Biosynthesis of fatty acids & Eicosanoids

University of Anbar/College of Pharmacy

Second semester 2020-2021 / Biochemistry II / 3rd stage

References :

1- Harper’s Illustrated Biochemistry

2- Lehninger Principles of Biochemistry

By

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BIOMEDICAL IMPORTANCE

Fatty acids are synthesized by an extramitochondrial system, which is responsible for the complete synthesis of palmitate from acetyl-CoA in the cytosol. In most mammals, glucose is the primary substrate for lipogenesis, the main fuel molecule they obtain from the diet.

Unsaturated fatty acids in phospholipids of the cell membrane are important in maintaining membrane fluidity. A high ratio of polyunsaturated fatty acids to saturated fatty acids (P:S ratio) in the diet is considered to be beneficial in preventing coronary heart disease.

Animal tissues have limited capacity for desaturating fatty acids, and require certain dietary polyunsaturated fatty acids derived from plants.

These essential fatty acids are used to form eicosanoic (C20) fatty acids, which give increase to the eicosanoids prostaglandins, thromboxanes, leukotrienes, and lipoxins.

Prostaglandins mediate inflammation, pain, induce sleep, and also regulate blood coagulation and reproduction. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen act by inhibiting prostaglandin synthesis.

Leukotrienes have muscle contractant and chemotactic properties and are important in allergic reactions and inflammation.

THE MAIN PATHWAY FOR DE NOVO SYNTHESIS OF FATTY ACIDS (LIPOGENESIS) OCCURS IN THE CYTOSOL

This system is present in many tissues, including liver, kidney, brain, lung, mammary gland, and adipose tissue. Its cofactor requirements include NADPH, ATP, Mn^{2+}, biotin, and HCO_3^- (as a source of CO_2). AcetylCoA is the immediate substrate, and free palmitate is the end produc

Production of Malonyl-CoA Is the Initial & Controlling Step in Fatty Acid Synthesis

Bicarbonate as a source of CO_2 is required in the initial reaction for the carboxylation of acetyl-CoA to malonyl-CoA in the presence of ATP and acetyl-CoA carboxylase. This enzyme has a major role in the regulation of fatty acid synthesis. Acetyl-CoA carboxylase has a requirement for the B vitamin biotin and is a multienzyme protein containing biotin, biotin carboxylase, biotin carboxyl carrier protein, and a carboxyl transferase, as well as a regulatory allosteric site.

The reaction takes place in two steps:

(1)Carboxylation of biotin involving ATP

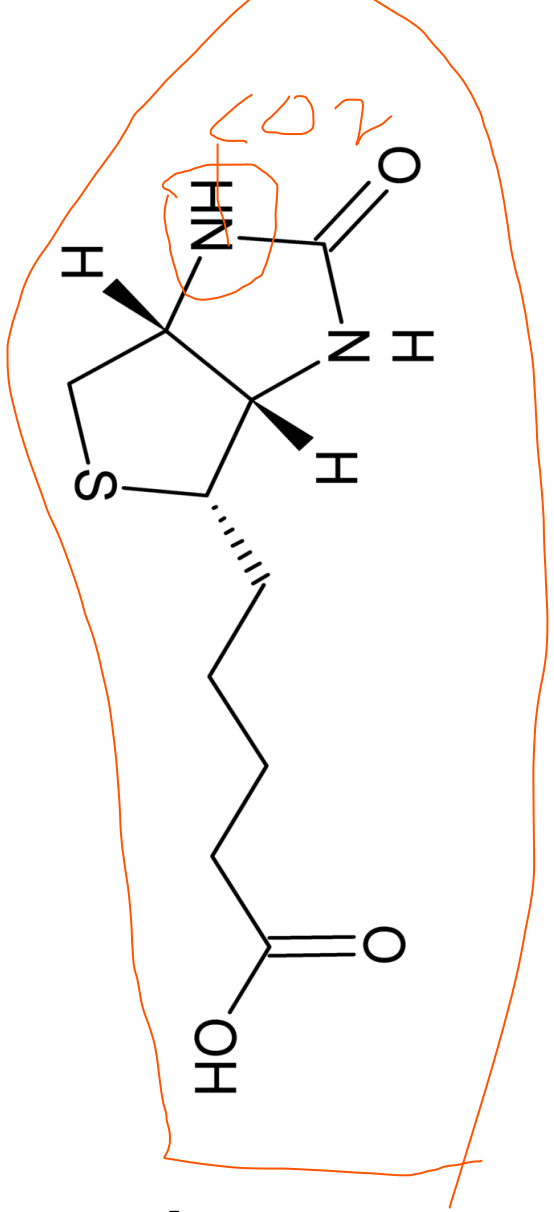
(2) Transfer of the carboxyl group to acetyl-CoA to form malonyl-CoA.

Acetyl carboxylase is a multienzyme complex containing two enzymes, biotin carboxylase (E1) and a carboxyltransferase (E2) and the biotin carrier protein (BCP). Biotin is covalently linked to the BCP.

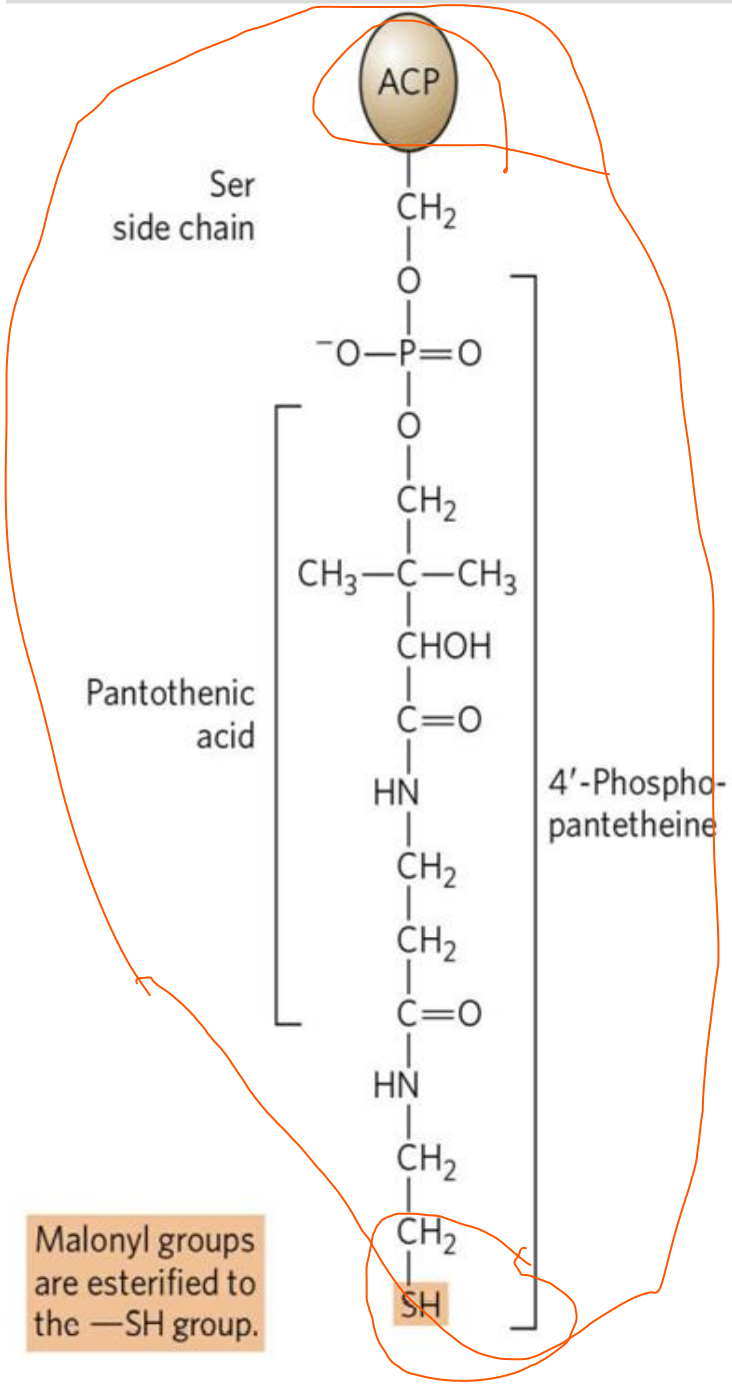
The reaction proceeds in two steps.

