Biosynthesis of Fatty Acids

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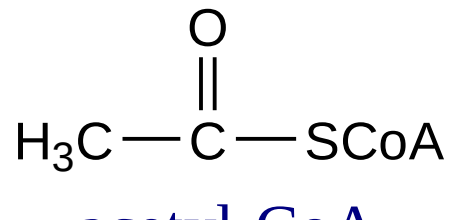
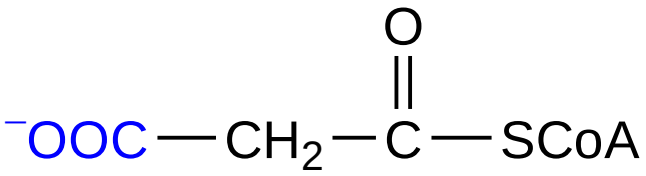
The input to fatty acid synthesis is **acetyl-CoA**, a two carbon compound, which is carboxylated to three carbon compound **malonyl-CoA**.



acetyl-CoA



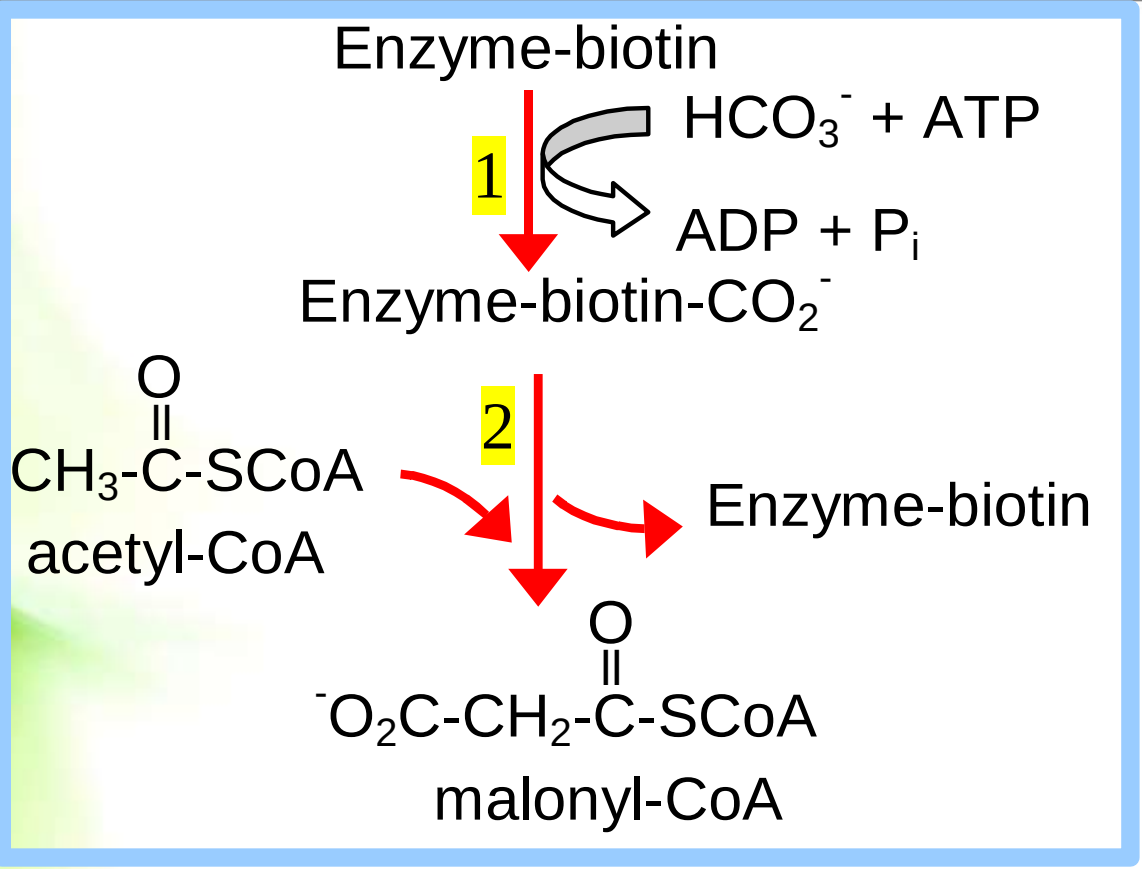
malonyl-CoA

ATP-dependent carboxylation provides energy input.

