BIOCHEMISTRY

Lipid Metabolism

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Chapter Description

- Overview

  Lipid metabolism covers the synthesis and the various oxidation pathways of fatty acids.

- Expected Outcomes

  You should be able to understand some degree of lipid metabolism with fatty acid as a representative of this complex macromolecule.

- Other related Information

  Some relevant questions been provided for improving your understanding of the topic. You are expected to search for external sources for information to adequately answer the questions. All pictures and figures within this chapter categorized as creative commons for the purpose of education only.

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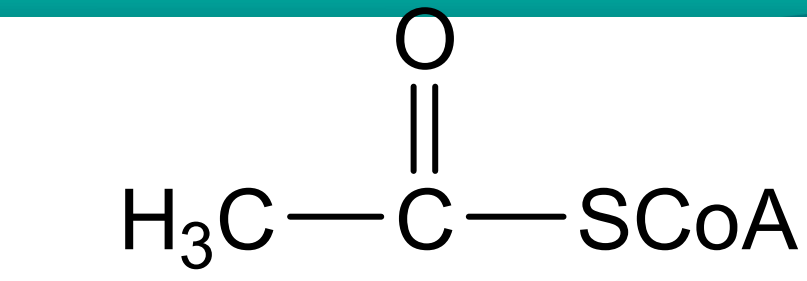
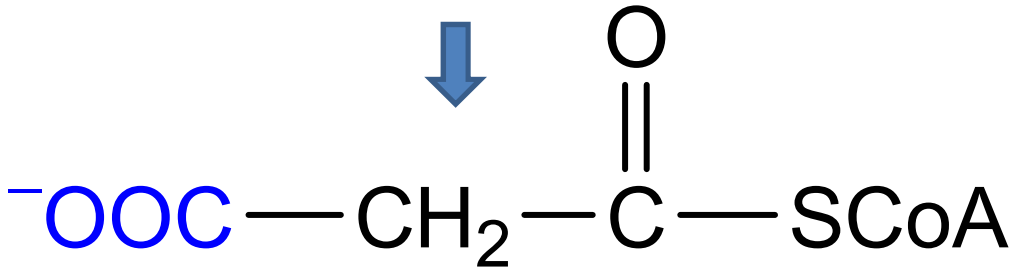
http://ocw.ump.edu.my/course/view.php?id=485

Anabolism of Fatty Acid

Initial step catalyzed by Acetyl-CoA Carboxylase (ACC):



acetyl-CoA



malonyl-CoA

- is the **committed step** of the fatty acid synthesis pathway.

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

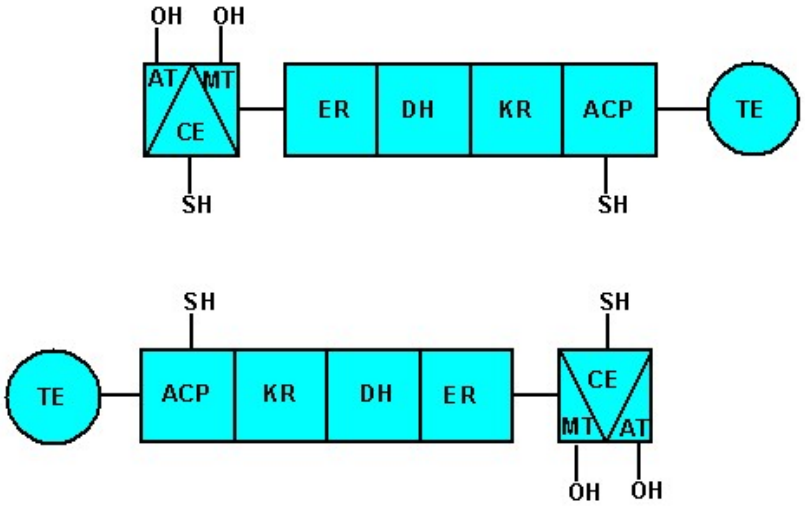
- in **bacteria** catalyzed by 6 **different** enzymes plus a separate acyl carrier protein (ACP)
- in **mammals** catalyzed by **individual** domains of a very large polypeptide that includes an ACP domain.

  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 Synthase (FAS)



AT: Acetyl transacylase

MT: Malonyl transacylase

CE : Condensing enzyme

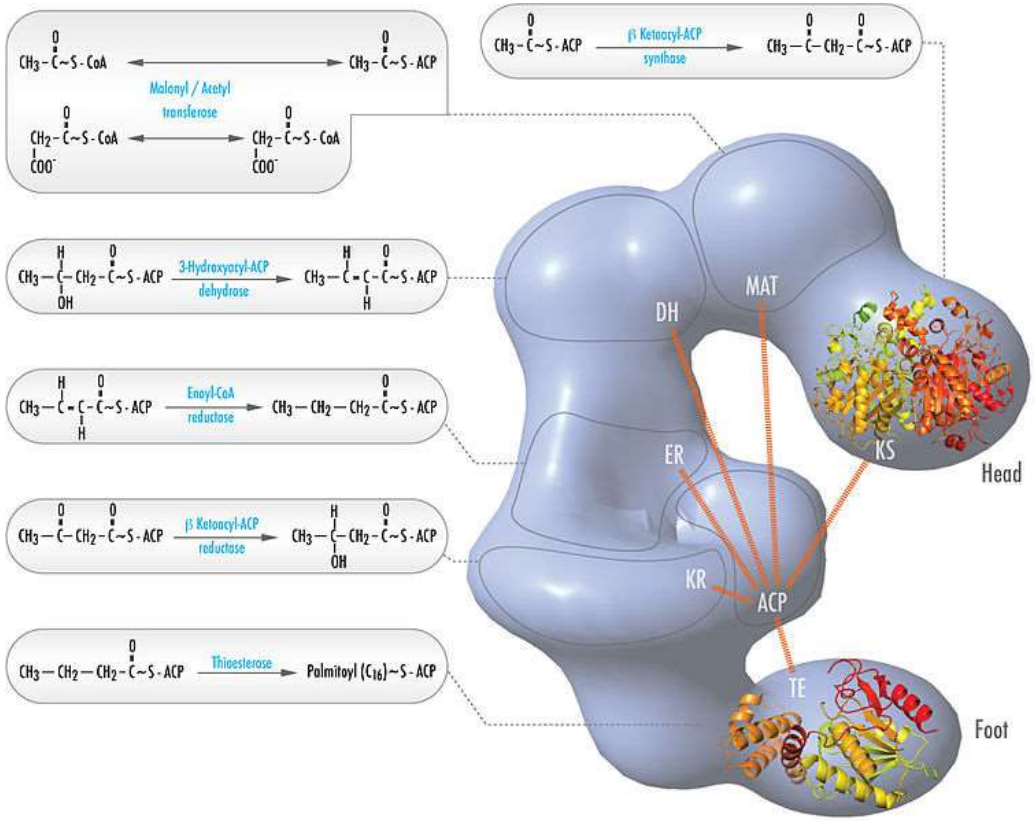
ACP: Acyl Carrier Protein

KR : 3-Ketoacyl reductase

DH : 3-Hydroxyacyl dehydratase

ER : Enoyl reductase

TE : Thioesterase



- **The active enzyme is a dimer of identical subunits**
- **FAS is a polypeptide chain with multiple domains, each with distinct enzyme activities required for fatty acid biosynthesis.**