step 1, catalysed by E1, biotin is carboxylated as it accepts a COO^- group from HCO_3^- and ATP is used.

step 2, catalyzed by E2, the COO^- is transferred to acetyl-CoA forming malonylCoA.



biotin



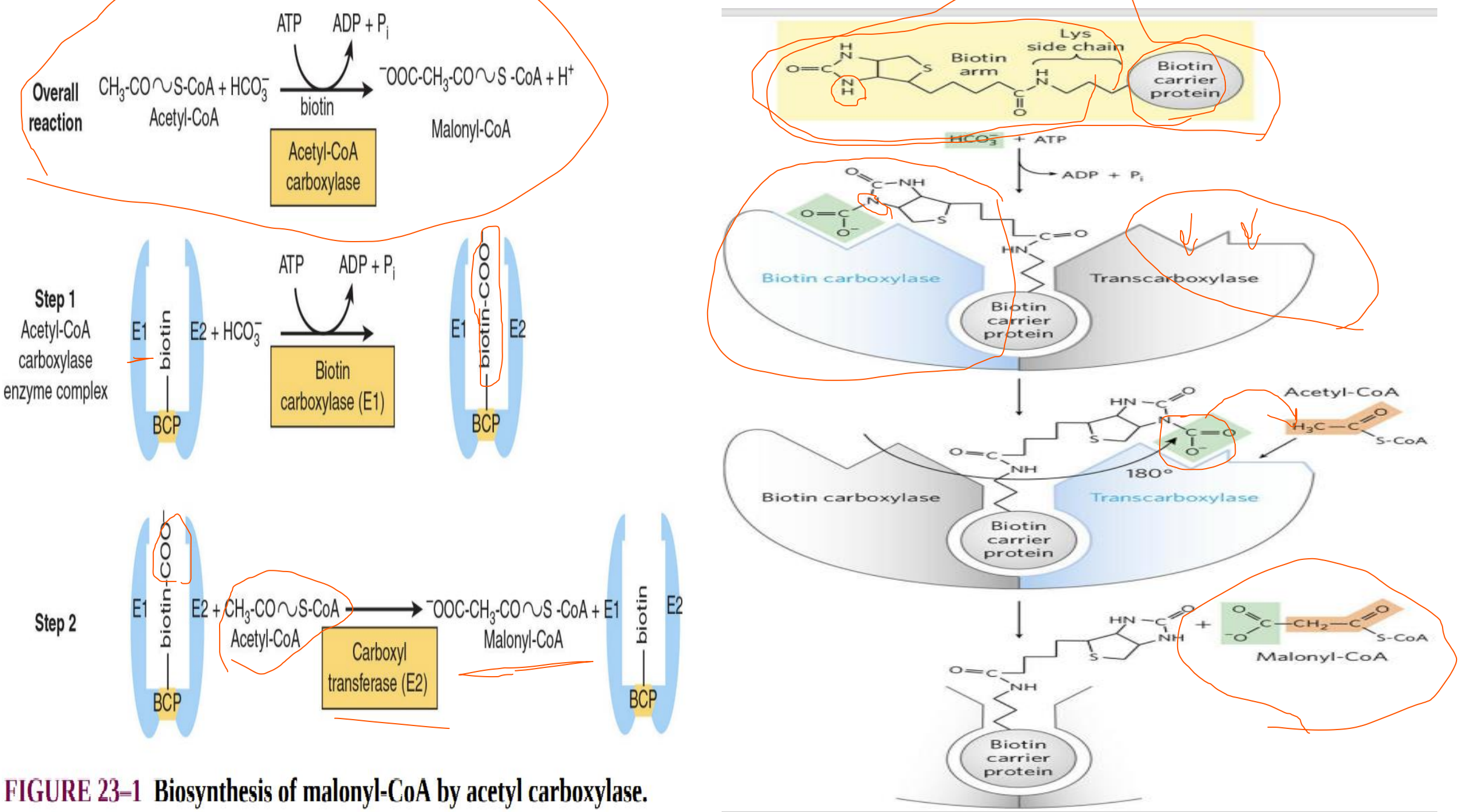
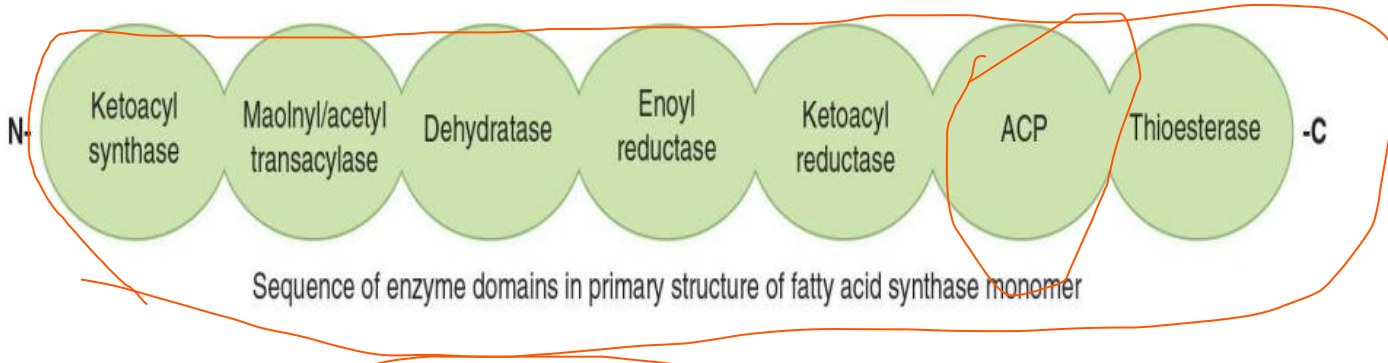


FIGURE 23–1 Biosynthesis of malonyl-CoA by acetyl carboxylase.