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

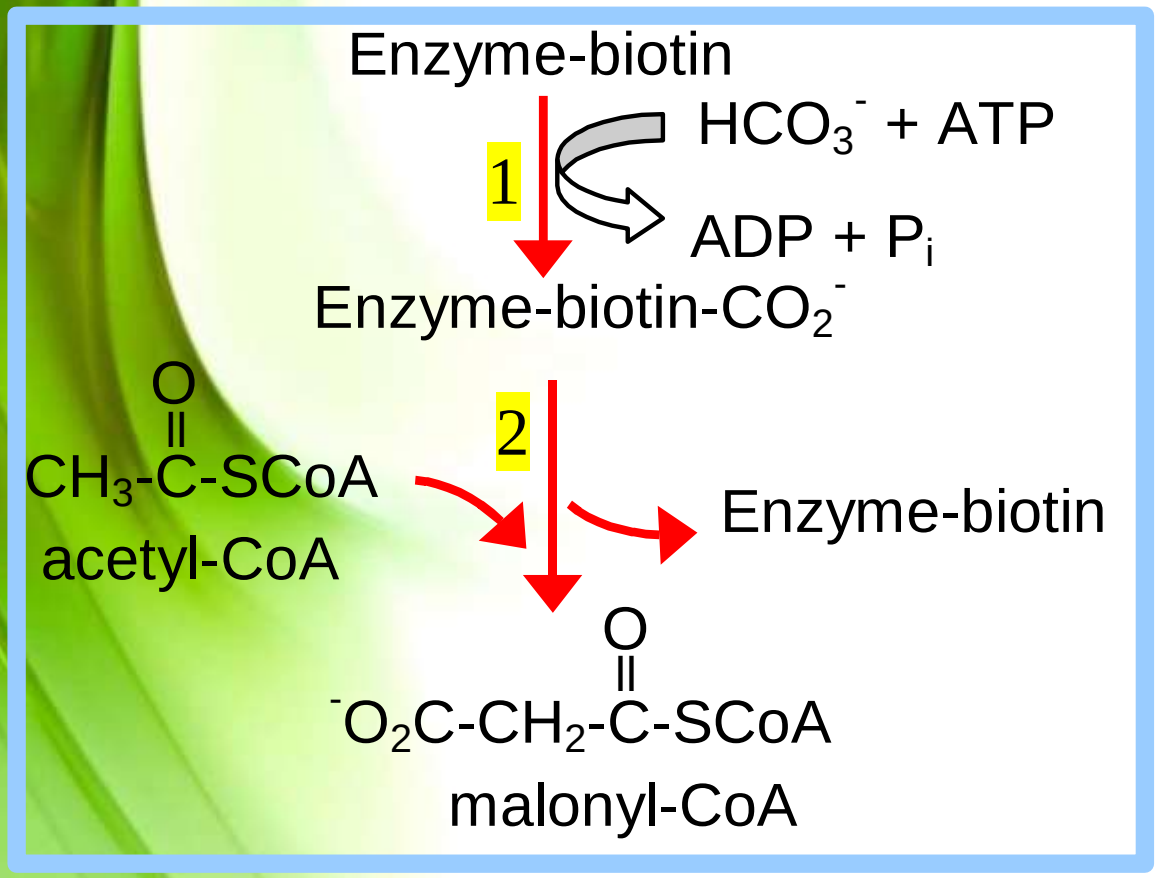
The spontaneous decarboxylation drives the condensation reaction.

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

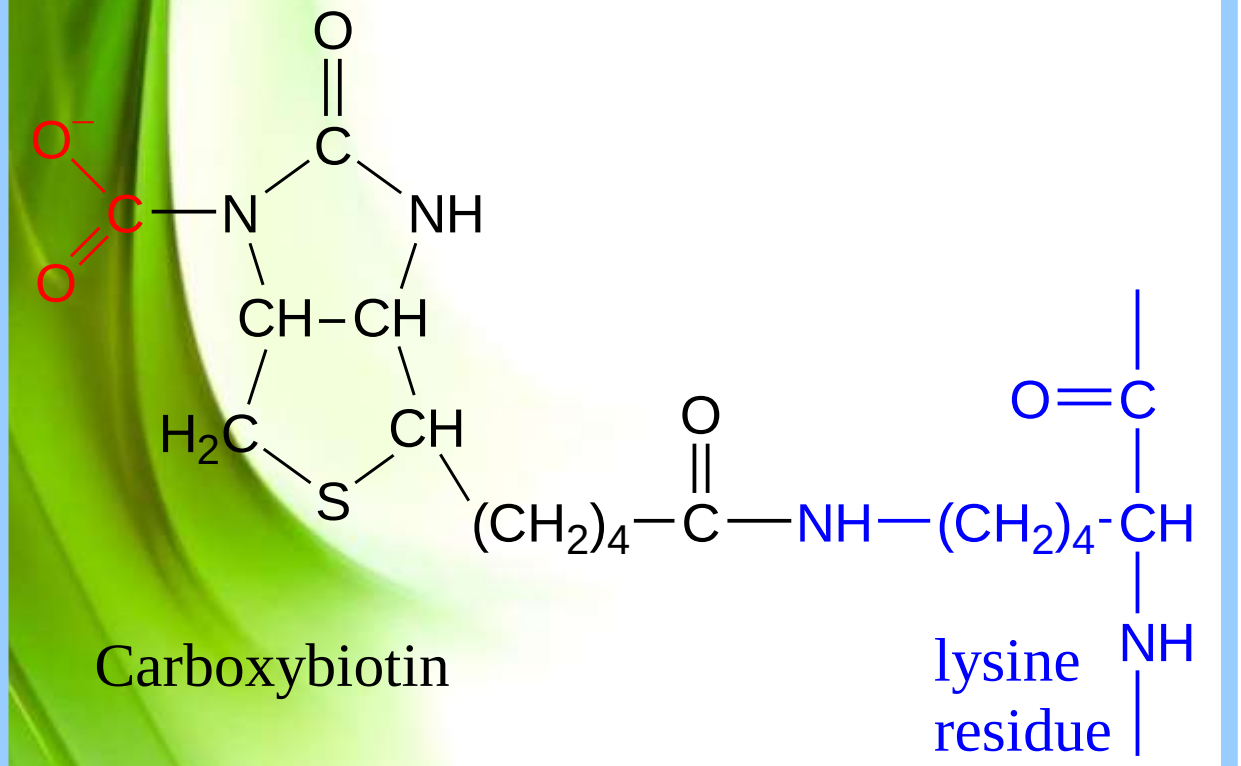


As with other carboxylation reactions, the enzyme prosthetic group is **biotin**.

ATP-dependent carboxylation of the biotin, carried out at one active site 1 , is followed by transfer of the carboxyl group to acetyl-CoA at a second active site 2 .



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



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

Conformational changes associated with regulation:

- In the **active** conformation, Acetyl-CoA Carboxylase associates to form multimeric **filamentous** complexes.
- For non-active confirmation, It is dissociated to yield the **monomeric** form of the enzyme, Acetyl-CoA Carboxylase as (protomer).

AMP functions as an **energy sensor** and regulator of metabolism.