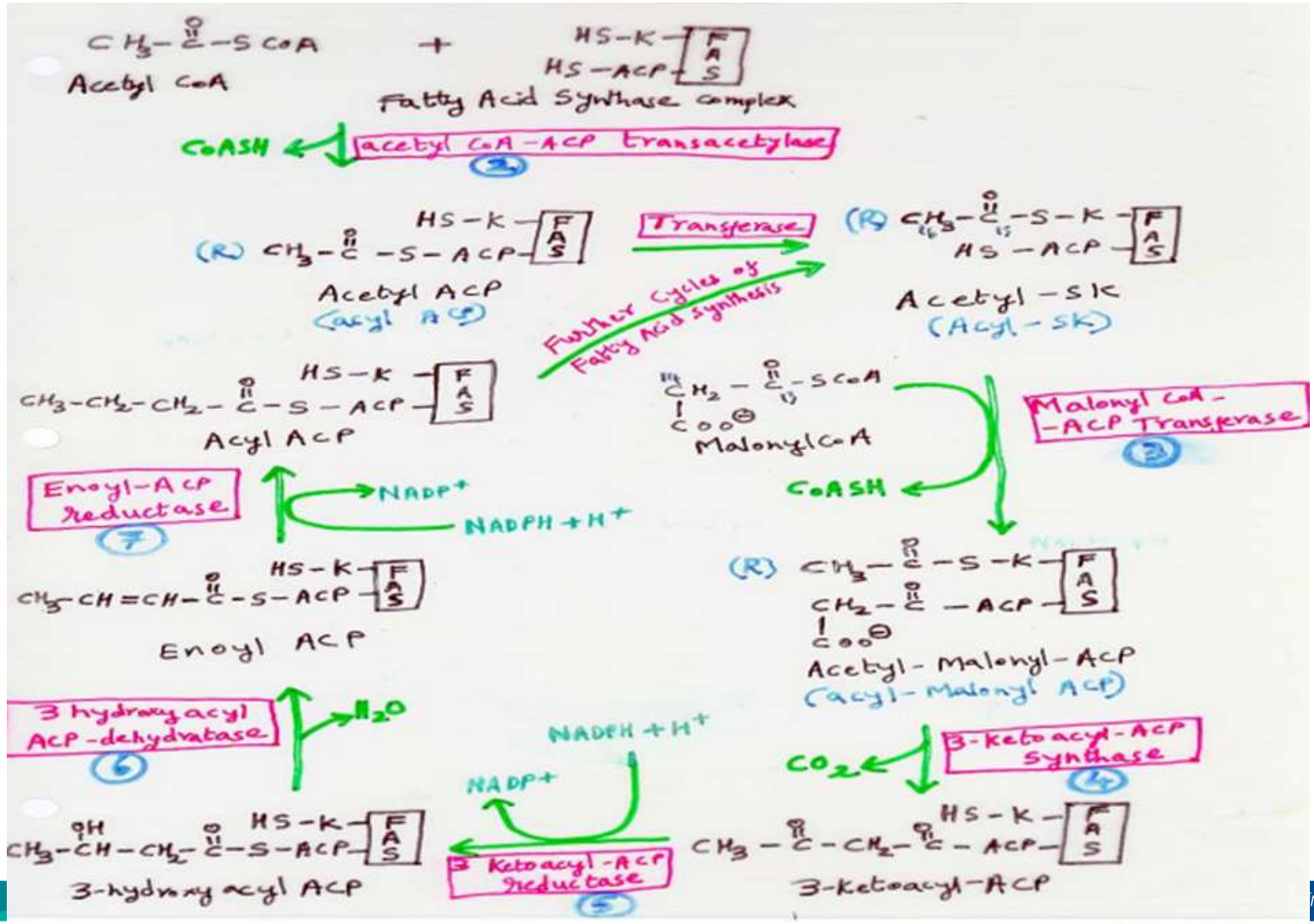
Fatty Acid Synthesis

- **ACP:** Fatty acid biosynthesis, the activator is a protein called the acyl carrier protein (ACP). It is part of the FAS complex. The acyl groups get anchored to the CoA group of ACP by a thioester linkage (ACP-SH).

- **Condensing enzyme** (CE)/β-ketoacyl synthase (K-SH). Also part of FAS, K has a cysteine SH that participates in thioester linkage with the carboxylate group of the fatty acid

- During FA biosynthesis, the **growing FA chain** alternates between **K-SH and ACP-SH**

An overview sketch on fatty acid synthesis:



Fatty Acid Synthesis Stepwise:

- The acetyl group gets transferred to ACP by acetyl CoA-ACP transacylase.
- The acetyl (acyl) group next gets transferred to the K arm of FAS complex.
- Next, the malonyl group gets transferred to ACP by malonyl CoA ACP transacylase. This results in both arms of FAS occupied forming acyl-malonyl-ACP.
- The COO group of malonyl ACP is removed as CO2, the acetyl group gets transferred to the alpha carbon of malonyl ACP. This results in 3-keto acyl ACP

Fatty Acid Synthesis Stepwise (cont):

- The 3-keto group is converted to a -CH2- by a series of reactions **reverse to FA β-oxidation**:

  1. **reduction** to hydroxyl group. Enz: 3-keto acyl ACP Reductase
  2. **dehydration** to form a 2,3 double bond and Enz: 3-hydroxy acyl ACP dehydratase
  3. a second **reduction** to remove the double bond. Enz: Enoyl ACP reductase

- Both reduction reactions require the reduced cofactor NADPH.

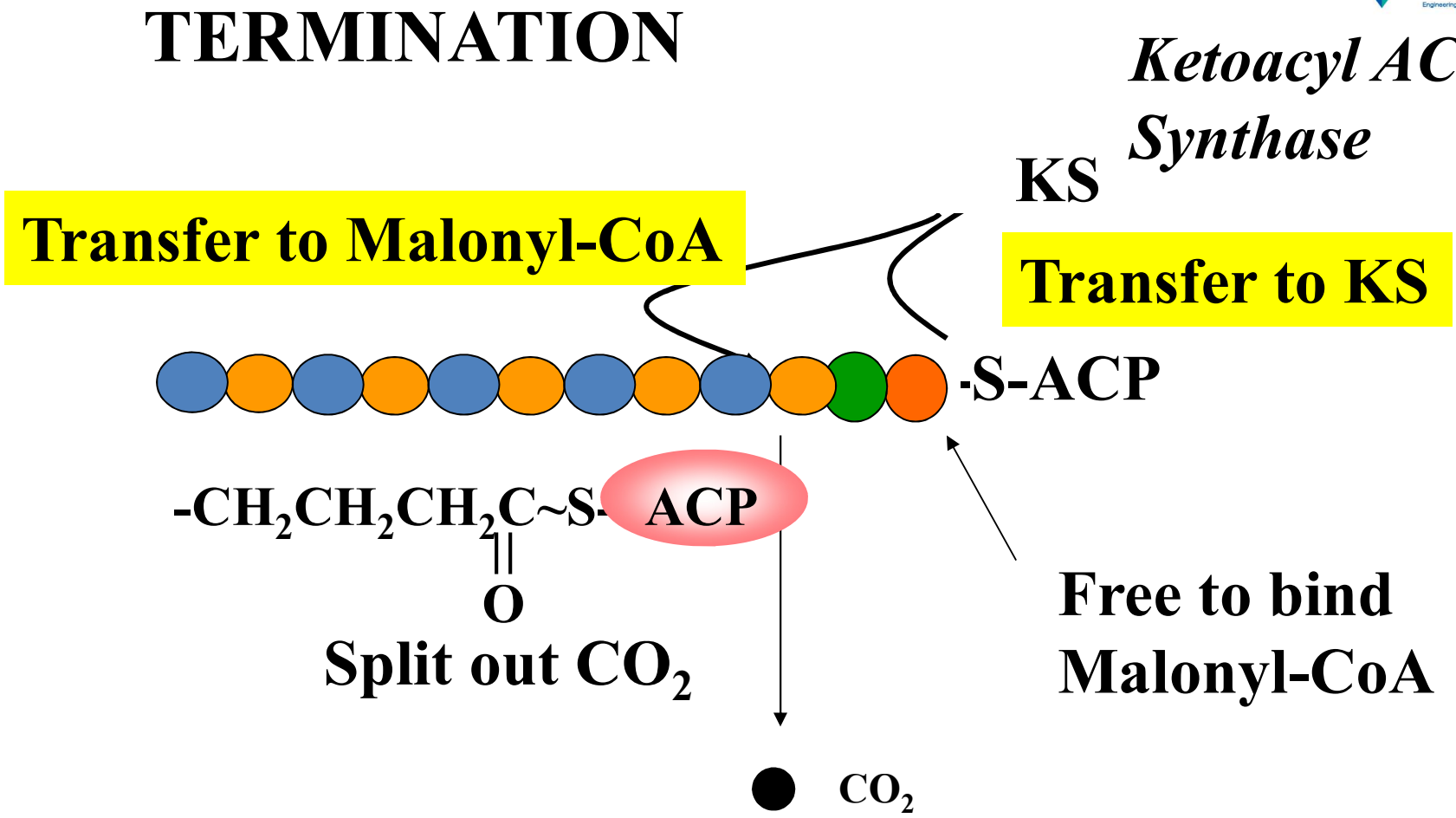
Repeated cycles for chain extension:

- The result of the first cycle of fatty acid biosynthesis is a four carbon chain associated to the ACP arm
- This chain gets transferred to the K arm.
- A new malonyl CoA is introduced on the ACP arm
- The reactions proceed as before. For each cycle the acyl group transferred to the α-carbon of malonyl CoA is 2-carbons longer the previous cycle

Product release:

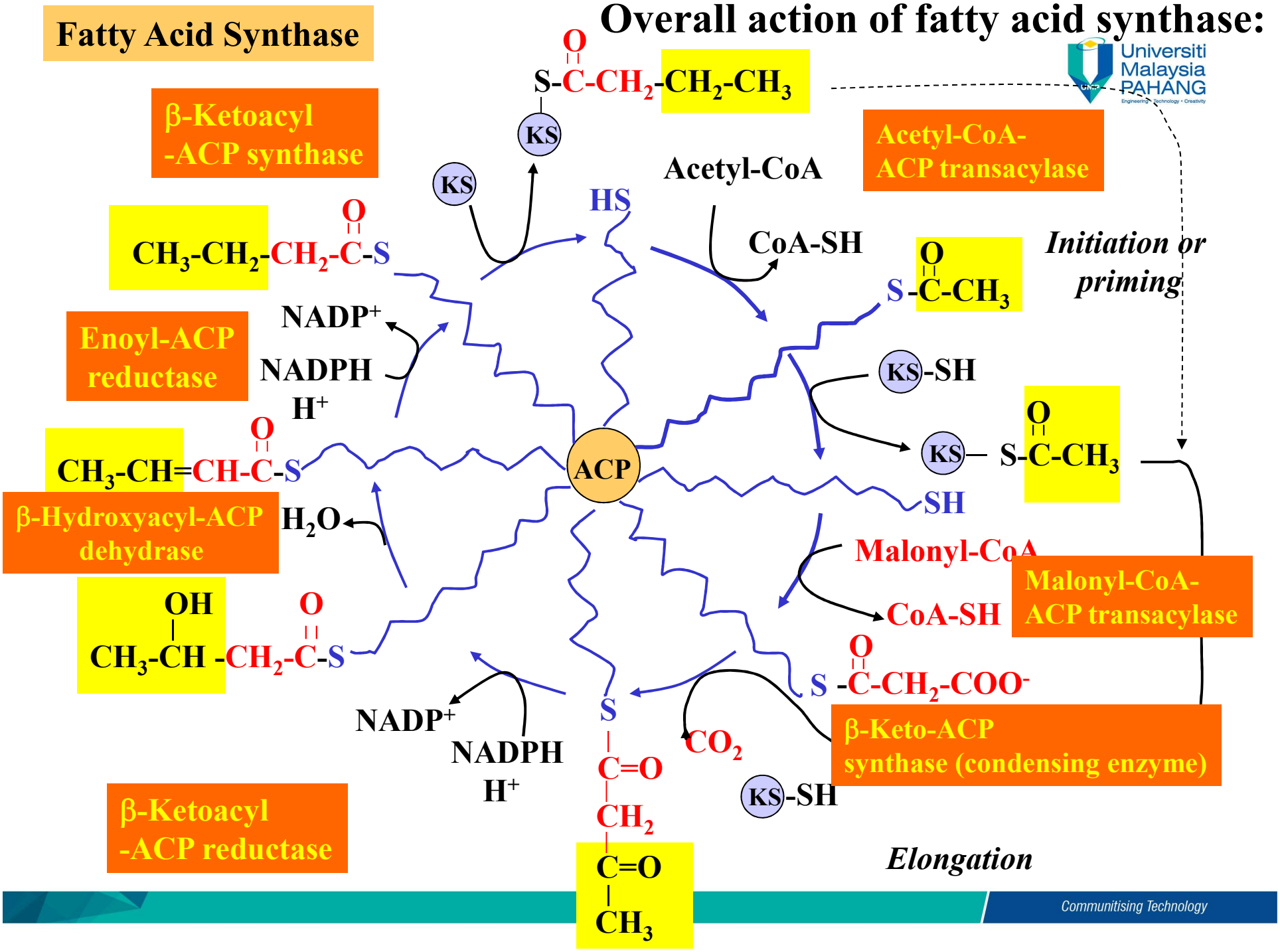
- At the end of 7 cycles a 16 carbon chain is attached to the ACP arm (palmitoyl ACP)
- The C16 unit is hydrolyzed from ACP yielding free palmitate
- When the fatty acid is 16 carbon atoms long, a **Thioesterase** domain catalyzes hydrolysis of the thioester linking the fatty acid to phosphopantetheine.
- The **16-C** saturated fatty acid **palmitate** is the final product of the Fatty Acid Synthase complex.

TERMINATION



When C16 stage is reached, instead of transferring to KS, the transfer is to H_2O and the fatty acid is released

Fatty Acid Synthase



Palmitate, a 16-C saturated fatty acid, is the final product of the Fatty Acid Synthase reactions.

Summary (ignoring H^+ & water):

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

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

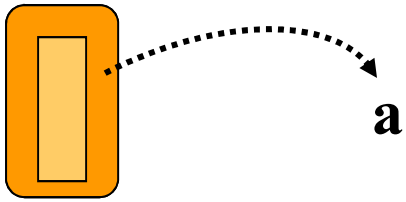
Accounting for ATP-dependent synthesis of malonate:

8 acetyl-CoA + 14 NADPH + 7 ATP →

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

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 biosynthesis takes place in the cytosol. Acetyl-CoA is mainly in the Mitochondria



acetyl-CoA

How is acetyl-CoA made available to the cytosolic fatty acyl synthase?

SOLUTION

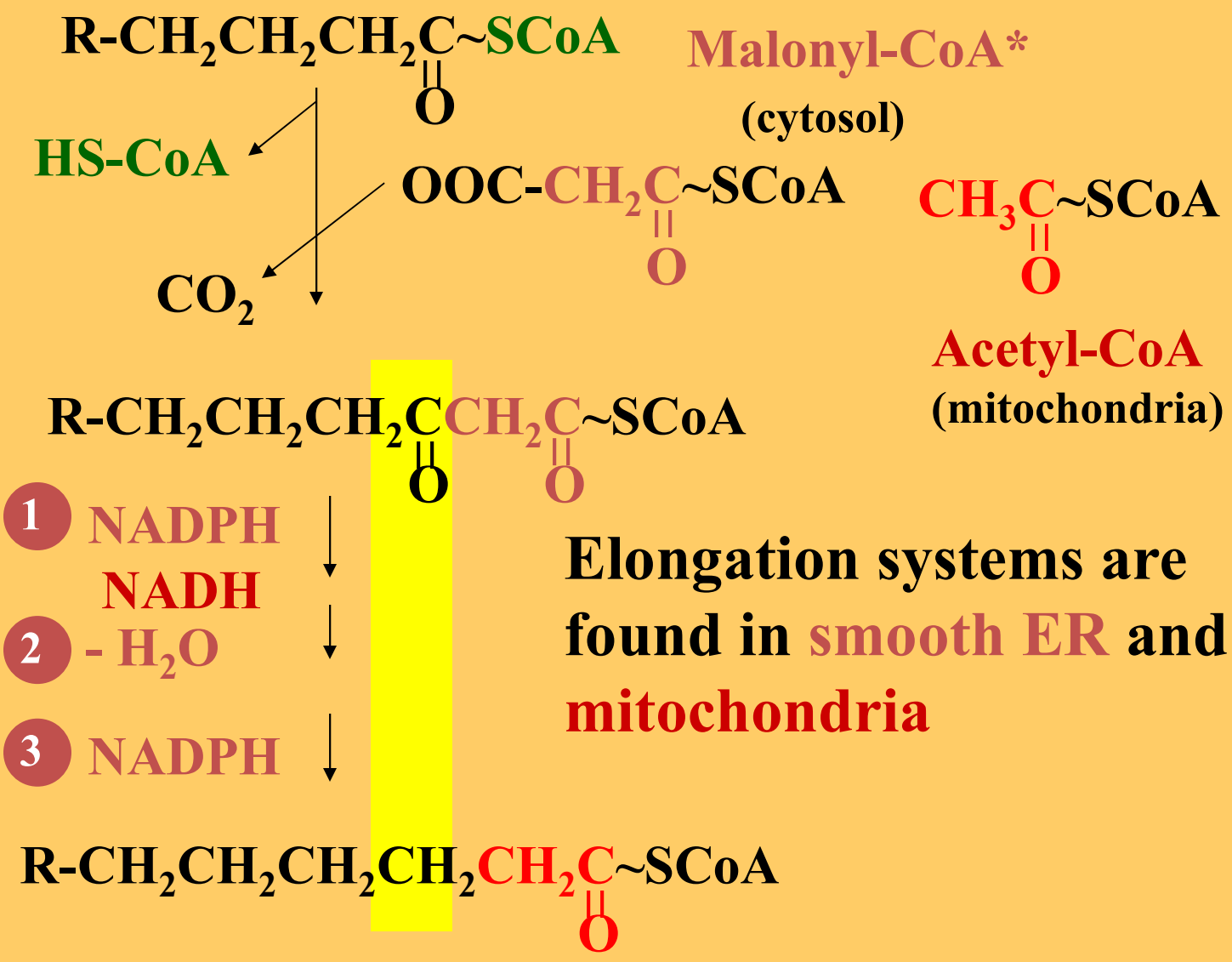
Acetyl-CoA is delivered to cytosol from the mitochondria as CITRATE

Elongation beyond the 16-C length of the palmitate product of Fatty Acid Synthase occurs in mitochondria and endoplasmic reticulum (ER).

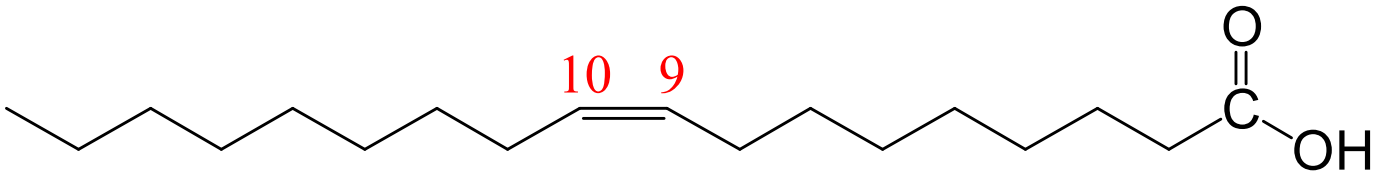
- Fatty acid elongation within **mitochondria** involves the **β-oxidation pathway** running in reverse, but NADPH serves as electron donor for the final reduction step.
- Polyunsaturated fatty acids esterified to CoA are substrates for the **ER elongation machinery**, which uses malonyl-CoA as donor of 2-carbon units.

  The reaction sequence is similar to Fatty Acid Synthase but individual steps are catalyzed by **separate proteins**.