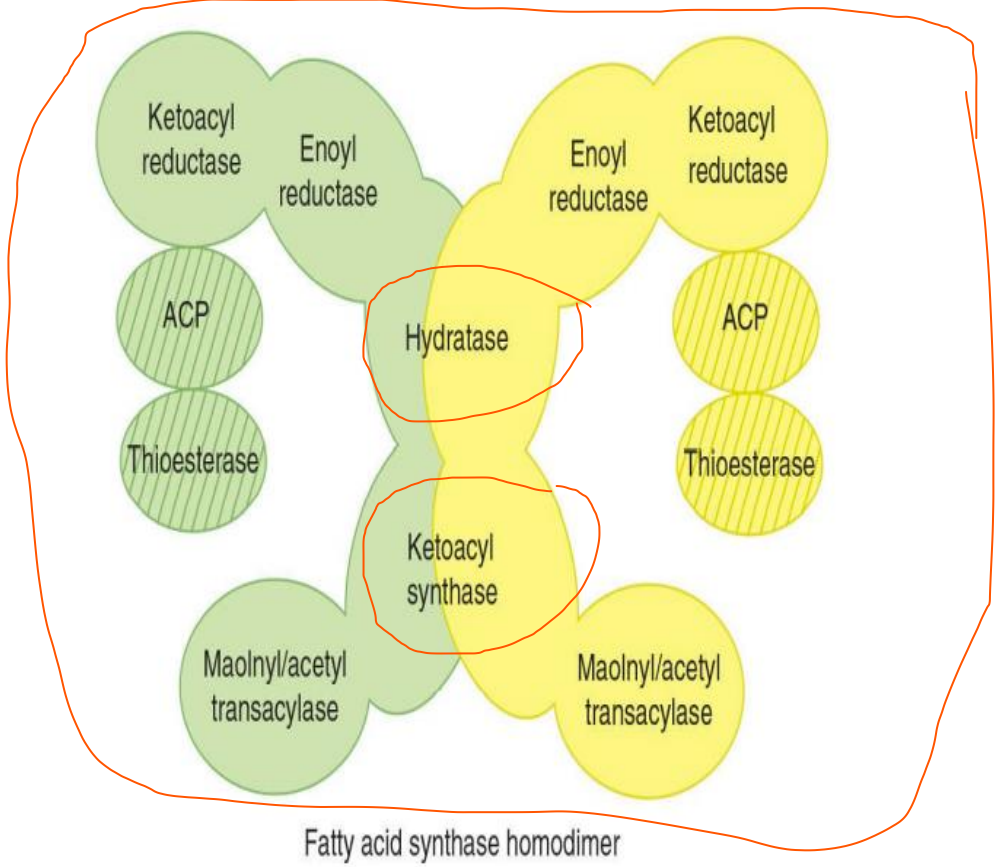
The Fatty Acid Synthase Complex Is a Homodimer of Two Polypeptide Chains Containing Six Enzyme Activities and the Acyl Carrier Protein

$$CH_3CO{-}S{-}CoA + 7HOOCCHCO{-}S{-}CoA + 14NADPH + 14H^+ \rightarrow CH_3(CH_2)_{14}COOH + 7CO_2 + 6H_2O + 8CoA{-}SH + 14NADP^+$$



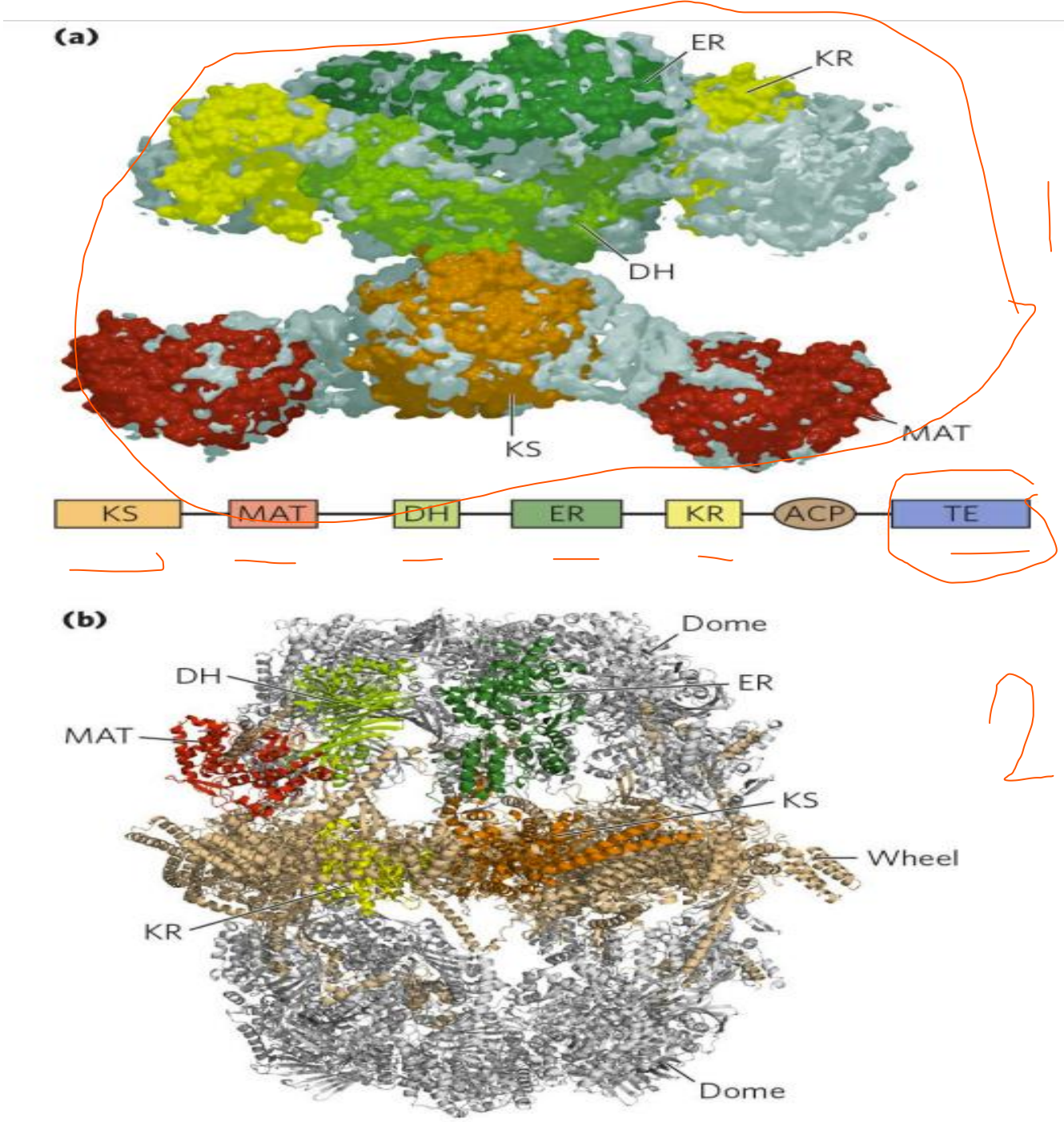
Sequence of enzyme domains in primary structure of fatty acid synthase monomer

After the formation of malonyl-CoA, fatty acids are formed by the fatty acid synthase enzyme complex. This individual enzymes required for fatty acid synthesis are linked in this multienzyme polypeptide complex that incorporates the acyl carrier protein (ACP), which has a similar function to that of CoA in the β-oxidation pathway It contains the vitamin pantothenic acid in the form of 4′- phosphopantetheine