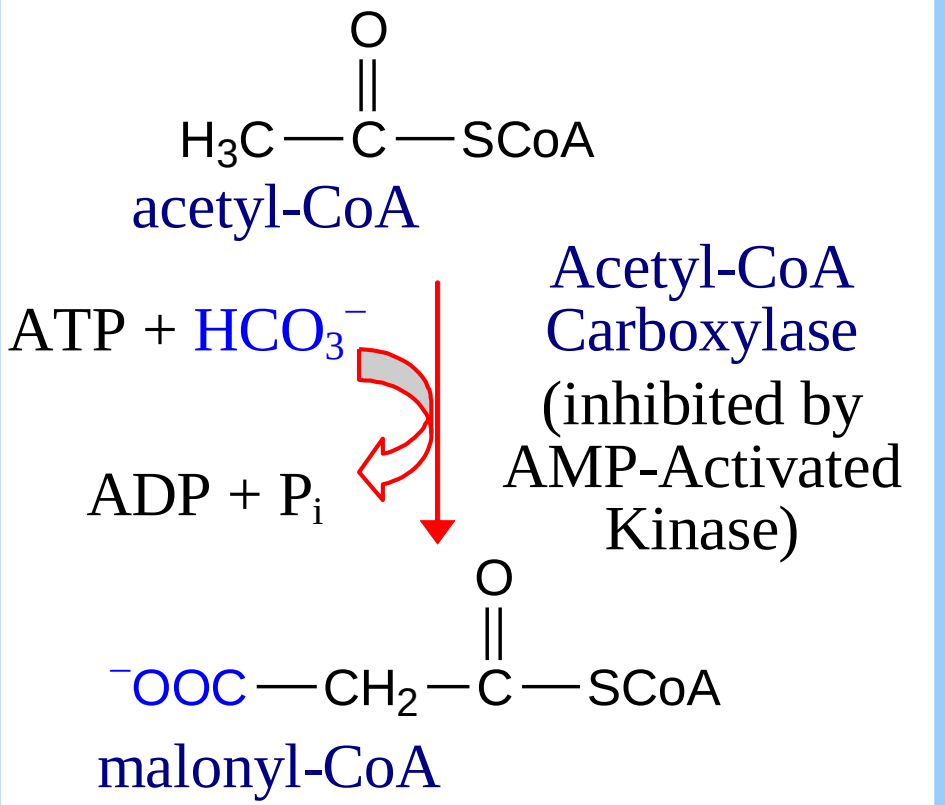
When ATP production does not keep up with needs, a higher portion of a cell's adenine nucleotide pool is in the form of AMP (adenosine nucleotide mono-phosphate).

- **AMP promotes catabolic pathways** that lead to synthesis of ATP.
- **AMP inhibits** energy-utilizing **synthetic pathways**.

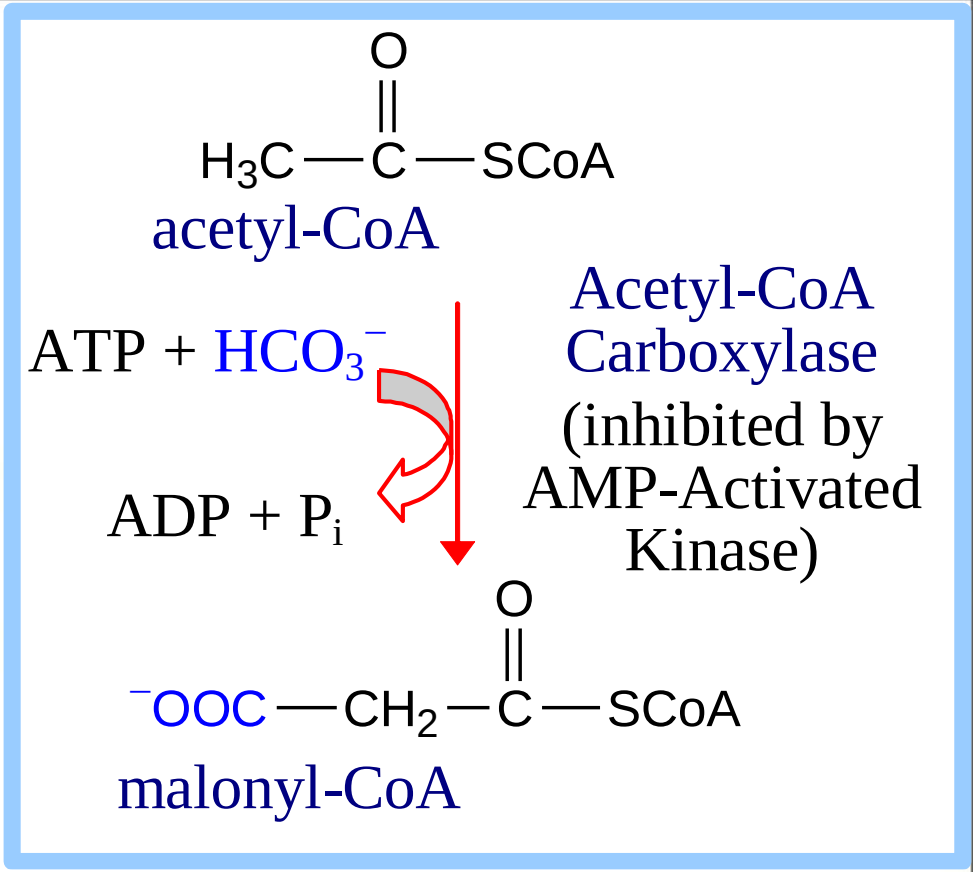
E.g., AMP regulates fatty acid synthesis and catabolism by controlling availability of **malonyl-CoA**.

AMP-Activated Kinase catalyzes phosphorylation of **Acetyl-CoA Carboxylase**.

This causes **inhibition** of ATP-utilizing of **malonyl-CoA** production.