Elongation of Chain (two systems)



Elongation systems are found in smooth ER and mitochondria

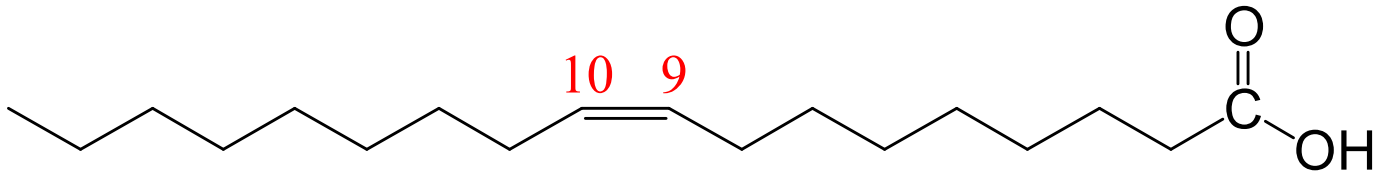


oleate 18:1 cis Δ^9

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.

Desaturation

Rules:

The fatty acid desaturation system is in the membranes of the smooth endoplasmic reticulum

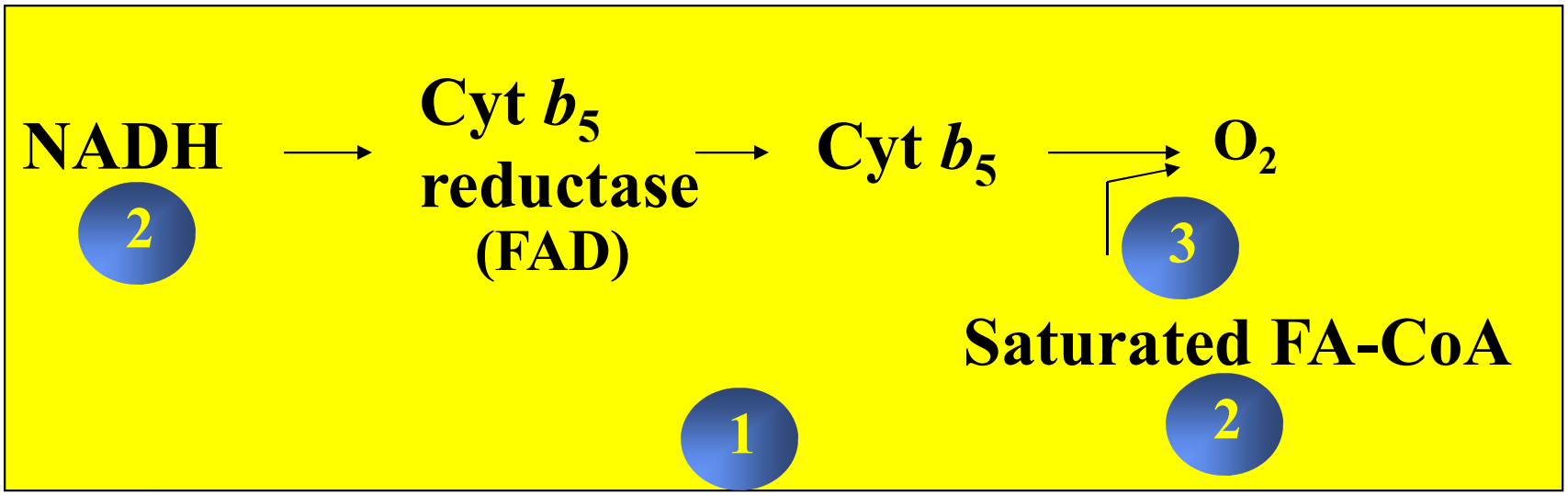
There are 4 fatty acyl desaturase enzymes in mammals designated Δ^9 , Δ^6, Δ^5, and Δ^4 *fatty acyl-CoA desaturase*

Mammals cannot incorporate a double bond beyond Δ^9; plants can.

Mammals can synthesize long chain unsaturated fatty acids using desaturation and elongation

Rule:

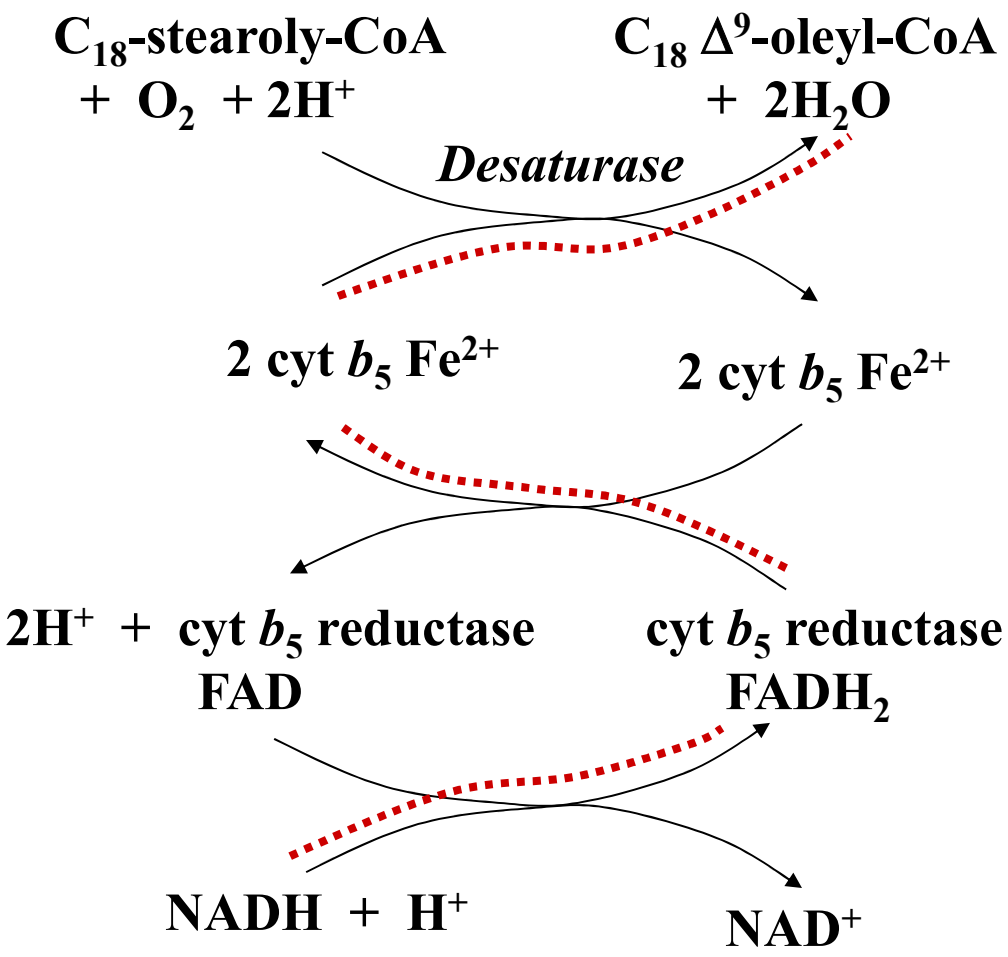
The Desaturase System requires O_2 and resembles an electron transport system

