Metabolism of lipids

Biosynthesis of fatty acids and triacylglycerols

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Main characteristics of the fatty acid biosynthesis (1)

- Takes place in the *majority of animal cells* (mainly in the liver, adipocytes and in the lactating mammary gland)
- Occurs at times of *caloric food abundance* – to build fuel reserves for future demands
- Takes place *in the cytosol* , outside mitochondria x fatty acid degradation, which takes place in mitochondria
- Many of the enzymes of FA synthesis in higher organisms are organized into a *multienzyme complex* called *fatty acid synthase*
- *Intermediates of the synthesis* are covalently linked to the *acyl carrier protein = ACP,* one of the component of the fatty acid synthase complex, and not to CoA as during the FA degradation

Main characteristics of the fatty acid biosynthesis (2)

- **Biosynthetic reactions are catalyzed by *enzymes different from those catalyzing the degradation processes* despite the fact that the intermediates are similar to those produced during the degradation process**
- **The FAs are built by *sequential addition of two-carbon units* derived from acetyl CoA. The activated donor of the two-carbon units in the elongation step is *malonyl-ACP* (a three-carbon unit) but during the elongation, CO_2 is released. This drives the reaction**
- **The reducing agent is *NADPH*.**
- Elongation by FA synthase complex ***stops upon formation of C16 palmitate*.** Further elongation and the insertion of double bonds (by desaturases) are carried out by other enzyme systems (in mt, ER)
- In ***bacteria***, FAs are primarily precursors of ***phospholipids***, not of fuels

Fatty acid biosynthesis: precursors

acetyl-CoA

- **from *pyruvate (by oxidative decarboxylation)*, the main source is glucose**
- **from the degradation of *some amino acids***
- **from *fatty acids***

NADPH

- **from *pentose cycle* – the main source**
- **from decarboxylation of malate by *malate enzyme* ($NADP^+$-linked malate dehydrogenase in the cytosol)**

malate + $NADP^+$ ⟷ pyruvate + CO_2 + NADPH + H^+

- **from dehydrogenation of isocitrate to α–ketoglutarate by *isocitrate dehydrogenase* (NAD^+- linked enzyme is present in mt only, $NADP^+$- linked one is both in mt and in the cytosol)**

Transport of acetyl CoA from the mitochondrial matrix to the cytosol

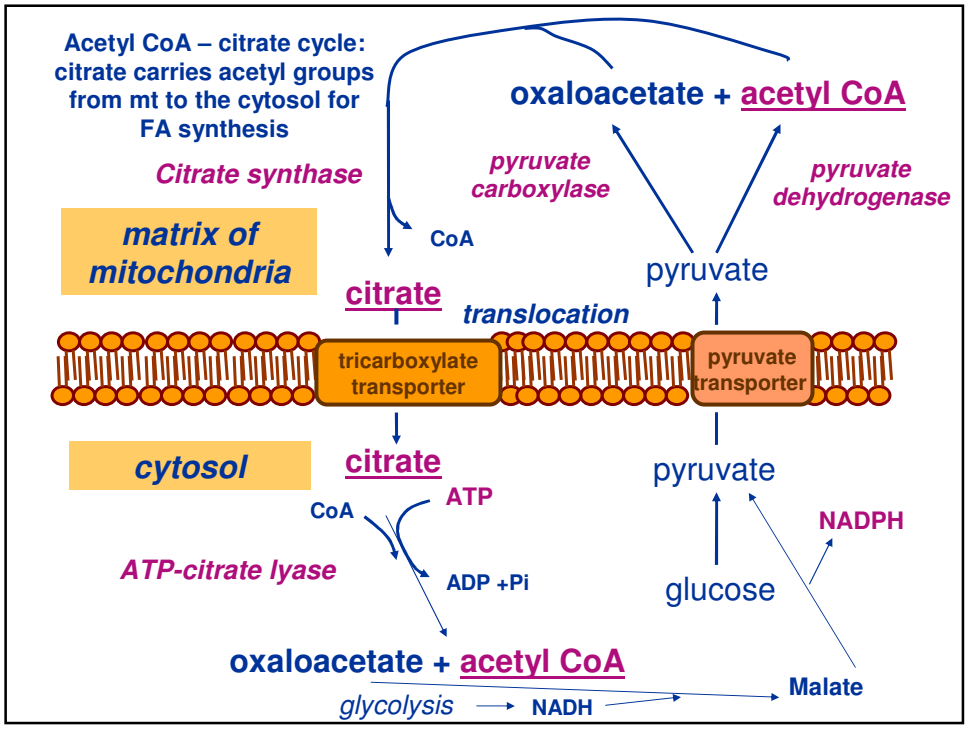
The inner mt membrane is *not permeable* for *acetyl-CoA*