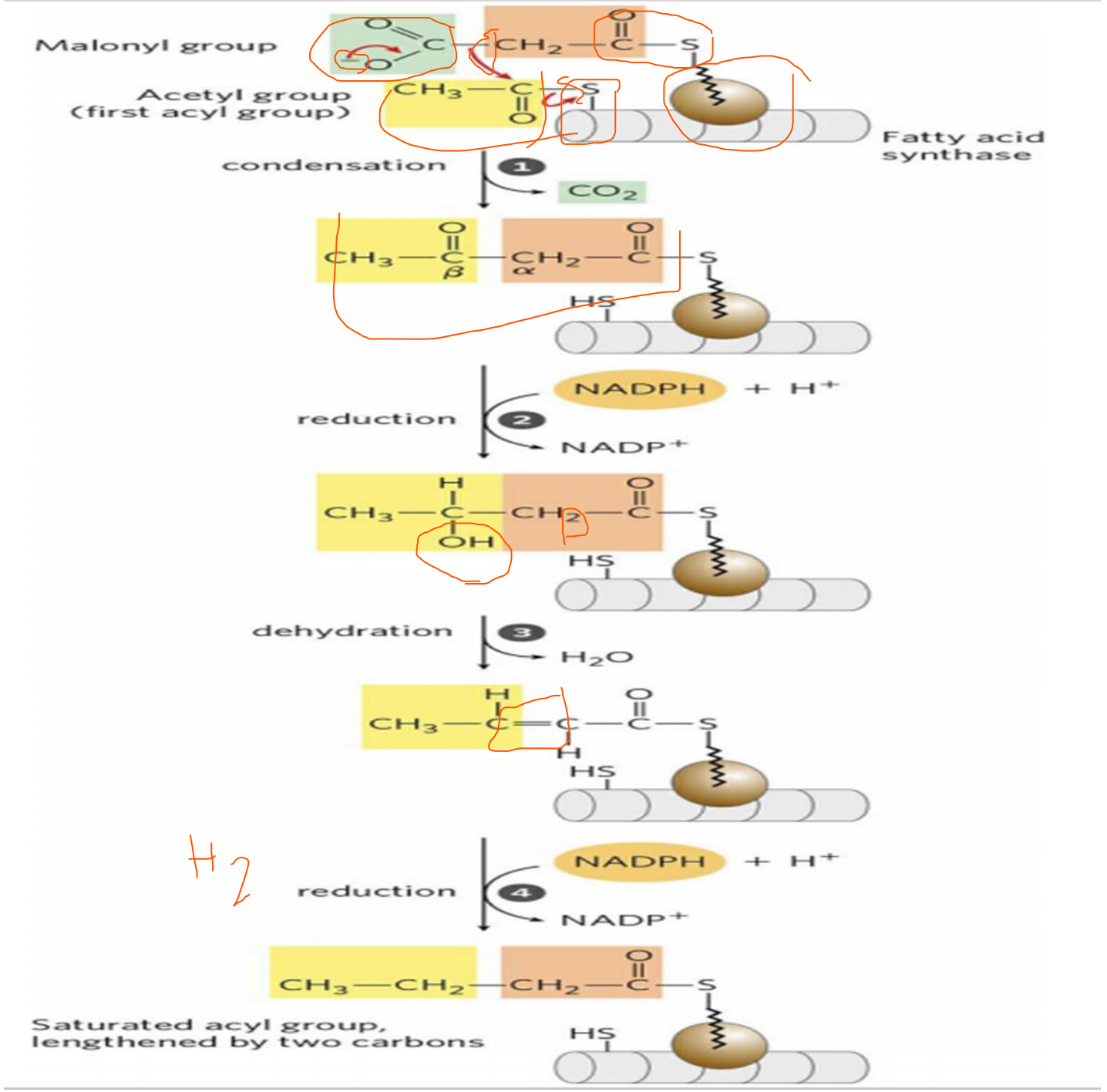
Fatty acid synthase homodimer

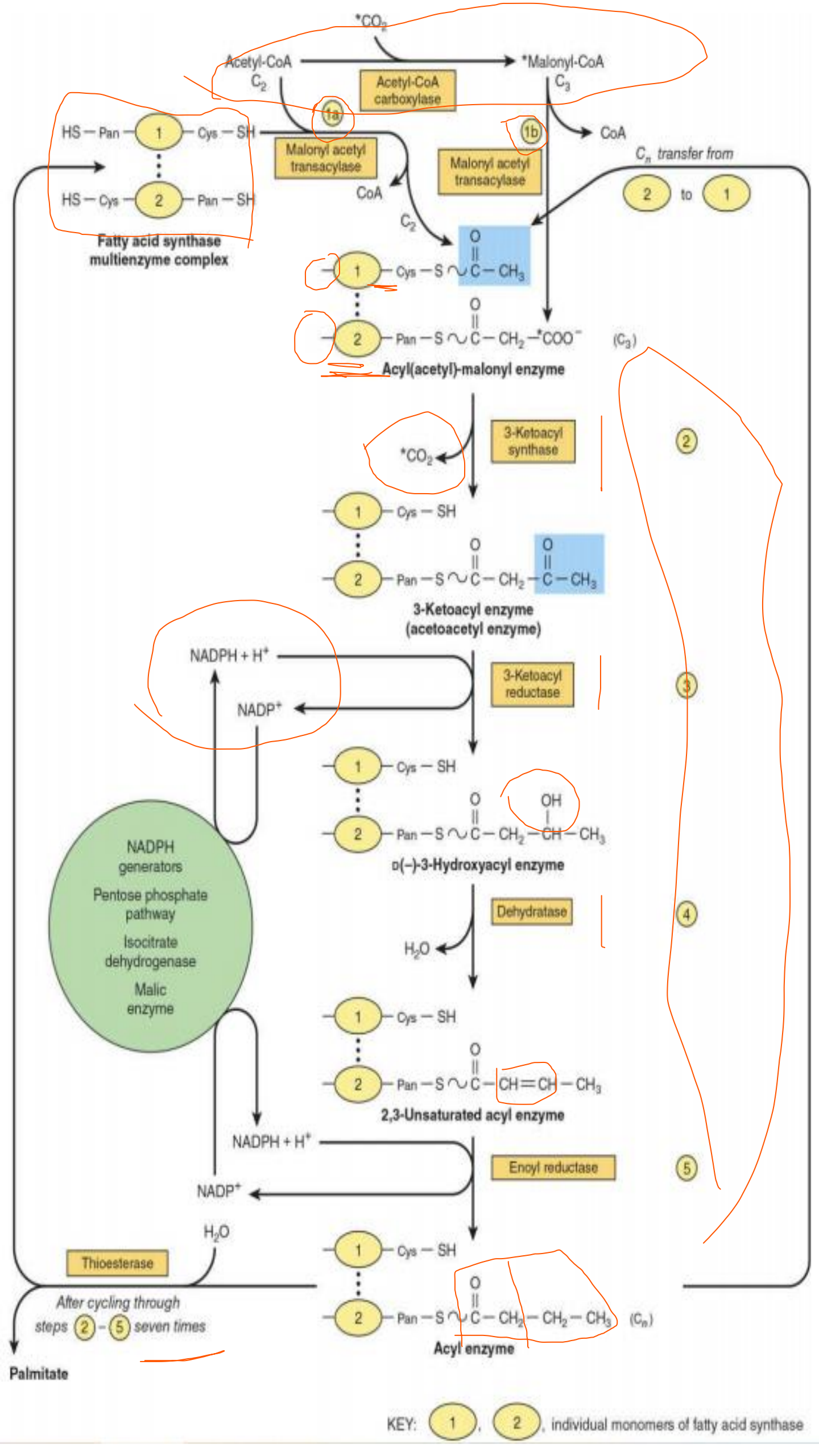
The structure of fatty acid synthase. Shown here are low-resolution structures of (a) the mammalian (porcine) and (b) fungal enzyme systems. (a) All of the active sites in the mammalian system are located indifferent domains within a single large polypeptide chain. The different enzymatic activities are:
β-ketoacyl-ACP synthase (KS)
malonyl/acetyl-CoA–ACP transferase (MAT)
β-hydroxyacyl-ACP dehydratase (DH)
enoyl-ACP reductase (ER)
β-ketoacyl-ACP reductase (KR)
ACP is the acyl carrier protein.
The linear arrangement of the domains in the polypeptide is shown the structure. The seventh domain is a thioesterase (TE) that releases the palmitate product from ACP when synthesis is completed. The ACP and TE domains are disordered in the crystal and are therefore not shown in the structure