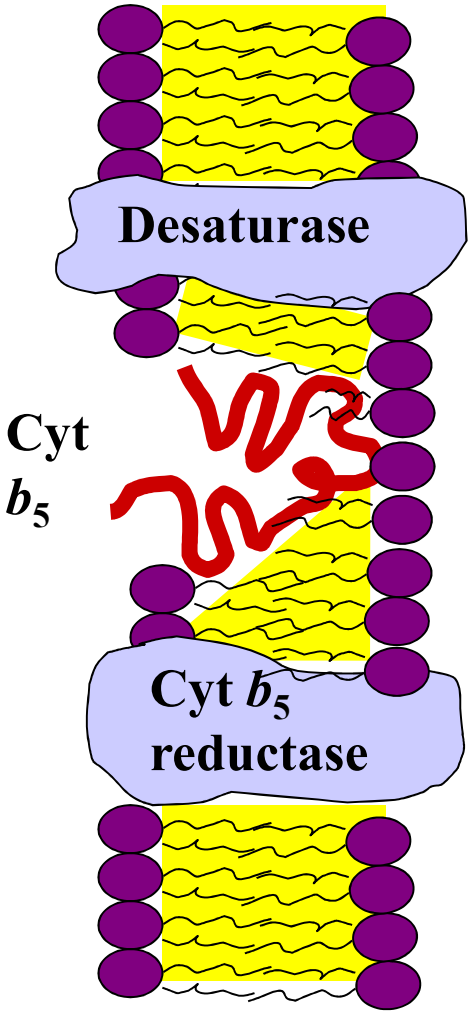


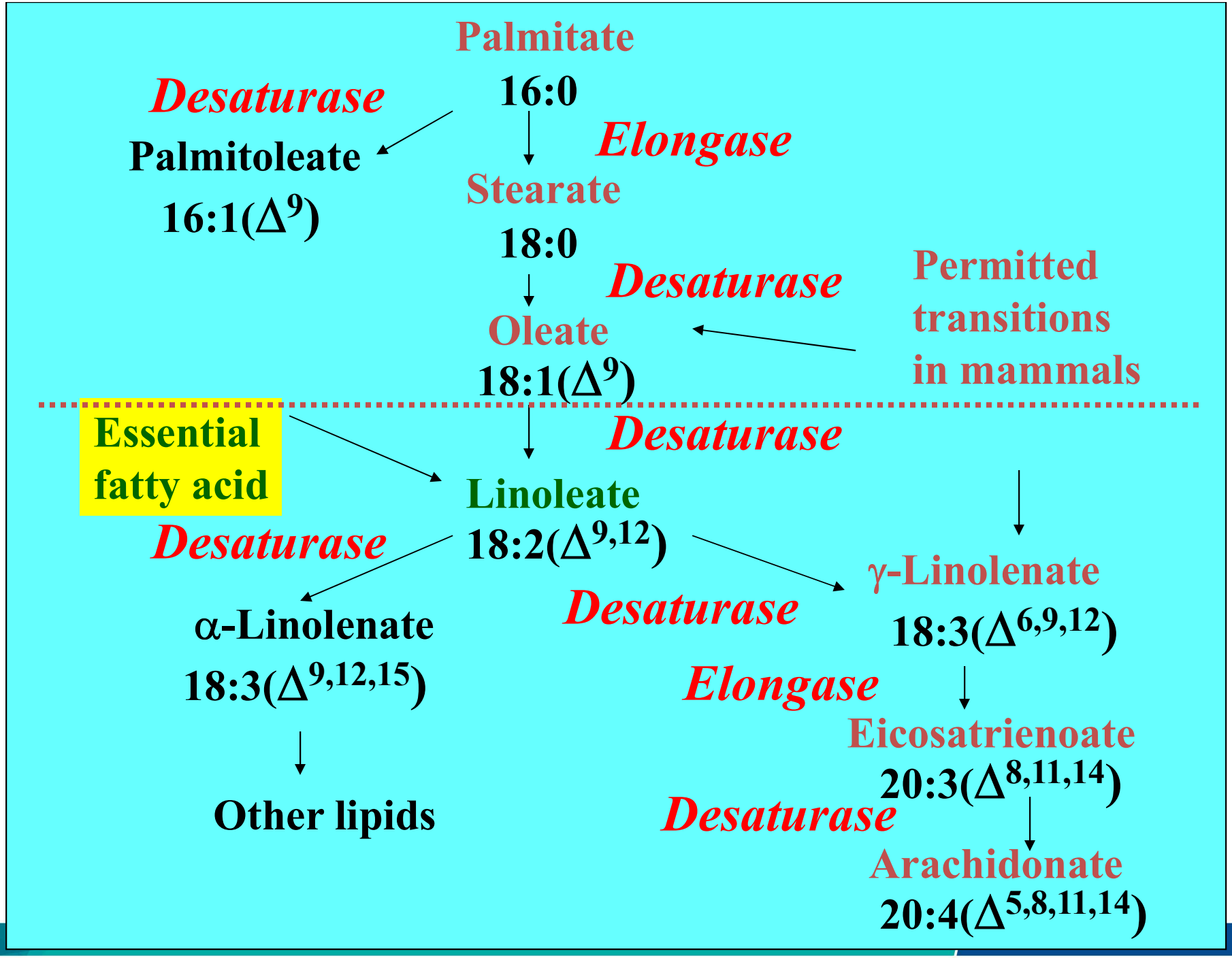
NOTE:

1. **System is in ER membrane**
2. **Both NADH and the fatty acid contribute electrons**
3. **Fatty acyl desaturase is considered a mixed function oxidase**

Fatty acid desaturation system






Palmitate
16:0
Desaturase
Palmitoleate
16:1(Δ9)
Elongase
Stearate
18:0
Desaturase
Oleate
18:1(Δ9)
Permitted transitions in mammals
Essential fatty acid
Desaturase
Linoleate
18:2(Δ9,12)
Desaturase
γ-Linolenate
18:3(Δ6,9,12)
Desaturase
α-Linolenate
18:3(Δ9,12,15)
Elongase
Eicosatrienoate
20:3(Δ8,11,14)
Other lipids
Desaturase
Arachidonate
20:4(Δ5,8,11,14)

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Catabolism of Fatty acids

Transport of FA s in blood

- Free fatty acids—also called unesterified (UFA) or nonesterified (NEFA) fatty acids—are fatty acids that are in the **unesterified state.**

- In plasma, longer-chain FFA are combined with **albumin,** and in the cell they are attached to a **fatty acid-binding protein,** so that in fact they are never really "free."

- Shorter-chain fatty acids are more water-soluble and exist as the unionized acid or as a fatty acid anion.

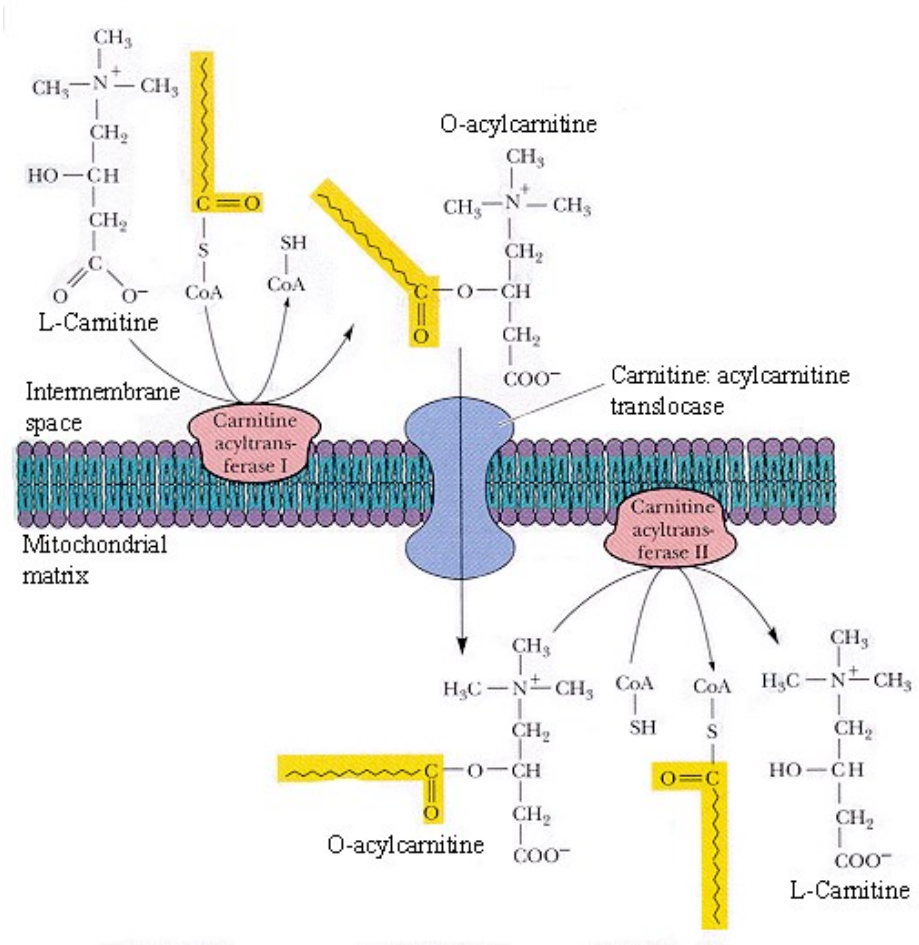
Activation of FA s

- Fatty acids must first be converted to an active intermediate before they can be catabolized. This is the only step in the complete degradation of a fatty acid that requires energy from ATP.

- In the presence of ATP and coenzyme A, the enzyme **acyl-CoA synthetase (thiokinase)** catalyzes the conversion of a fatty acid to an "**active fatty acid**" or **acyl-CoA**, which uses one high-energy phosphate with the formation of AMP and PP_i

- Acyl-CoA synthetases are found in the endoplasmic reticulum, peroxisomes, and inside and on the outer membrane of mitochondria