↓

The transport into the cytosol requires *conversion* of acetyl CoA (+ oxaloacetate) into *citrate* which has a *transporter* in the inner mt membrane

↓

In the cytosol takes place a *back conversion* of the citrate *into acetyl CoA* (+ oxaloacetate)



Biosynthesis of fatty acids: reaction steps and enzymes

(1) Formation of malonyl CoA

acetyl CoA-carboxylase

(2) Synthesis of the hydrocarbon chain (up to C16)

fatty acid synthase (FAS) complex – cytosol

(3) hydrocarbon chain further prolongation (>C16)

elongation systems - mitochondria, endoplasmic reticulum ER

(4) double bond formation – unsaturated FA

desaturation systems - endoplasmic reticulum ER

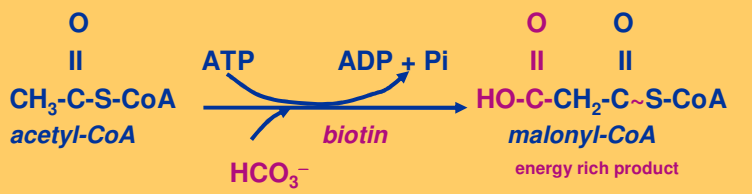
1. Formation of malonyl CoA

Carboxylation of acetyl CoA to *malonyl CoA*

This reaction is irreversible and the commited step = rate limiting step in FA biosynthesis

- Acetyl CoA has not enough energy for the condensation with the growing FA hydrocarbon chain
- It is „activated" by ATP-driven carboxylation catalyzed by *acetyl-CoA carboxylase* and the following elongation reaction is driven by the release of CO_2
- Acetyl CoA carboxylase: two subunits, each has covalently bound *biotin* prosthetic group via ε- amino group of lysine residues of the protein; *biotin is a carboxyl group carrier*

The reaction is catalyzed by acetyl CoA carboxylase - a biotin enzyme:

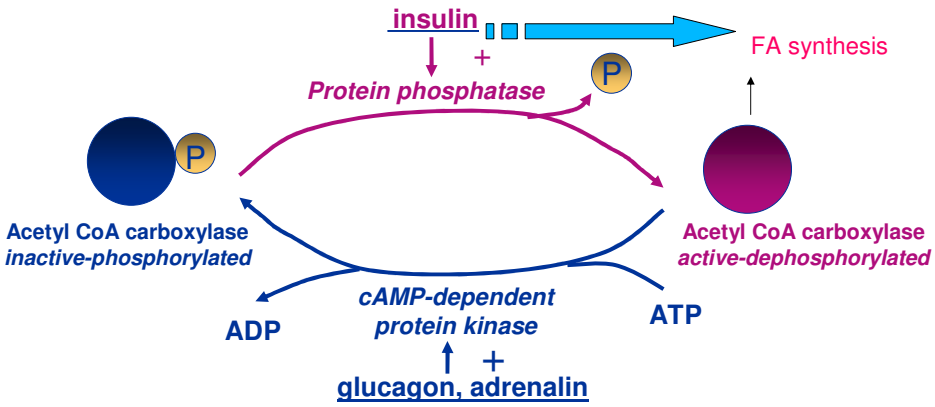


Regulation of acetyl CoA carboxylase activity by phosphorylation

I. short time

– *reversible phosphorylation*

- active enzyme ⟶ dephosphorylated (effect of insulin)
- inactive enzyme ⟶ phosphorylated (effect of glucagon, adrenalin)