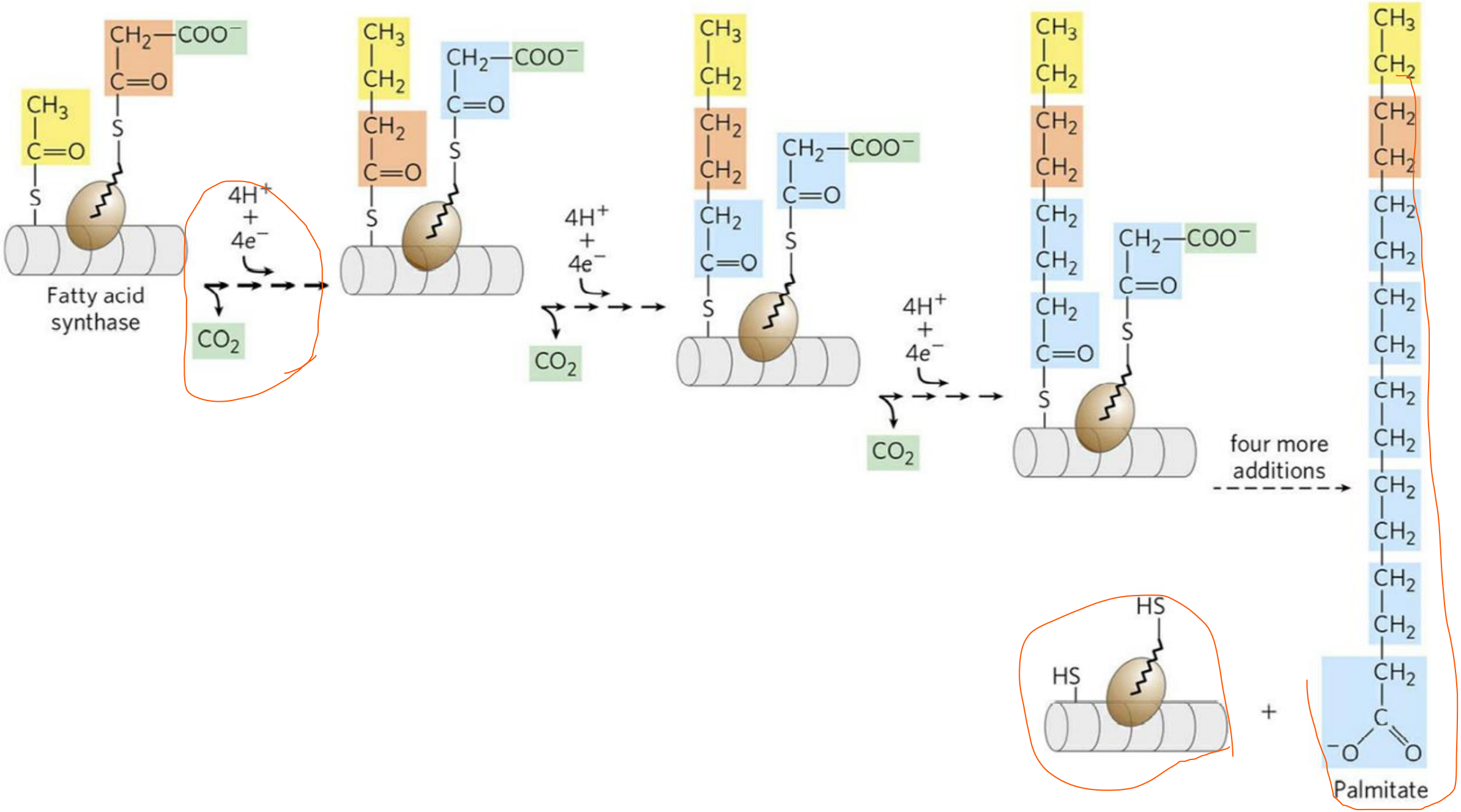


Addition of two carbons to a growing fatty acyl chain: a four-step sequence.

- Condensation of an activated 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.
- β-keto group is reduced to an alcohol
- Elimination of H_2O creates a double bond
- The double bond is reduced to form the corresponding saturated fatty acyl group