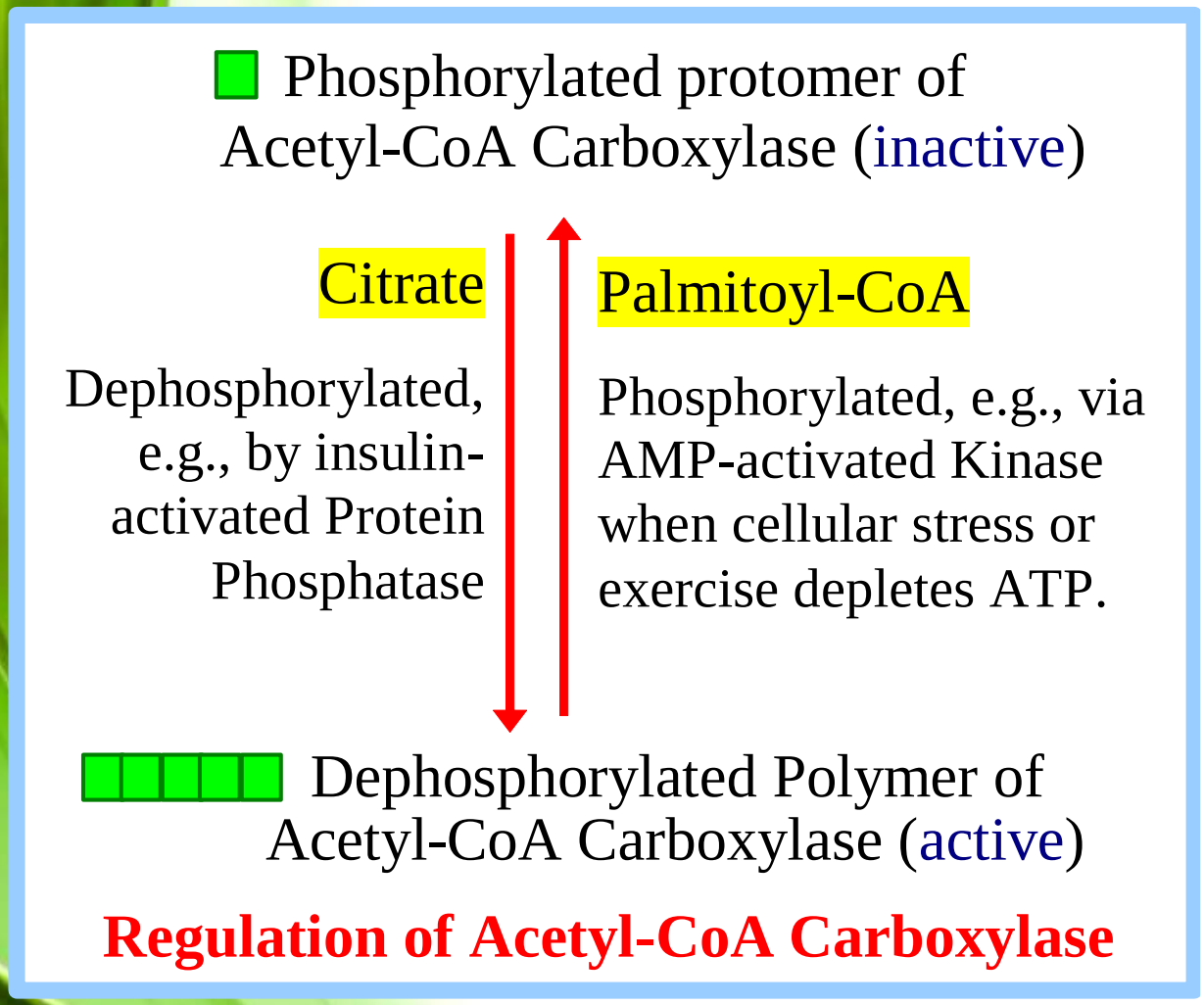


- **Fatty acid synthesis** is **diminished** by lack of the substrate malonyl-CoA.
- As discussed earlier, **fatty acid oxidation** is **stimulated** due to decreased inhibition by malonyl-CoA of transfer of fatty acids into mitochondria.



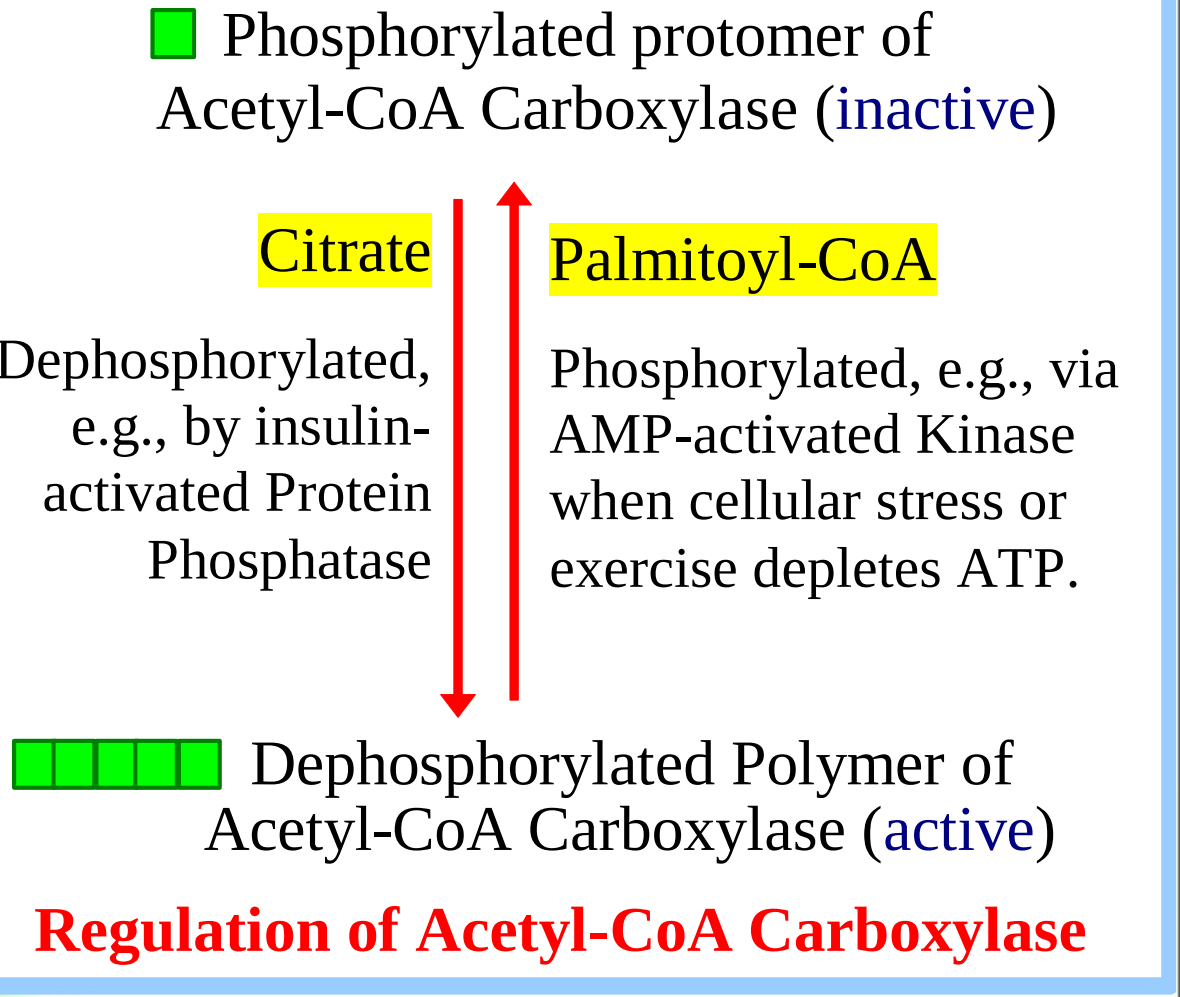
A **cAMP** cascade, activated by glucagon & epinephrine when blood glucose is low, may also result in phosphorylation of Acetyl-CoA Carboxylase via **cAMP-Dependent Protein Kinase**.