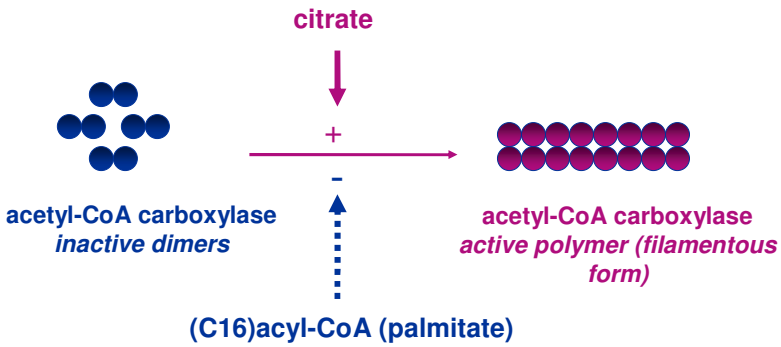
Regulation of acetyl CoA carboxylase (ACC) activity by polymerization

II. short-term: two forms of ACC

Favoured by dephoshorylation

– *Allosteric regulation*

- Activation by citrate: ⟶ shift to ACC *polymerization*
- Inactivation by palmitoyl-CoA: ⟶ shift to inactive dimer form



Regulation of acetyl CoA carboxylase activity by gene expression

III. long-term – adaptation

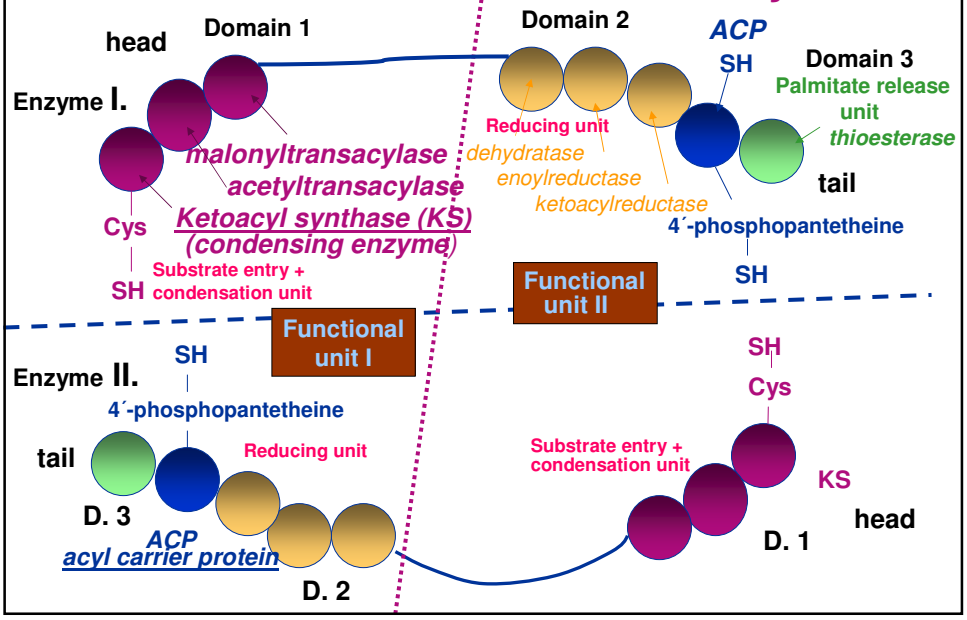
- Prolonged intake of energy-rich food (saccharides in particular) induces high expression of acetyl CoA carboxylase resulting in increased rate of FA synthesis
- Low-caloric diet or starvation suppress expression of acetyl CoA carboxylase resulting in the decrease of FA synthesis

Palmitate biosynthesis

Fatty acid synthase (FAS) is a single multifunctional protein with seven different catalytic activities