Carnityl acyl transferase

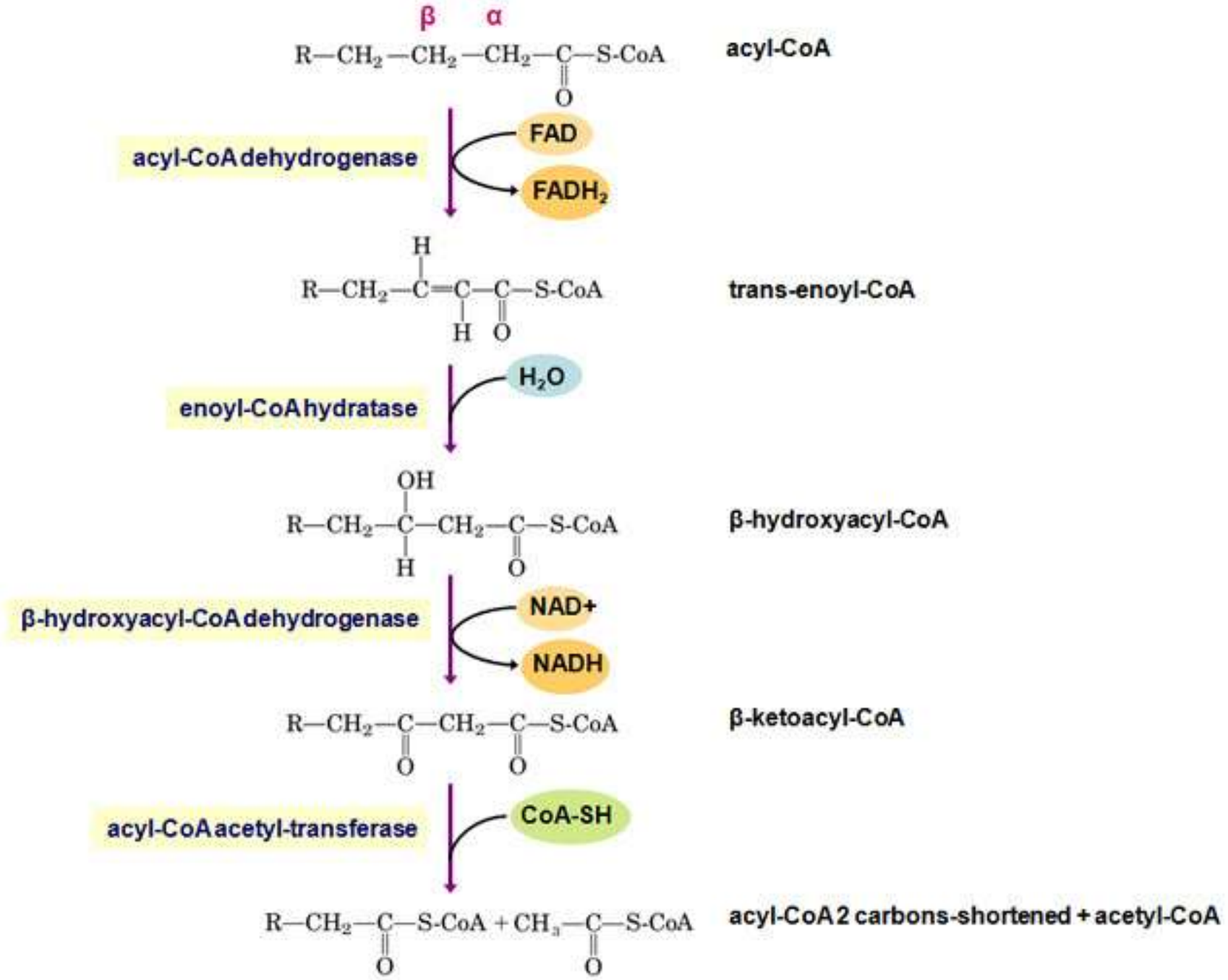


β - oxidation

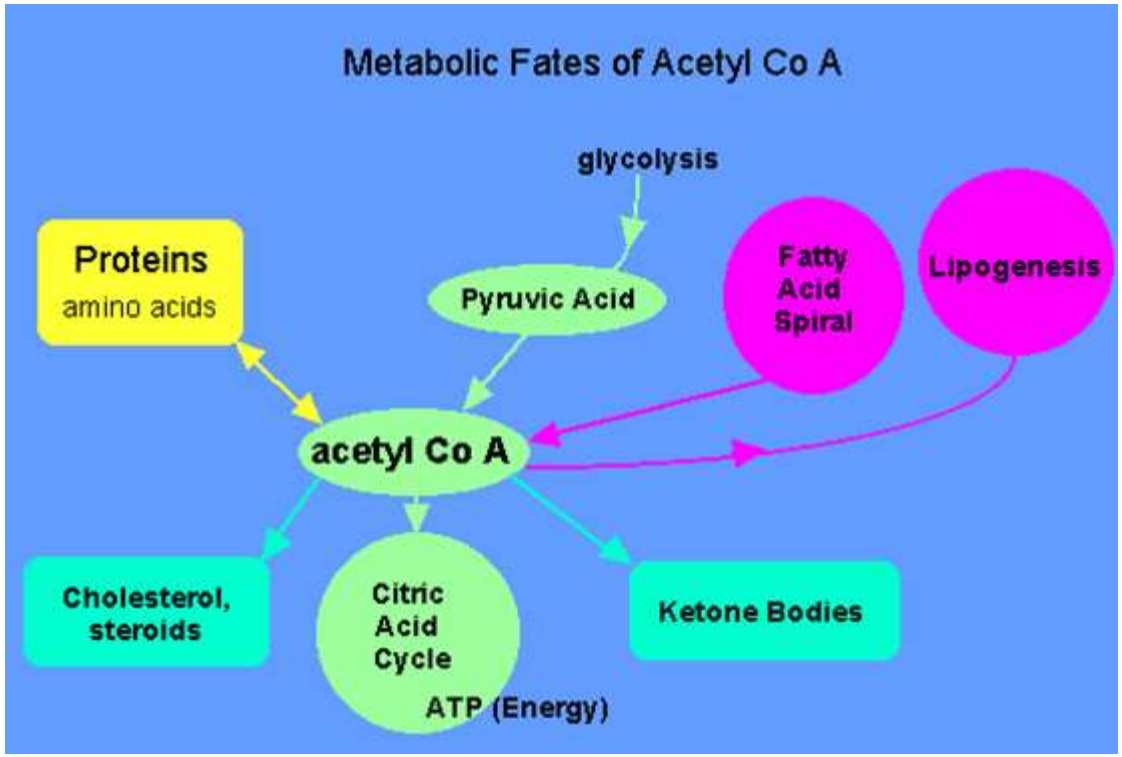
- In β-oxidation , **two carbons** at a time are cleaved from acyl-CoA molecules, starting at the carboxyl end.

- The chain is broken between the (**2**)- and (**3**)-carbon atoms—hence the name - **β** oxidation.

- The two-carbon units formed are acetyl-CoA; thus, palmitoyl-CoA forms eight acetyl-CoA molecules


β α
R—CH2—CH2—CH2—C—S-CoA
O
acyl-CoA
acyl-CoA dehydrogenase
FAD
FADH2
H
R—CH2—C=C—C—S-CoA
H O
trans-enoyl-CoA
enoyl-CoA hydratase
H2O
OH
R—CH2—C—CH2—C—S-CoA
H O
β-hydroxyacyl-CoA
β-hydroxyacyl-CoA dehydrogenase
NAD+
NADH
R—CH2—C—CH2—C—S-CoA
O O
β-ketoacyl-CoA
acyl-CoA acetyl-transferase
CoA-SH
R—CH2—C—S-CoA + CH3—C—S-CoA
O O
acyl-CoA 2 carbons-shortened + acetyl-CoA

- Since **acetyl-CoA** can be oxidized to CO_2 and water via the citric acid cycle (which is also found within the mitochondria), the complete oxidation of fatty acids is achieved.



Oxidation of a Fatty Acid with an **Odd Number** of Carbon Atoms

- Fatty acids with an odd number of carbon atoms are oxidized by the pathway of β- oxidation, producing acetyl-CoA, until a three-carbon (**propionyl-CoA**) residue remains. This compound is converted to **succinyl-CoA**, a constituent of the citric acid.

- Hence, **the propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic**

Energetics

- Transport in the respiratory chain of electrons from **$FADH_2$ and NADH** leads to the synthesis of five high-energy phosphates for each of the seven cycles needed for the breakdown of palmitate to acetyl-CoA (**7 x 5 = 35**).

Energetics cont…

- A total of **8 mol of acetyl-CoA** is formed, and each gives rise to **12 mol** of ATP on oxidation in the citric acid cycle, making **8 x 12 = 96 mol**.

- Two must be subtracted for the initial activation of the fatty acid, yielding a net gain of **129 mol of ATP per mole of palmitate**. This represents 68% of the free energy of combustion of palmitic acid.

Hexanoic acid ($C_6H_{12}O_2$)

Hexanoic acid →

Hexanoyl-CoA -1 ATP

Hexanoyl-CoA →

3 Acetyl-CoA	**36 ATP**
2 $FADH_2$	**4 ATP**
2 NADH + H^+	**6 ATP**
	45 ATP

Mwt = 116

ATP per Gram = 0.4

Glucose ($C_6H_{12}O_6$)