With Acetyl-CoA Carboxylase inhibited, acetyl-CoA remains available for synthesis of ketone bodies, the alternative metabolic fuel used when blood glucose is low.



The antagonistic effect of **insulin**, produced when blood glucose is high, is attributed to activation of Protein Phosphatase.

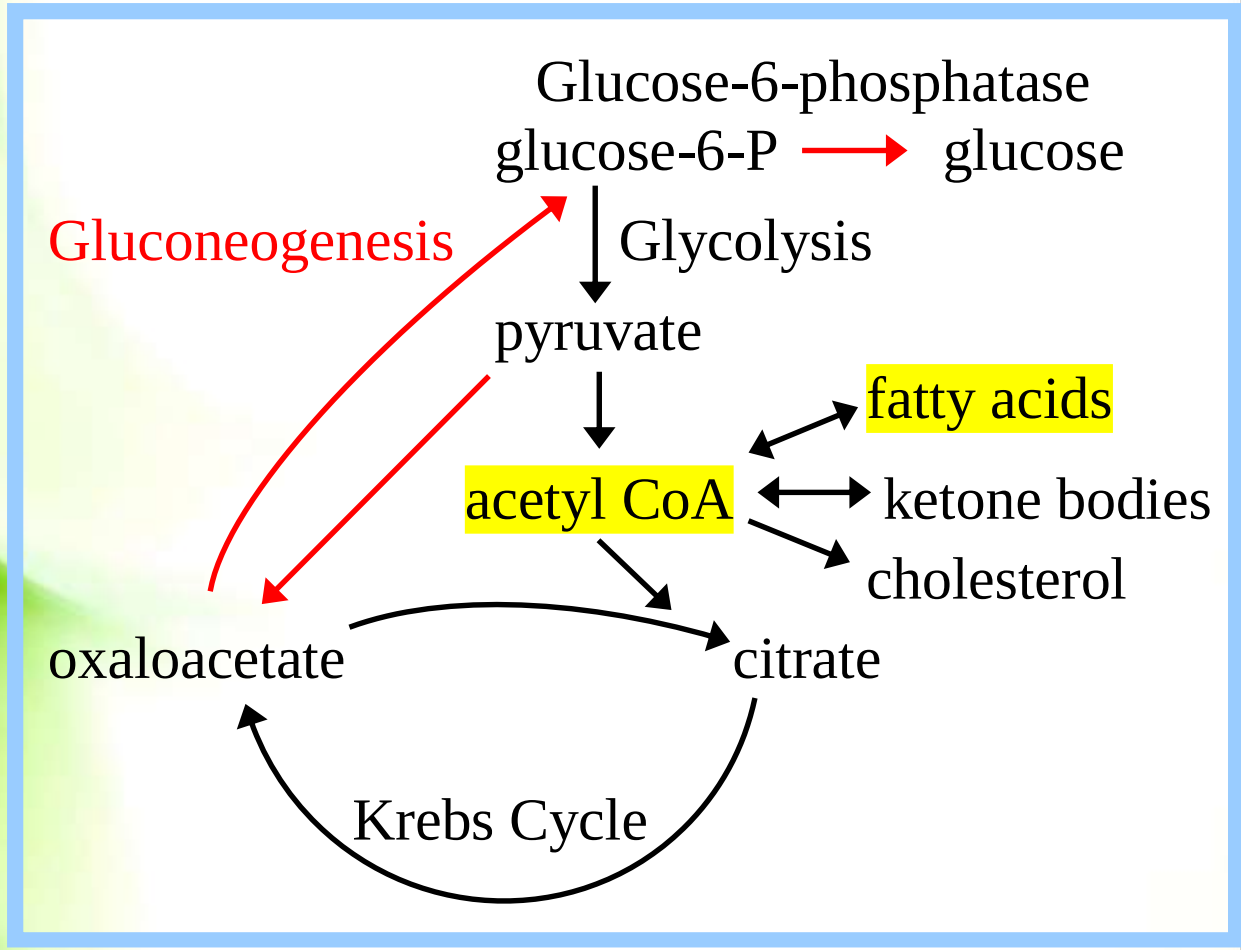
Regulation of Acetyl-CoA Carboxylase by **local metabolites**:



Regulation of Acetyl-CoA Carboxylase

Palmitoyl-CoA (product of Fatty Acid Synthase) promotes the **inactive** conformation, diminishing production of malonyl-CoA, the precursor of fatty acid synthesis.

This is an example of **feedback inhibition**.



Citrate allosterically **activates** Acetyl-CoA Carboxylase.