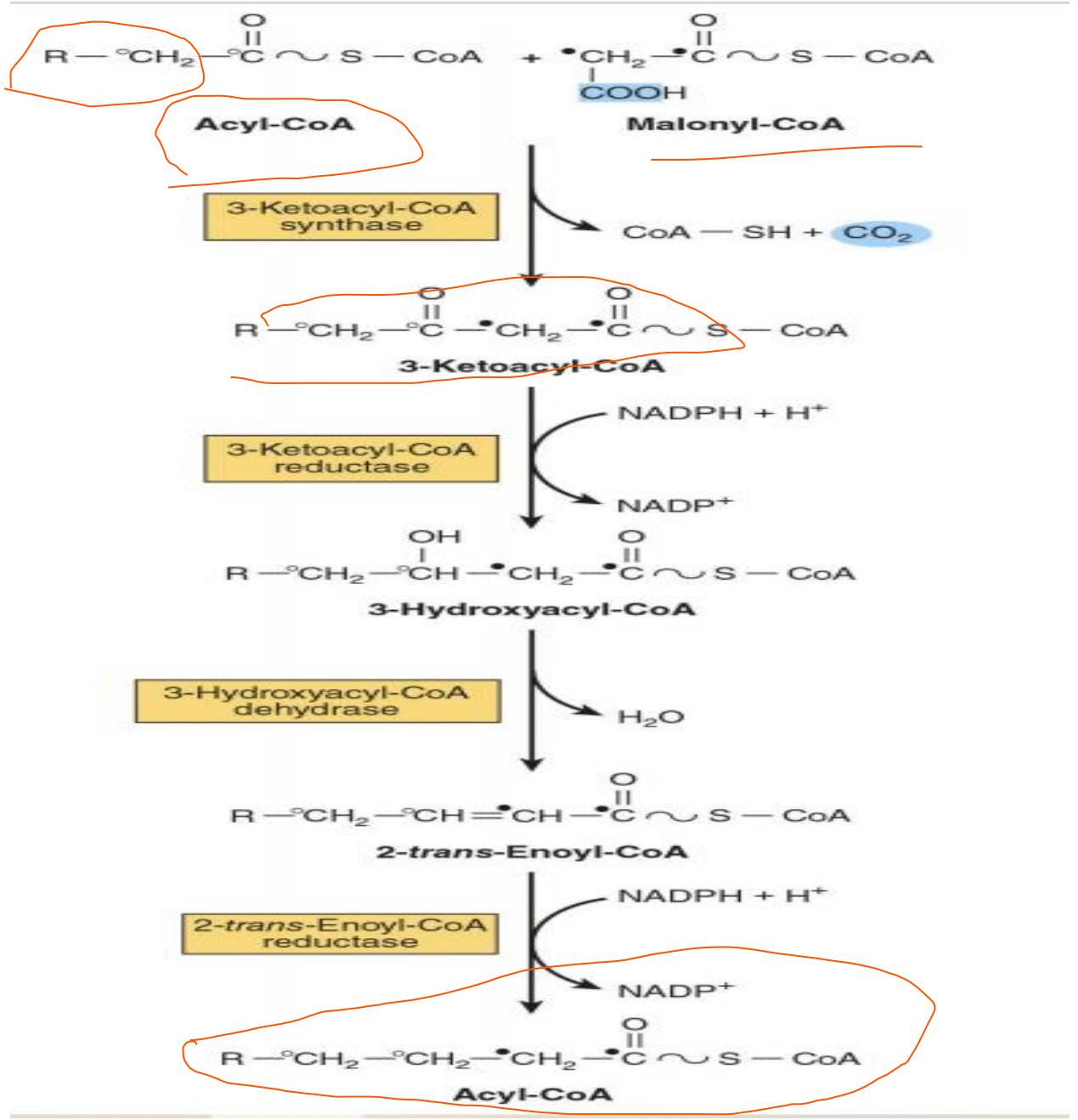



*CO2
Acetyl-CoA
C2
*Malonyl-CoA
C3
Acetyl-CoA carboxylase
1a
1b
CoA
Malonyl acetyl transacylase
Malonyl acetyl transacylase
Cn transfer from
2 to 1
HS — Pan — 1 — Cys — SH
HS — Cys — 2 — Pan — SH
Fatty acid synthase multienzyme complex
C2
Cys — S
CH3
Pan — S
CH2 — *COO−
(C3)
Acyl(acetyl)-malonyl enzyme
*CO2
3-Ketoacyl synthase
Cys — SH
CH2
CH3
3-Ketoacyl enzyme (acetoacetyl enzyme)
NADPH + H+
NADP+
3-Ketoacyl reductase
OH
CH
D(−)-3-Hydroxyacyl enzyme
NADPH generators
Pentose phosphate pathway
Isocitrate dehydrogenase
Malic enzyme
H2O
Dehydratase
CH=CH
2,3-Unsaturated acyl enzyme
NADPH + H+
NADP+
Enoyl reductase
H2O
Thioesterase
After cycling through steps 2 – 5 seven times
CH2 — CH2 — CH3
(Cn)
Acyl enzyme
Palmitate
2
3
4
5
KEY: 1, 2, individual monomers of fatty acid synthase


Fatty acid synthase
4H+ + 4e−
CO2
four more additions
HS
HS
Palmitate

Elongation of Fatty Acid Chains Occurs in the Endoplasmic Reticulum

This pathway (the "microsomal system") elongates saturated and unsaturated fatty acyl-CoAs (from C10 upward) by two carbons, using malonyl-CoA as the acetyl donor and NADPH as the reductant, and is catalyzed by the microsomal fatty acid elongase system of enzymes



THE NUTRITIONAL STATE REGULATES LIPOGENESIS

Excess carbohydrate is stored as fat in many animals in anticipation of periods of caloric deficiency such as starvation, hibernation, and to provide energy .

Lipogenesis converts excess glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat, assisting the anabolic phase of this feeding cycle.

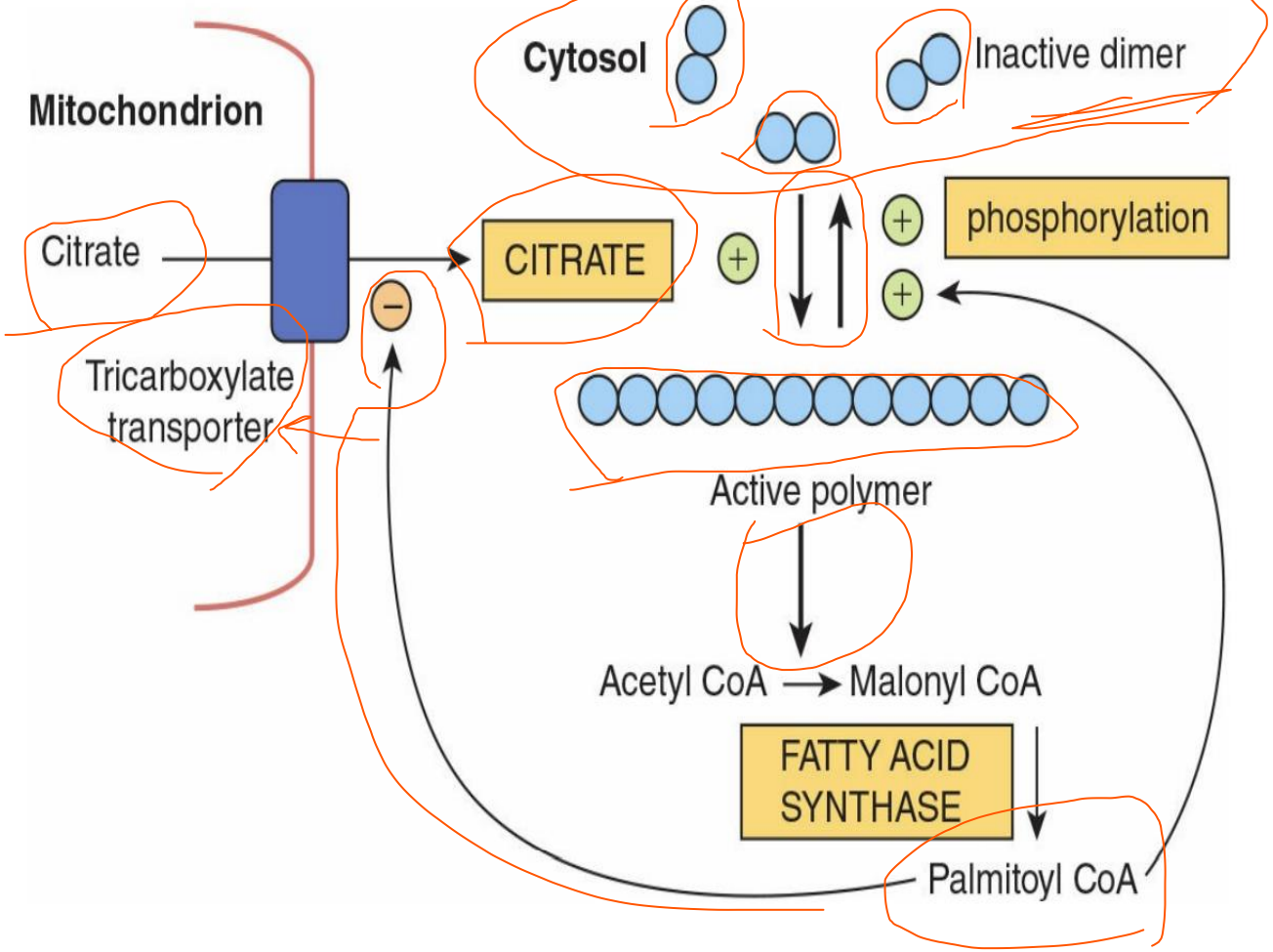
The nutritional state of the organism is the main factor regulating the rate of lipogenesis. Thus, the rate is high in the well-fed animal whose diet contains a high proportion of carbohydrate.