- *Active form of the FAS is a dimer* formed by two identical FAS molecules arranged in a configuration *head to tail*
- Each molecule of the FAS is arranged into *three domains* and involves *seven different catalytic activities + a carrier activity (ACP)* to bind *acyl intermediate* of the synthesis:
  - *Seven different catalytic sites are arranged on one polypeptide chain + acyl intermediate binding site*
- *Two molecules* of a fatty acid are synthesized *simultaneously*

Fatty acid synthase (FAS) - a multidomain multifunctional enzyme



Palmitate biosynthesis – steps (1)

Outside the fatty acid synthase (FAS):

(1) Formation of *acetyl CoA* and *malonyl CoA*

On the fatty acid synthase (FAS):

(2) Formation of *acetyl-ACP* and *malonyl-ACP*:
Acetyl CoA + ACP $\rightleftharpoons$ acetyl-ACP + CoA;
Malonyl CoA + ACP $\rightleftharpoons$ malonyl-ACP + CoA

(3) *Transfer of acetyl group* from acetyl-ACP *on* the cystein SH group of *ketoacyl synthase* (*KS*): CH_3-CO-S-*KS*

ACP = Acyl Carrier Protein *(4'-phosphopantetheine-SH)*

Palmitate biosynthesis – steps (2)

(4) Coupling (condensation) of *CH_3-CO from KS with malonyl-ACP* to form *acetoacetyl-ACP*: *CH_3-CO-CH_2-CO-S-ACP*. In the condensation reaction, a *four carbon unit is formed* from a two carbon unit and a three carbon unit, and CO_2 is released

(5) Reduction I, hydration, reduction II to form *butyryl-ACP*

(6) *Transfer of the butyryl group to Cys-SH of KS*

Palmitate biosynthesis – steps (3)

(7) The *ACP is "reloaded"* with a *malonyl group* from malonyl CoA

(8) *Another cycle of elongation* of the growing fatty acid chain by two carbon atoms occurs