[Citrate] is high when there is adequate acetyl-CoA entering Krebs Cycle.

Excess acetyl-CoA is then converted via malonyl-CoA to fatty acids for storage.

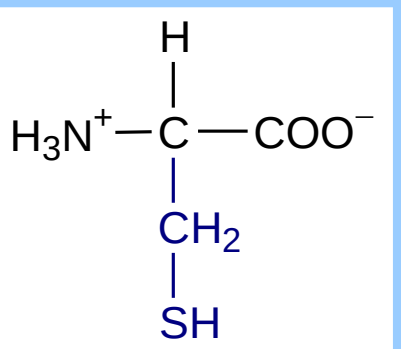
Fatty acid synthesis from acetyl-CoA & malonyl-CoA occurs by a series of reactions that are:

- 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.



cysteine

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.

Coenzyme A

β-mercaptoethylamine

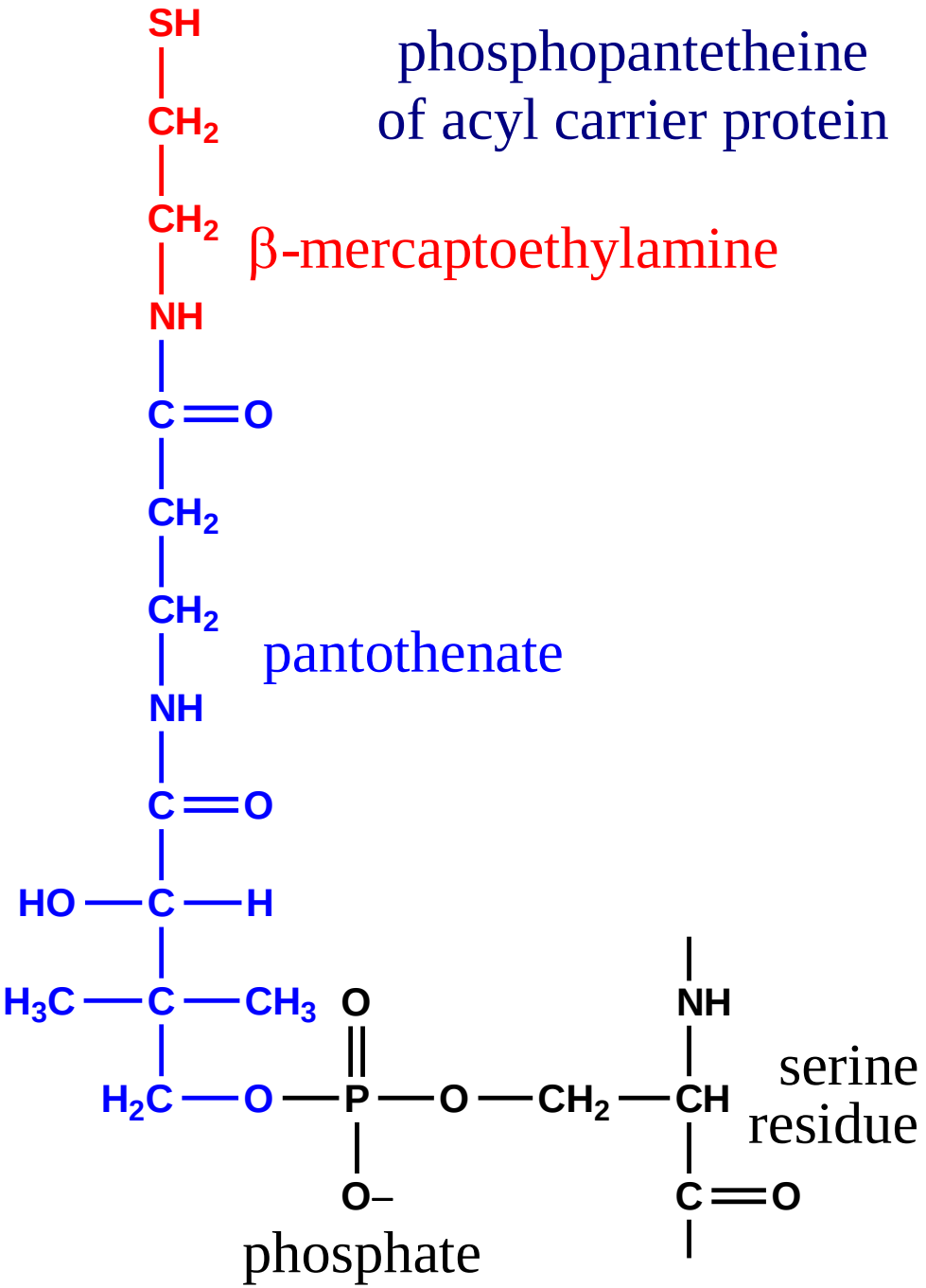
pantothenate

ADP-3'-phosphate

phosphopantetheine

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.