Glucose →

2 pyruvates	**2 ATP**
2 NADH + H^+	**6 ATP**

2 pyruvates →

2 Acetyl-CoA	**24 ATP**
2 NADH + H^+	**6 ATP**
	38 ATP

Mwt = 180

ATP per Gram = 0.22

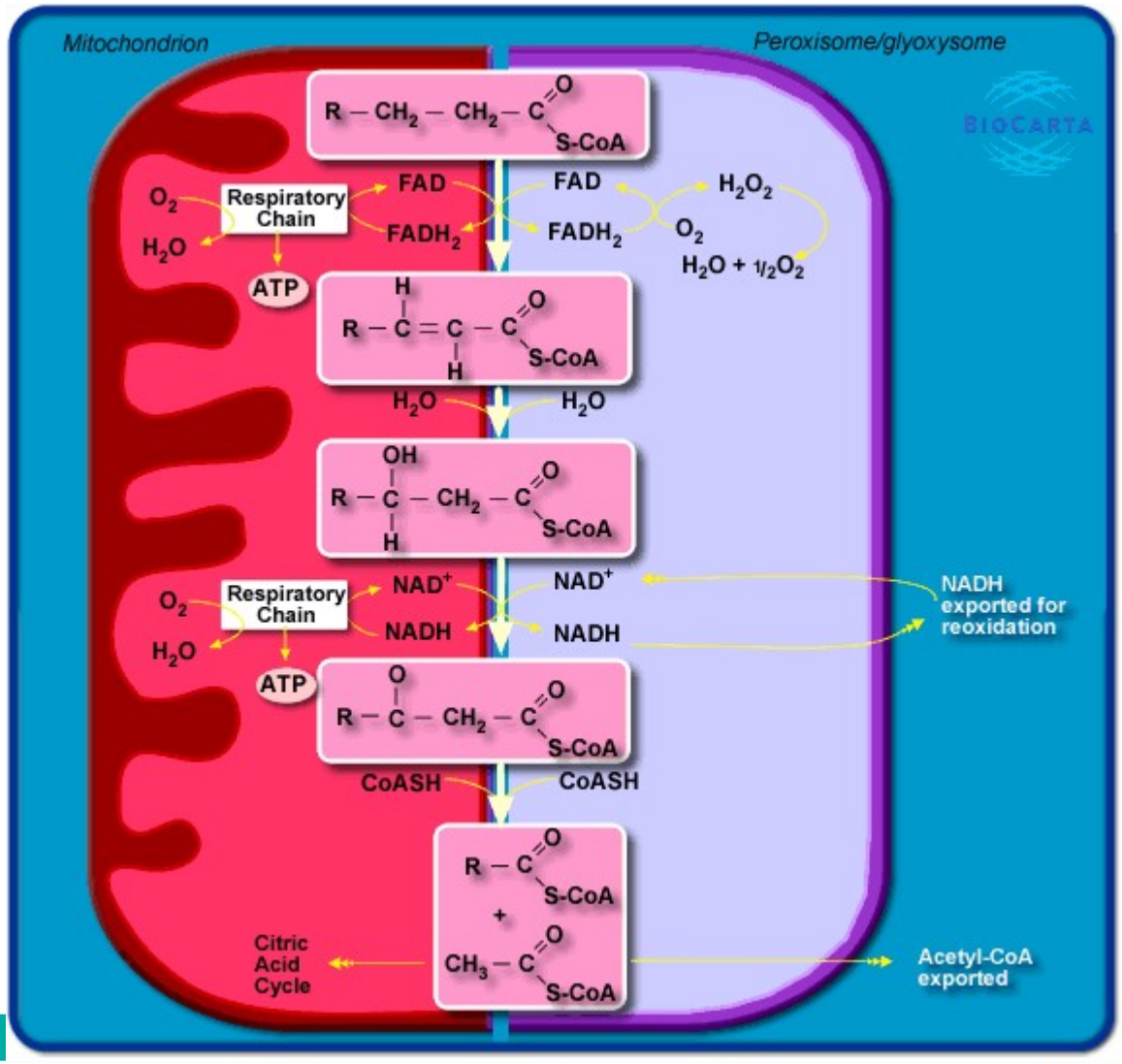
Oxidation of Long chain fatty acids

- Modified form of β-oxidation is found in **peroxisomes** and leads to the formation of acetyl-CoA and H_2O_2 (from the flavoprotein-linked dehydrogenase step), which is broken down by catalase .

- Thus, this dehydrogenation in peroxisomes is not linked directly to phosphorylation and the generation of ATP. The system facilitates the oxidation of very long chain fatty acids (eg, **C_{20}, C_{22}**).

- These enzymes are induced by **high-fat diets** and in some species by **hypolipidemic drugs** such as clofibrate.

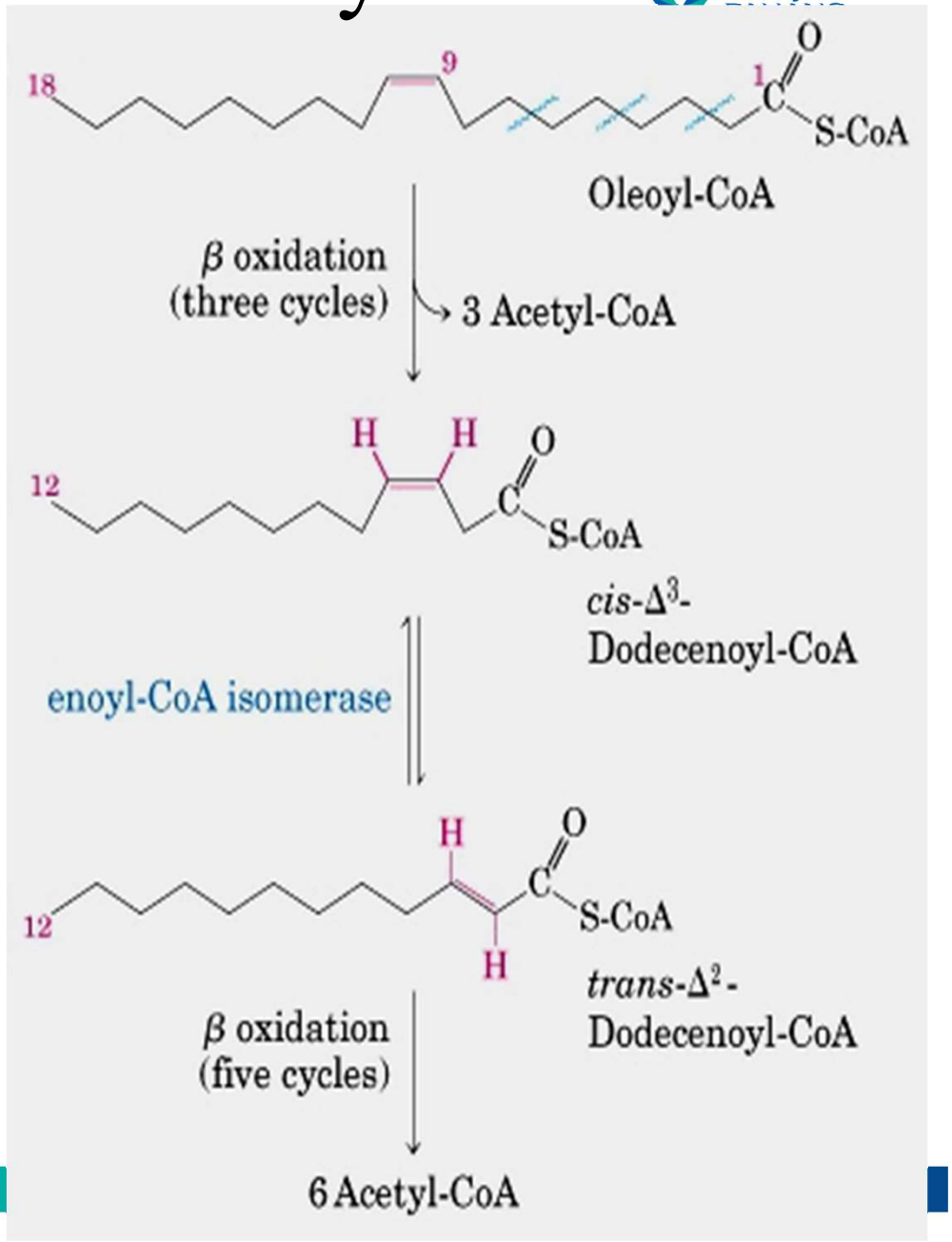
Oxidation of Long chain fatty acids



Oxidation of Unsaturated Fatty Acids

All the steps are same except, and additional enzyme called enoyl-CoA isomerase is required to convert the cis-double bond to trans double bond that can be recognized by enoyl-CoA hydratase.

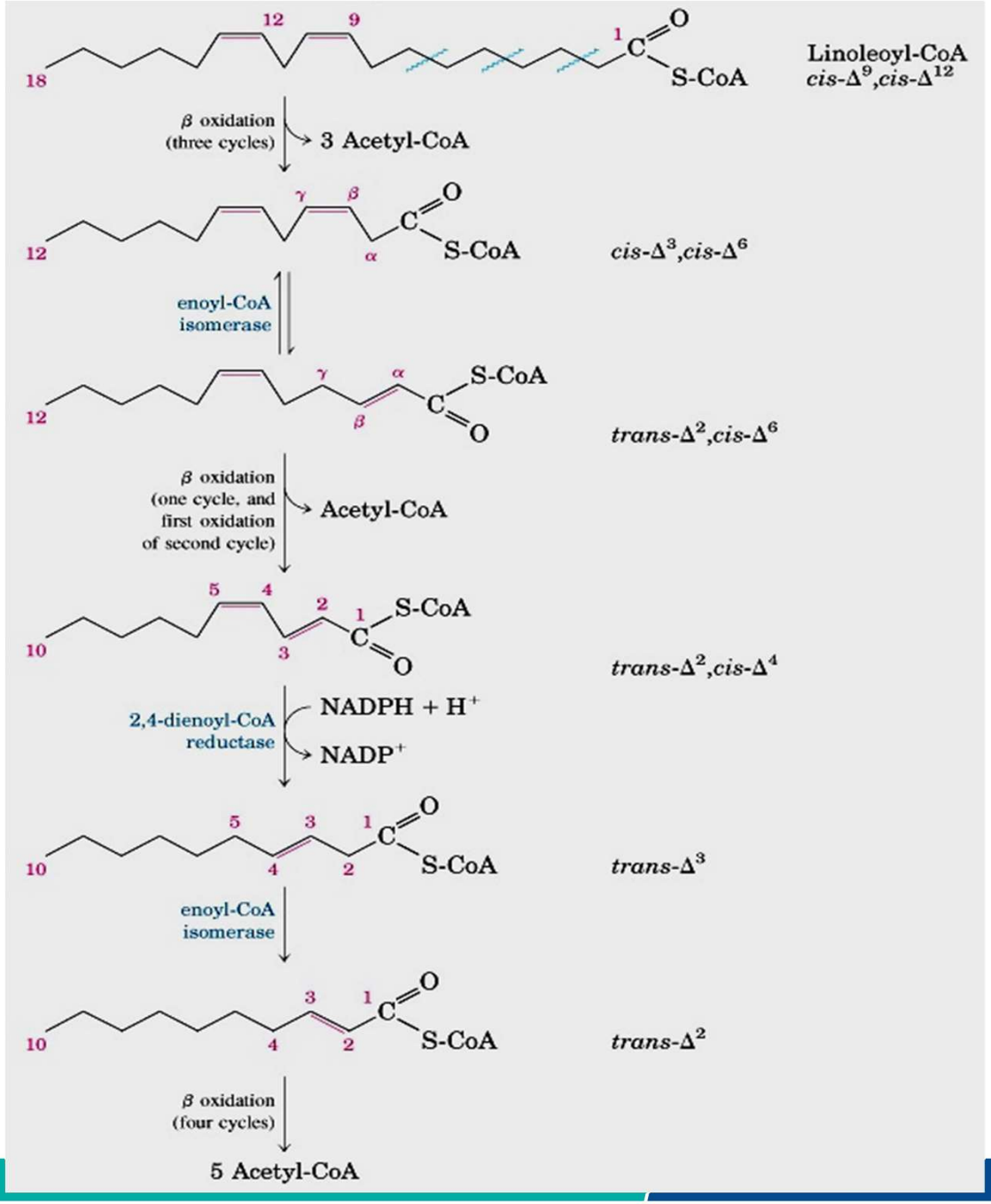
Now the rest of the chain can be oxidized as described before.