(9) The whole process is repeated *seven times to yield palmitoyl-ACP*

(10) Palmitoyl-ACP is *hydrolyzed* to yield palmitate and free ACP

Reaction steps catalyzed by FA synthase complex

- Substrates entry
- Condensation
- Reduction 1
- Dehydration
- Reduction 2

Repetition 7x

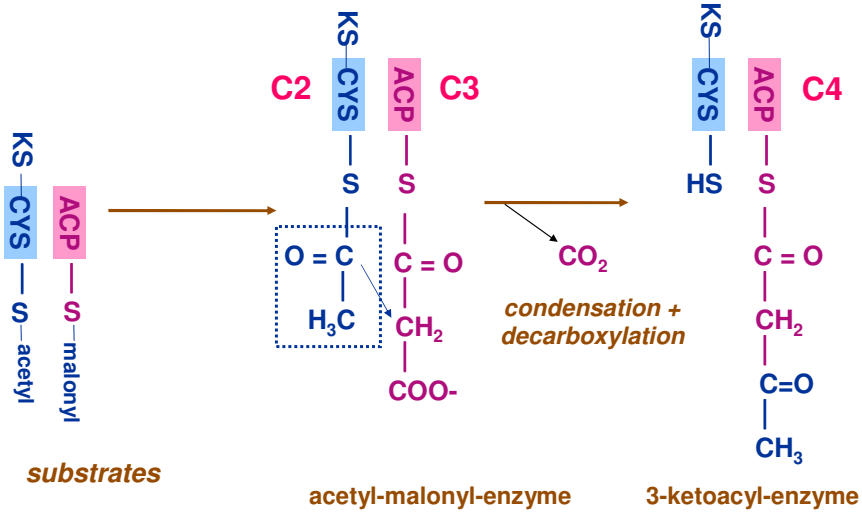
palmitoyl-enzyme

- Hydrolysis

palmitate

Fatty acid biosynthesis

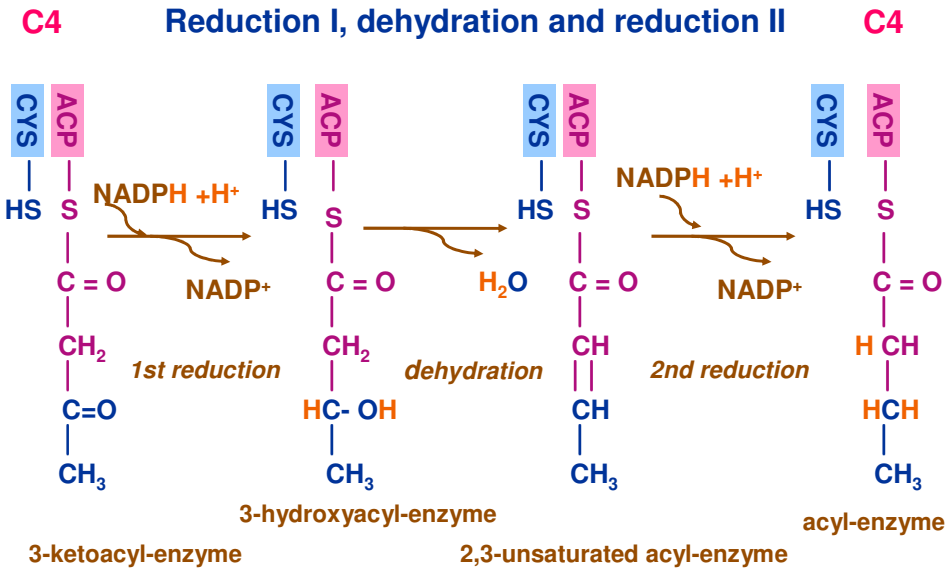
Condensation reaction



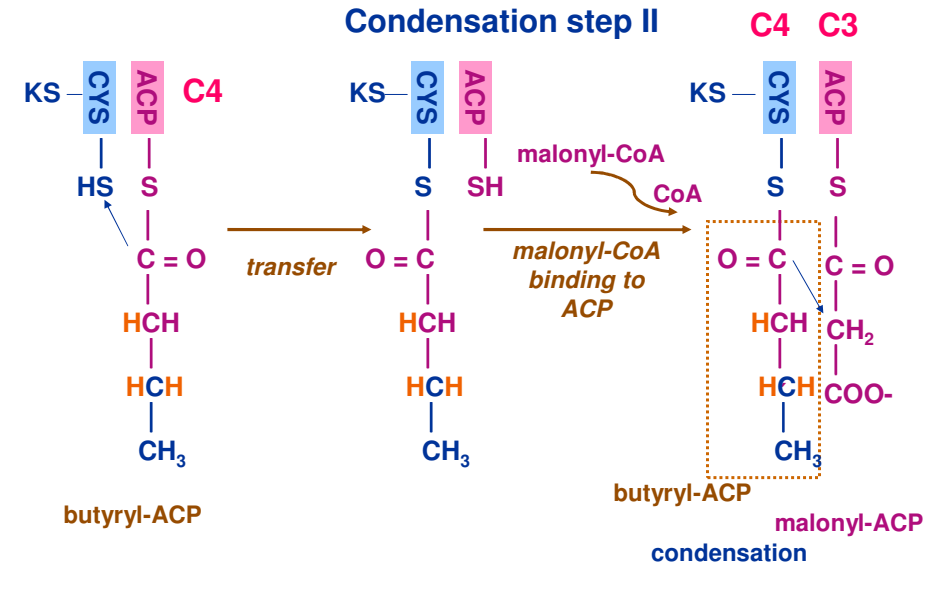
substrates acetyl-malonyl-enzyme 3-ketoacyl-enzyme

A four-carbon unit is formed from a two-carbon unit and a three-carbon unit, and CO_2 is released. The reaction is driven indirectly by ATP.