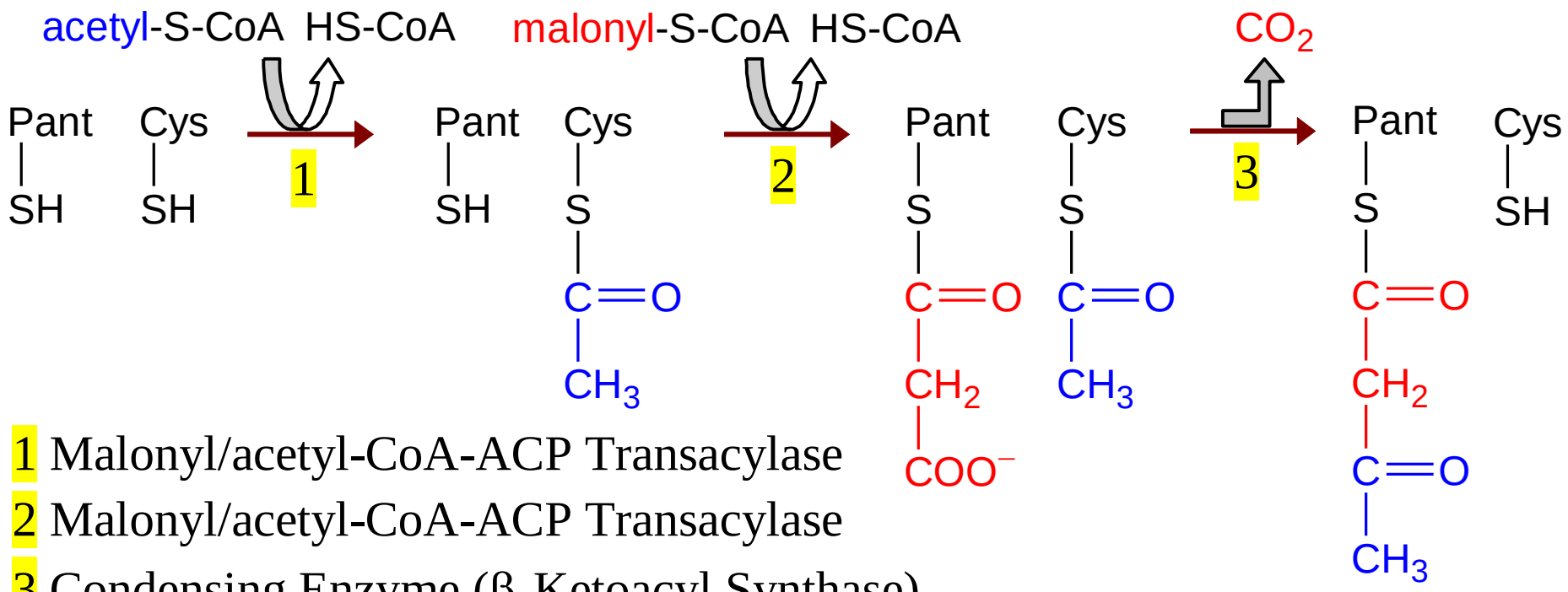


Order of domains in primary structure of mammalian Fatty Acid Synthase

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.

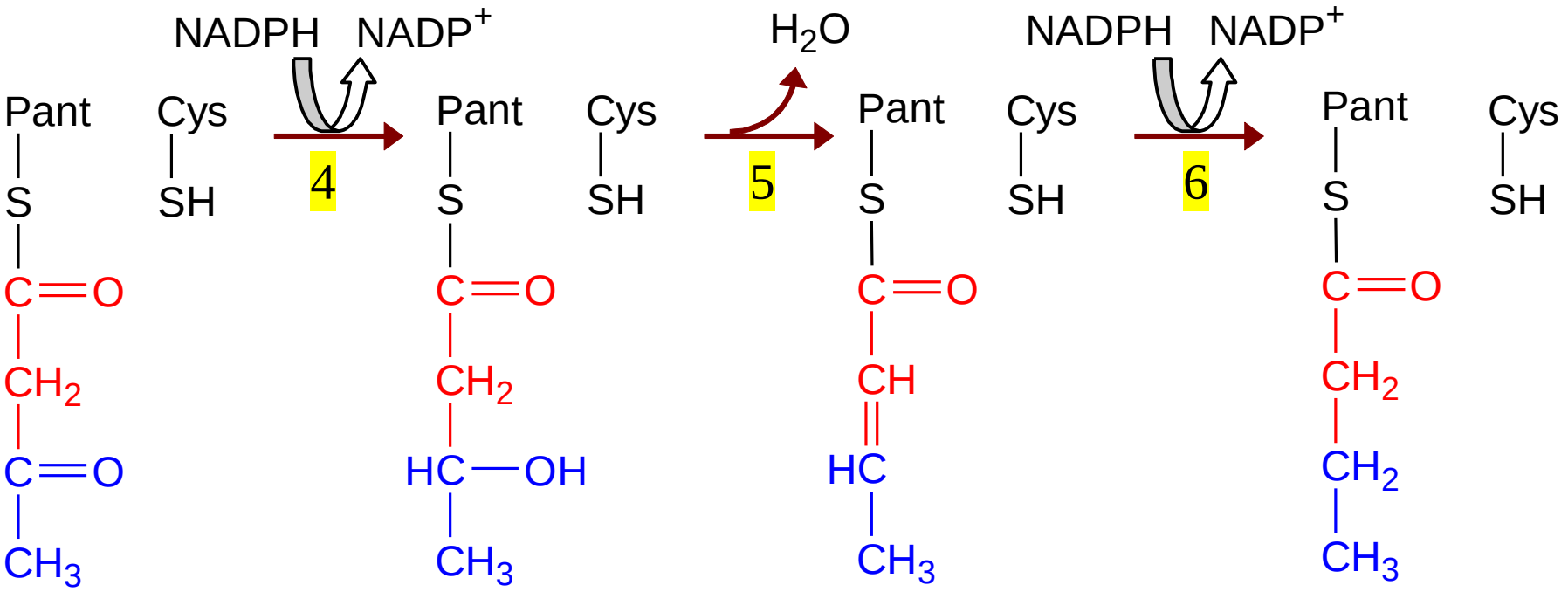


1 Malonyl/acetyl-CoA-ACP Transacylase

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.