When more than one bonds are unsaturated, then one more additional enzyme is used to saturate the second double bond using NADPH.

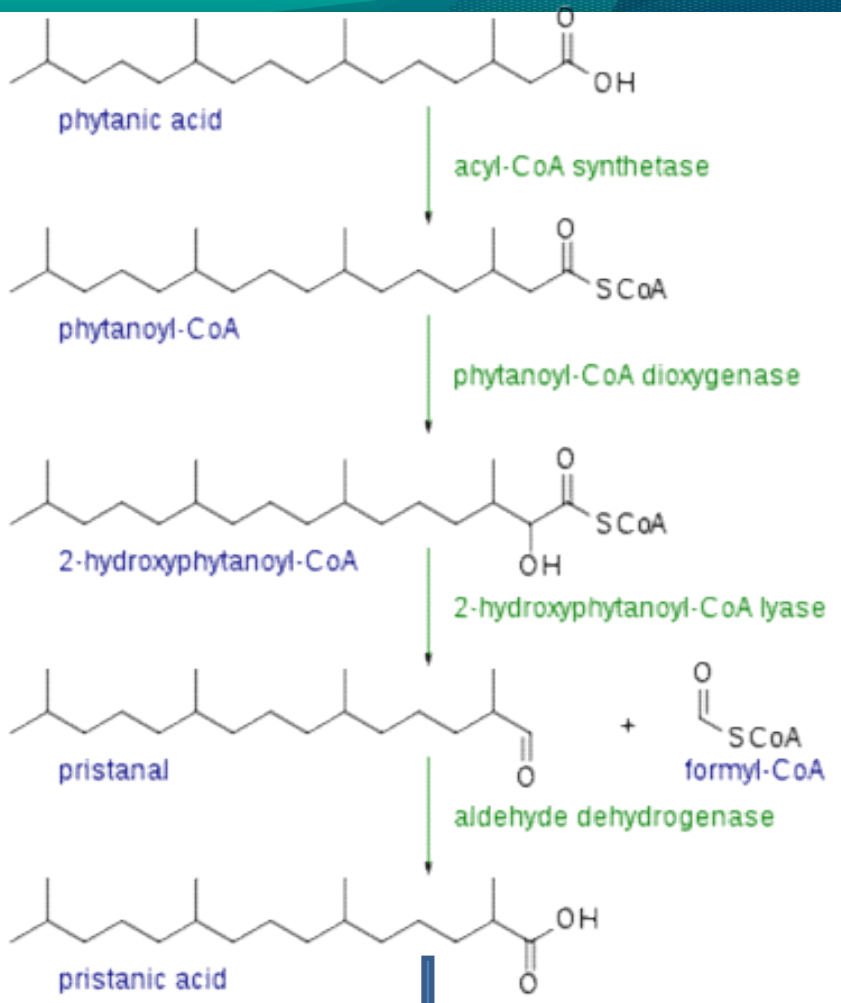
This enzyme is called 2,4 dienoyl reductase.

This is followed by isomerization reaction and β-oxidation.

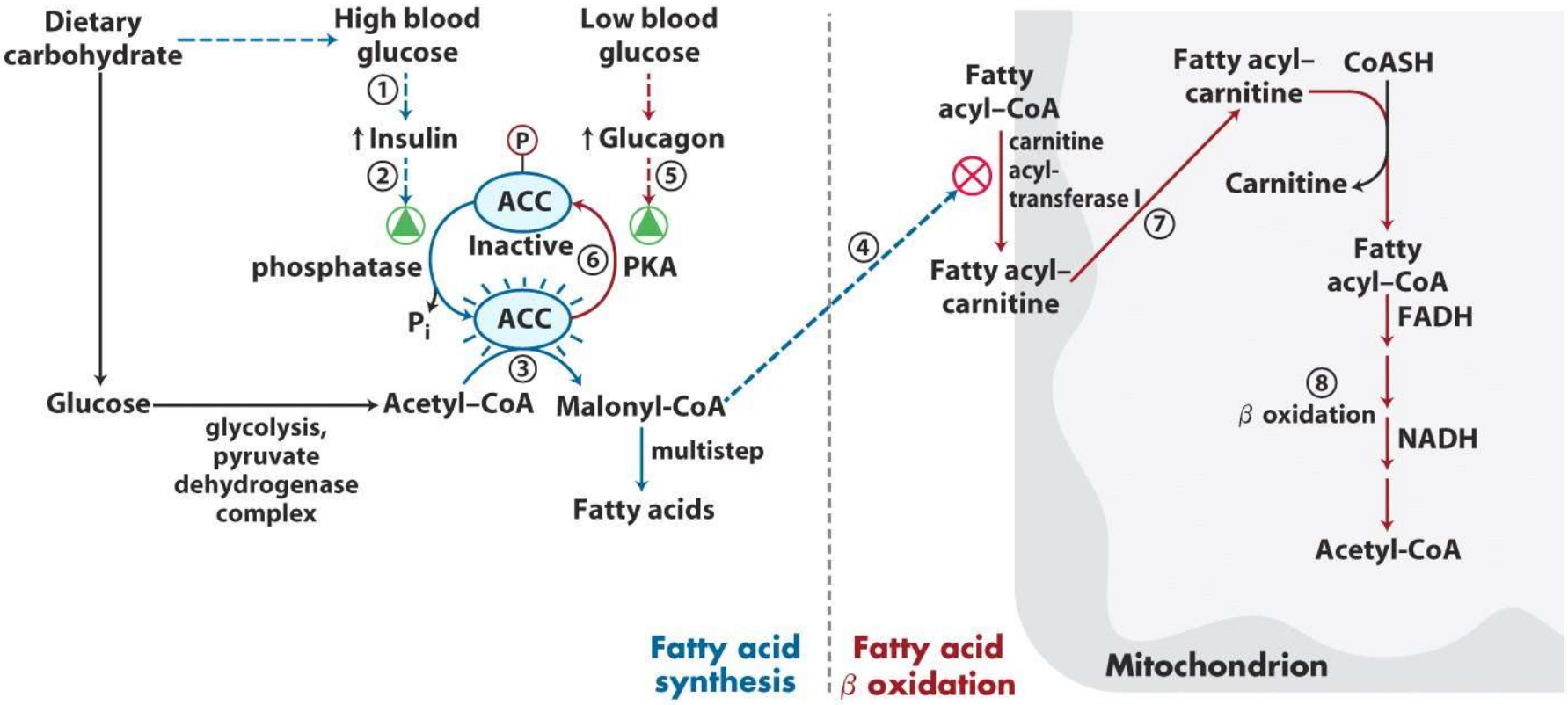


ά- Oxidation

- β-oxidation cannot react with a phytanic acid having a methyl group branching at β carbon
- Sequential conversion takes place from phytanic acid to pristanic acid (α-oxidation)



Hormonal regulation of Fatty acid synthesis and catabolism



References:

Title/URL	Author	Publisher	Year
Biochemistry (6th edition)	Campbell, M.K. and Farre	Thompson Brooks/C	
Biochemistry.2010	Garret, R.H., Grisham, C.	Thompson Brooks	2007
Biochemistry	Hames,D	USA: Taylor and Fran	-
Color Atlas of Biochemistry	Koolman, J., Roehm, K.H	Thieme Stuttgart	2005
Biochemistry demystified	Walker, S.	New York, USA; McGr	2008
Biochemistry, 7th Edition	Stryer	W.H Freeman and C(	2010
Biochemistry, 4th Edition	Donald Voet and Judith C	Wiley and Co	2011
Google with keyword of biochemistr	Various Online Biochemi	various	
Concepts in Biochemistry, 2nd ed	Boyer, R	Brooks/Cole/Thomsc	2002