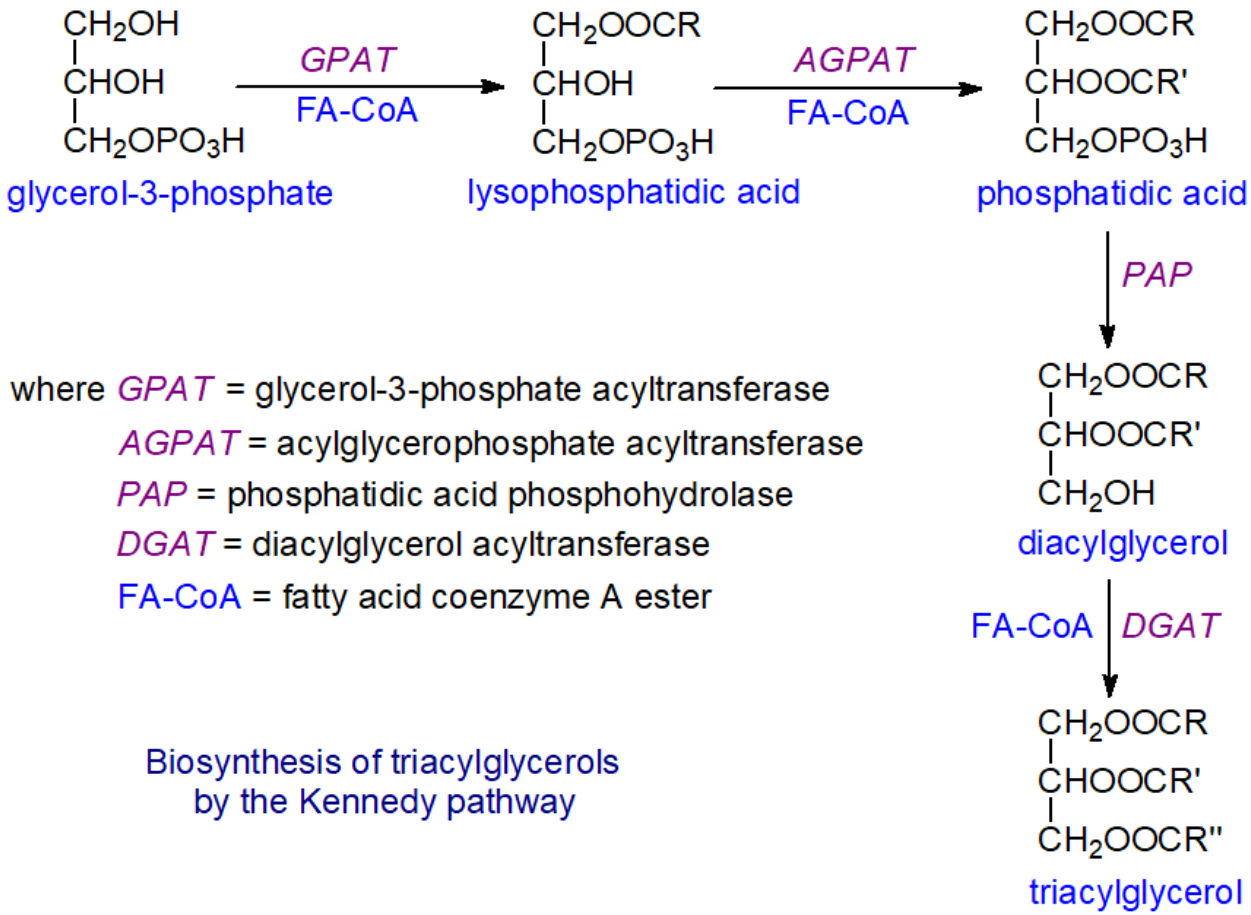
Phospholipid synthesis



Triglyceride synthesis



Biosynthesis of Triacyl Glycerols



where *GPAT* = glycerol-3-phosphate acyltransferase

AGPAT = acylglycerophosphate acyltransferase

PAP = phosphatidic acid phosphohydrolase

DGAT = diacylglycerol acyltransferase

FA-CoA = fatty acid coenzyme A ester

Biosynthesis of triacylglycerols
by the Kennedy pathway