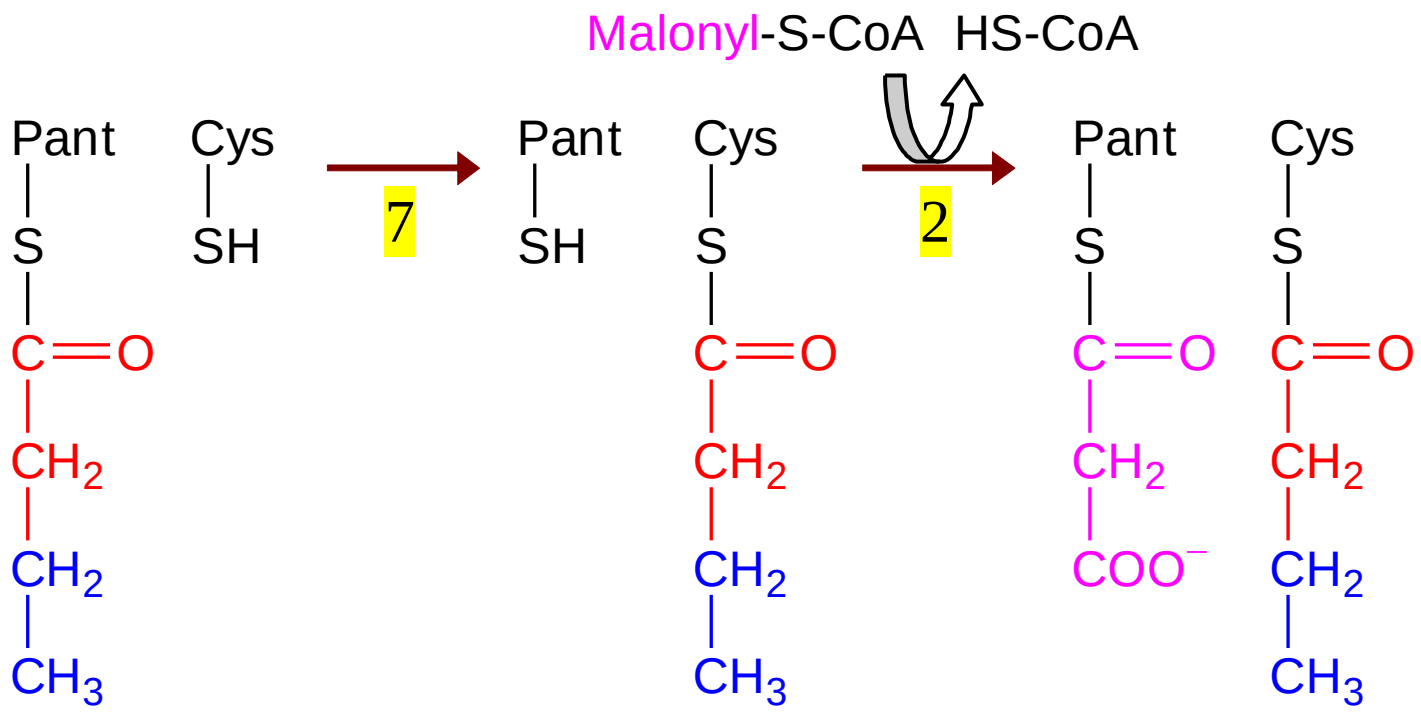


4 β-Ketoacyl-ACP Reductase

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.



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.

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.

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 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.

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.

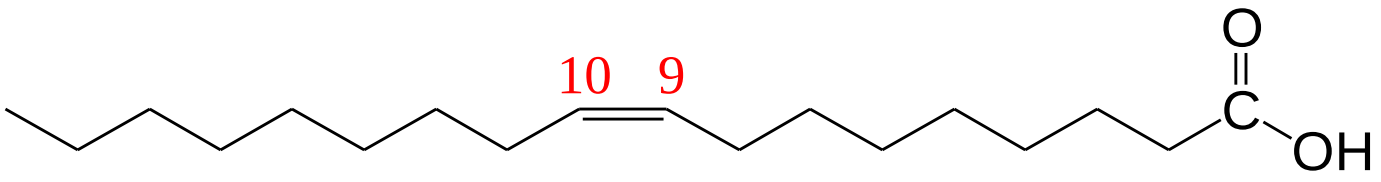
Elongation beyond the 16-C length of the palmitate product of Fatty Acid Synthase is mainly catalyzed by enzymes associated with the **endoplasmic reticulum** (ER).

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.

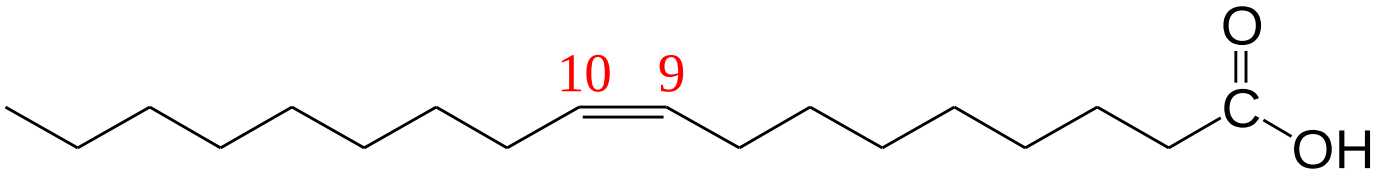


oleate 18:1 cis Δ^9

Desaturases introduce **double bonds** at specific positions in a fatty acid chain.

Mammalian cells are unable to produce double bonds at certain locations, e.g., Δ^{12}.

Thus some polyunsaturated fatty acids are **dietary essentials**, e.g., linoleic acid, 18:2 cis $\Delta^{9,12}$ (18 C atoms long, with cis double bonds at carbons 9-10 & 12-13).



oleate 18:1 cis Δ^9

Formation of a double bond in a fatty acid involves the following endoplasmic reticulum membrane proteins in mammalian cells:

- **NADH-cyt b_5 Reductase**, a flavoprotein with **FAD** as prosthetic group.
- **Cytochrome b_5**, which may be a separate protein or a domain at one end of the desaturase.
- **Desaturase**, with an active site that contains **two iron atoms** complexed by histidine residues.

The desaturase catalyzes a **mixed function oxidation** reaction.

There is a **4-electron** reduction of **O_2 → 2 H_2O** as a fatty acid is oxidized to form a double bond.

- **$2e^-$** pass from **NADH** to the desaturase via the FAD-containing reductase & cytochrome b_5, the order of electron transfer being:

  NADH → FAD → cyt b_5 → desaturase

- **$2e^-$** are extracted from the fatty acid as the double bond is formed.

E.g., the overall reaction for desaturation of stearate (18:0) to form oleate (18:1 cis Δ^9) is:

stearate + NADH + H^+ + O_2 → oleate + NAD^+ + 2H_2O

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