Lipogenesis is increased when sucrose is fed instead of glucose because fructose bypasses the phosphofructokinase control point in glycolysis and floods the lipogenic pathway

SHORT- & LONG-TERM MECHANISMS REGULATE LIPOGENESIS

Long-chain fatty acid synthesis is controlled in the short term by allosteric and covalent modification of enzymes and in the long term by changes in gene expression governing rates of synthesis of enzymes.

Regulation of acetyl-CoA carboxylase. Acetyl-CoA carboxylase is activated by citrate, which promotes the conversion of the enzyme from an inactive dimer to an active polymeric form. Inactivation is promoted by phosphorylation of the enzyme and by long-chain acyl-CoA molecules such as palmitoyl-CoA. In addition, acyl-CoA inhibits the tricarboxylate transporter, which transports citrate out of mitochondria into the cytosol, thus decreasing the citrate concentration in the cytosol and favoring inactivation of the enzyme.



Acetyl-CoA carboxylase is also regulated by hormones such as glucagon, epinephrine, and insulin via changes in its phosphorylation state

Regulation of acetyl-CoA carboxylase by phosphorylation/dephosphorylation. The enzyme is inactivated by phosphorylation by AMP-activated protein kinase (AMPK), which in turn is phosphorylated and activated by AMP-activated protein kinase kinase (AMPKK). Glucagon and epinephrine increase cAMP, and thus activate this latter enzyme via cAMP-dependent protein kinase. The kinase kinase enzyme is also believed to be activated by acyl-CoA. Insulin activates acetyl-CoA carboxylase via dephosphorylation of AMPK.