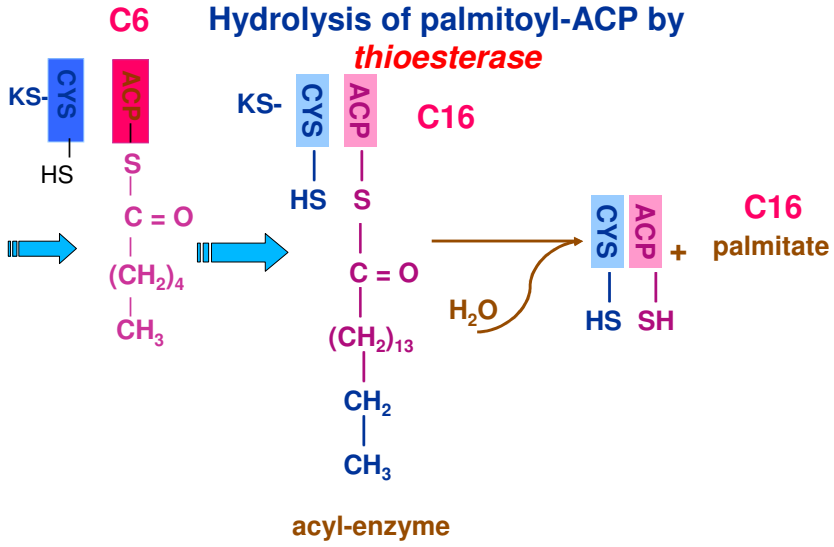
Pathway of fatty acid synthesis



Pathway of fatty acid synthesis



Pathway of fatty acid synthesis



Stoichiometry of C16 = palmitate biosynthesis

Synthesis of **malonyl-CoA**

$$7\ CH_3CO\text{-}S\text{-}CoA + 7\ ATP + 7\ CO_2 \longrightarrow 7\ HOOC\text{-}CH_2CO\text{-}S\text{-}CoA + 7\ ADP + 7\ Pi$$

Synthesis of **palmitate** (condensations and reductions)

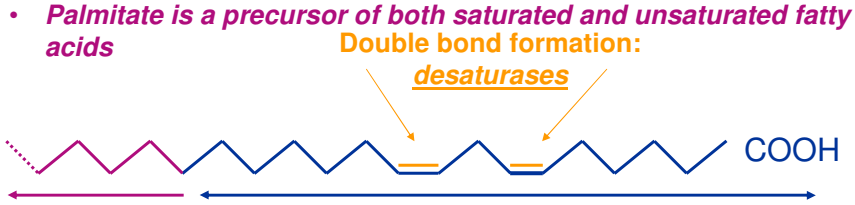
$$CH_3CO\text{-}S\text{-}CoA + 7\ HOOC\text{-}CH_2CO\text{-}S\text{-}CoA + 14\ NADPH + 14\ H^+ \longrightarrow CH_3\text{-}(CH_2)_{14}\text{-}COOH + 7\ CO_2 + 6\ H_2O + 8\ CoASH + 14\ NADP^+$$

Overall stoichiometry for the synthesis of palmitate from acetyl CoA

$$8\ CH_3CO\text{-}S\text{-}CoA + 7\ ATP + 14\ NADPH + 14\ H^+ \longrightarrow CH_3\text{-}(CH_2)_{14}\text{-}COOH + 7\ ADP + 7\ Pi + 6\ H_2O + 8\ CoASH + 14\ NADP^+$$

Further transformations of the FAs

- *A) Elongation – prolongation of the FA chain*
- *B) Desaturation – formation of polyunsaturated Fatty Acids*
- *C) Combination of A and B*
- *Palmitate is a precursor of both saturated and unsaturated fatty acids*



FA >C16
Chain prolongation:
Elongases

Palmitate:
FA synthase

- *D) Hydroxylation*

Elongation of Fatty Acids

***Endoplasmic reticulum* of the mammals:**

- **elongation of both saturated and unsaturated FAs**
- **fatty acyl-CoA (preferably C16:0-CoA) is elongated for two-carbon units in the addition reaction with *malonyl-CoA***
- ***synthesis of longer FA acid chains (up to C24) in the brain***

acyl-CoA $R\text{-}\overset{O}{\overset{\|}{C}}\text{-S-CoA} + {}^{-}O\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-S-CoA}$ *malonyl-CoA*

it takes place at the carboxyl end

$R\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-S-CoA} + CoA + CO_2$

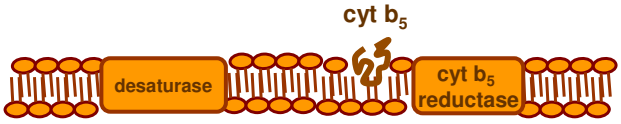
- **reduction with NADPH, etc.**

***Mitochondria* of the mammals:**

- **fatty acyl-CoA is elongated by the addition with *acetyl CoA***
- **both NADH and NADPH serve as electron donors**
- **essentially the reversal of the β-oxidation pathway**
- **primarily to elongate FAs shorter than C16**

Formation of polyunsaturated fatty acids

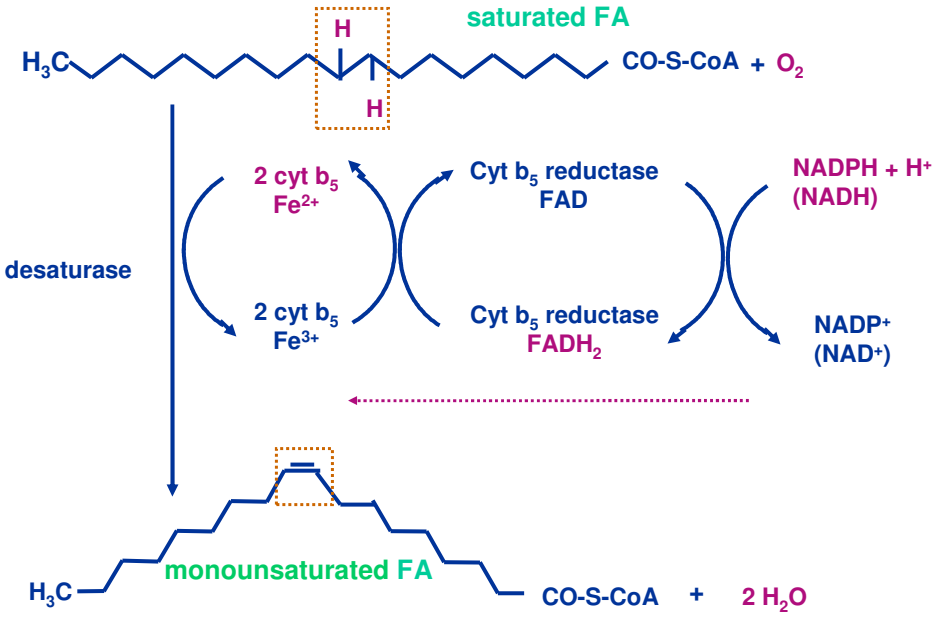
- **Components of the desaturation system**
  - **complexes of membrane-bound proteins in the endoplasmic reticulum of liver cells**
    - ***cytochrome b_5 reductase (flavoprotein)***
    - ***cytochrome b_5***
    - ***desaturase of fatty acyl-CoA*** **(monooxygenase system - it oxidizes two substrates simultaneously - NAD(P)H and FA)**



membrane of the endoplasmic reticulum

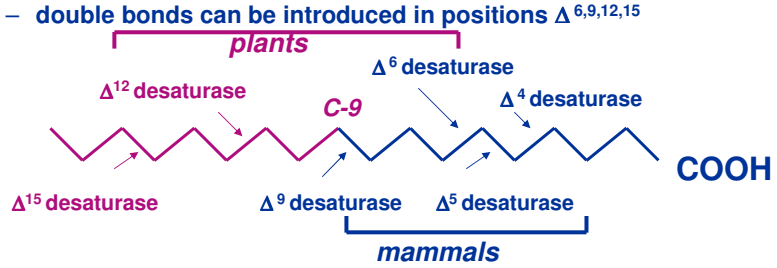
- **Requirements for double bond formation in FAs: inflow of electrons and of molecular oxygen**

stearoyl-CoA + NAD(P)H + H^+ + O_2 ⟶ oleoyl-CoA + $NAD(P)^+$ + 2 H_2O

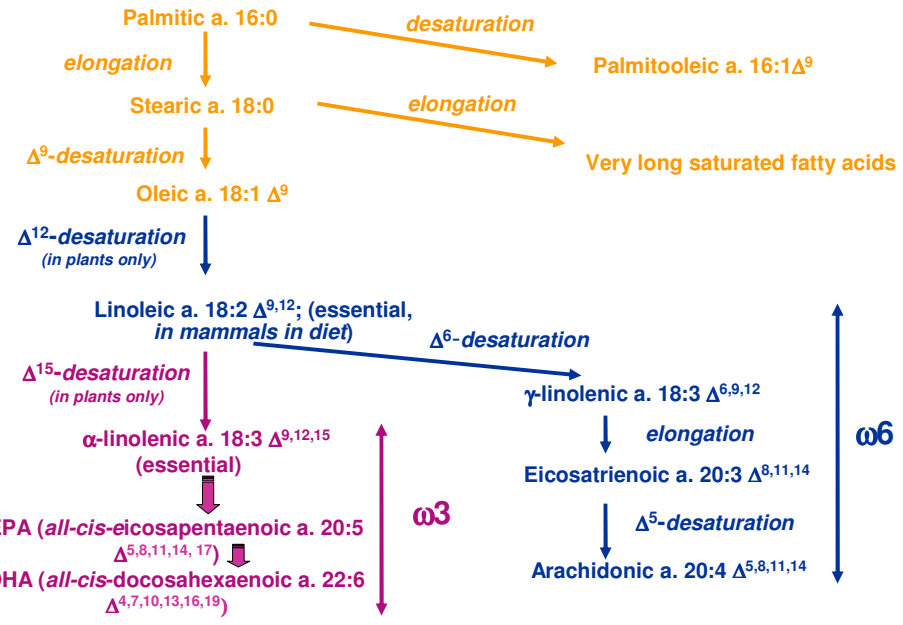


Introduction of double bonds - desaturation of fatty acids

- *MAMMALS*
  - four DESATURASES
  - double bonds can be introduced in positions $\Delta^{4,5,6,9}$
  - Absence of enzymes to introduce double bonds at carbon atoms beyond C-9 in the fatty acid chain
- MAMMALS cannot synthesize C18:2(9,12) linoleic (ω6) and C18:3 (9,12,15) linolenic (ω3) acids: essential FAs
- PLANTS and COLD WATER FISH: double bonds can be added beyond C-9, after Δ^9
  - double bonds can be introduced in positions $\Delta^{6,9,12,15}$



Transformations of the palmitate - Summary



Biosynthesis of triacylglycerols

glycerol-3-phosphate

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phosphatidate

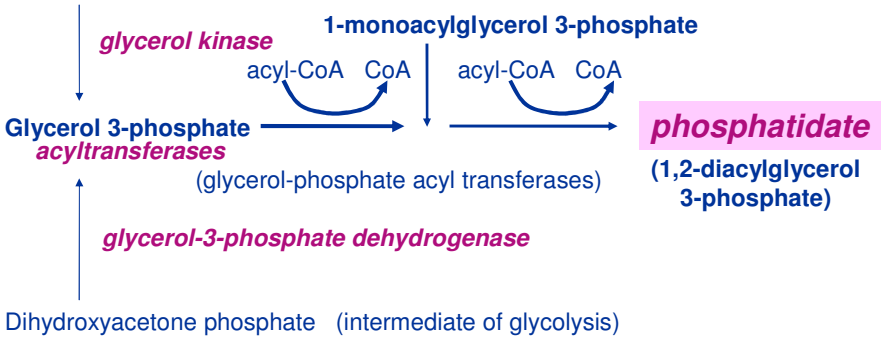
↓

triacylglycerol

Biosynthesis of phosphatidate

Common intermediate is ***glycerol 3-phosphate***

Glycerol (by-product of triacylglycerol mobilization, hydrolysis)



Dihydroxyacetone phosphate (intermediate of glycolysis)

Biosynthesis of triacylglycerols