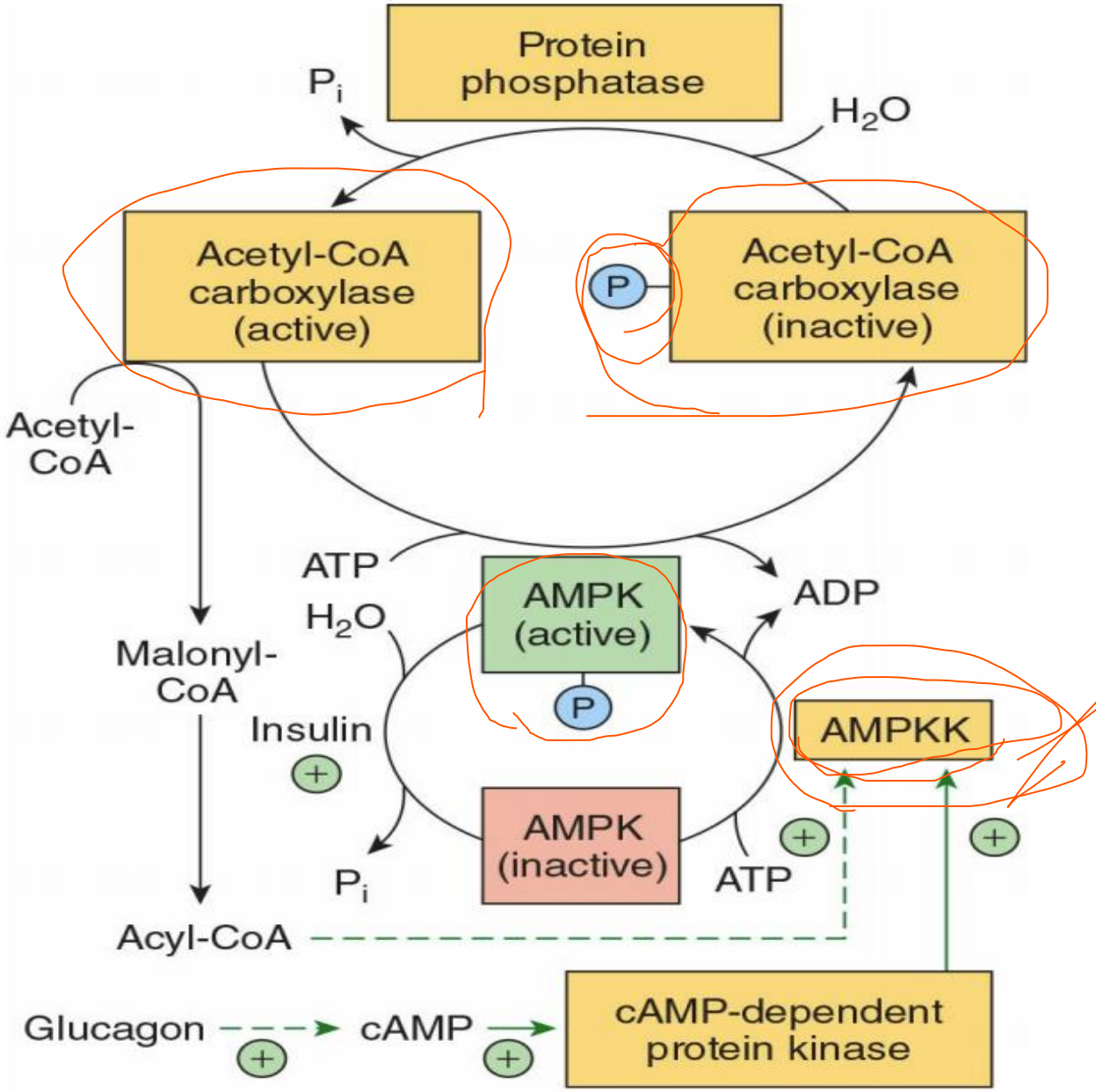


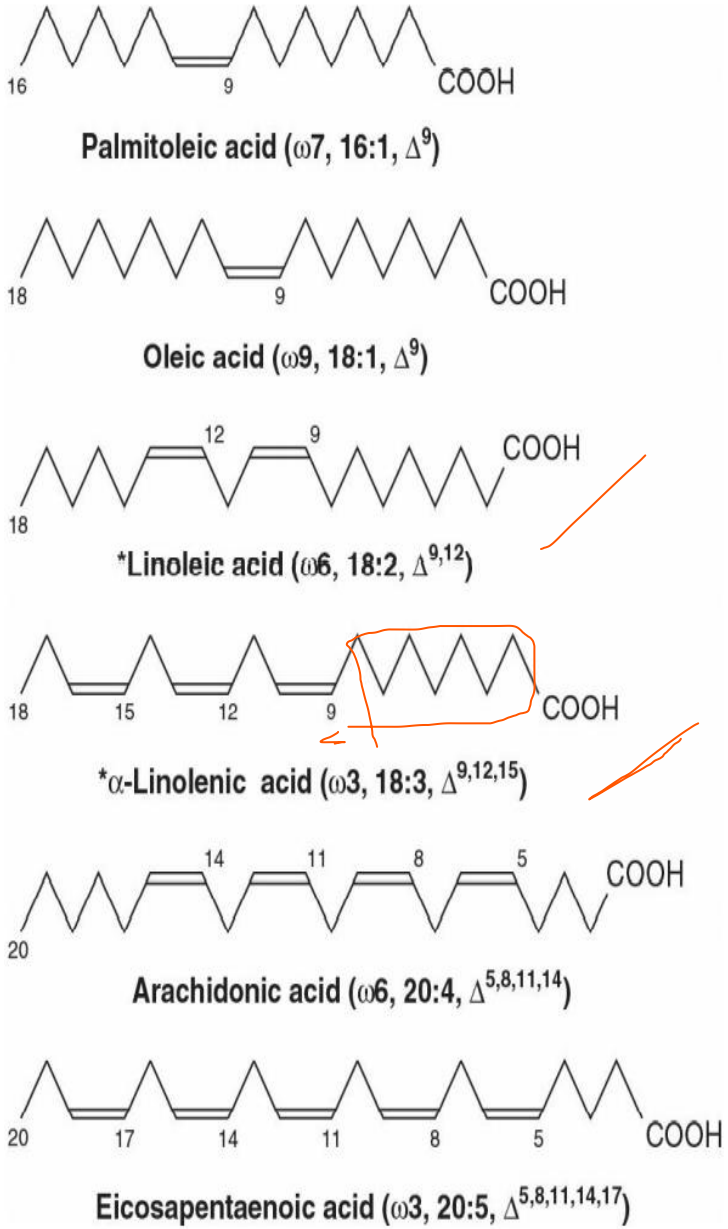
Table 14.3 Comparison of fatty acid synthesis and oxidation

		Fatty acid synthesis	*β-Oxidation*
1.	Major tissues	Liver, adipose tissue	Muscle, liver
2.	Subcellular site	Cytosol	Mitochondria
3.	Precursor/substrate	Acetyl CoA	Acyl CoA
4.	End product	Palmitate	Acetyl CoA
5.	Intermediates are bound to	Acyl carrier protein	Coenzyme A
6.	Coenzyme requirement	NADPH (supplying reducing equivalents)	FAD and NAD^+ (get reduced)
7.	Carbon units added/degraded	Malonyl CoA	Acetyl CoA
8.	Transport system	Citrate (mitochondria ⟶ cytosol)	Carnitine (cytosol ⟶ mitochondria)
9.	Inhibitor	Long chain acyl CoA (inhibits acetyl CoA carboxylase)	Malonyl CoA (inhibits carnitine acyltransferase I)
10.	The pathway increased	After rich carbohydrate diet	In starvation
11.	Hormonal status that promotes	High ratio of insulin/glucagon	Low ratio of insulin/glucagon
12.	Status of enzyme(s)	Multifunctional enzyme complex	Individual enzymes

SOME POLYUNSATURATED FATTY ACIDS CANNOT BE SYNTHESIZED BY MAMMALS & ARE NUTRITIONALLY ESSENTIAL

Certain long-chain unsaturated fatty acids of metabolic significance in mammals. Other C20 , C22 , and C24 polyenoic fatty acids may be derived from oleic, linoleic, and α-linolenic acids by chain elongation. Palmitoleic and oleic acids are **not** essential in the diet because the tissues can introduce a double bond at the Δ 9 position of a saturated fatty acid. **Linoleic** and **α-linolenic acids** are the only fatty acids known to be essential for the complete nutrition of many species of mammals

In humans and most other mammals, arachidonic acid can be formed from linoleic acid. Double bonds can be introduced at the Δ 4 , Δ 5 , Δ 6 , and Δ 9 positions in most animals, but never beyond the Δ 9 position. In contrast, plants are able to synthesize the nutritionally essential fatty acids by introducing double bonds at the Δ 12 and Δ 15 positions.



MONOUNSATURATED FATTY ACIDS ARE SYNTHESIZED BY A Δ 9 DESATURASE SYSTEM

Several tissues including the liver are considered to be responsible for the formation of nonessential monounsaturated fatty acids from saturated fatty acids.

The first double bond introduced into a saturated fatty acid is nearly always in the Δ 9 position

An enzyme system Δ 9 desaturase in the endoplasmic reticulum catalyzes the conversion of palmitoyl-CoA or stearoyl-CoA to palmitoleoyl-CoA or oleoyl-CoA, respectively.

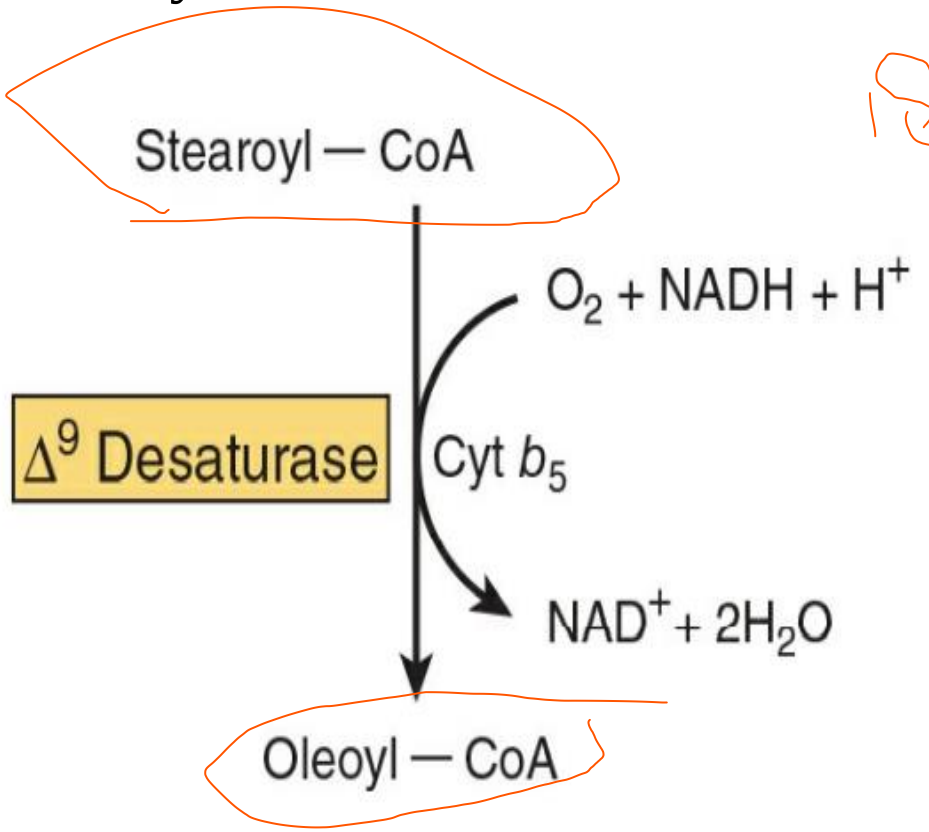
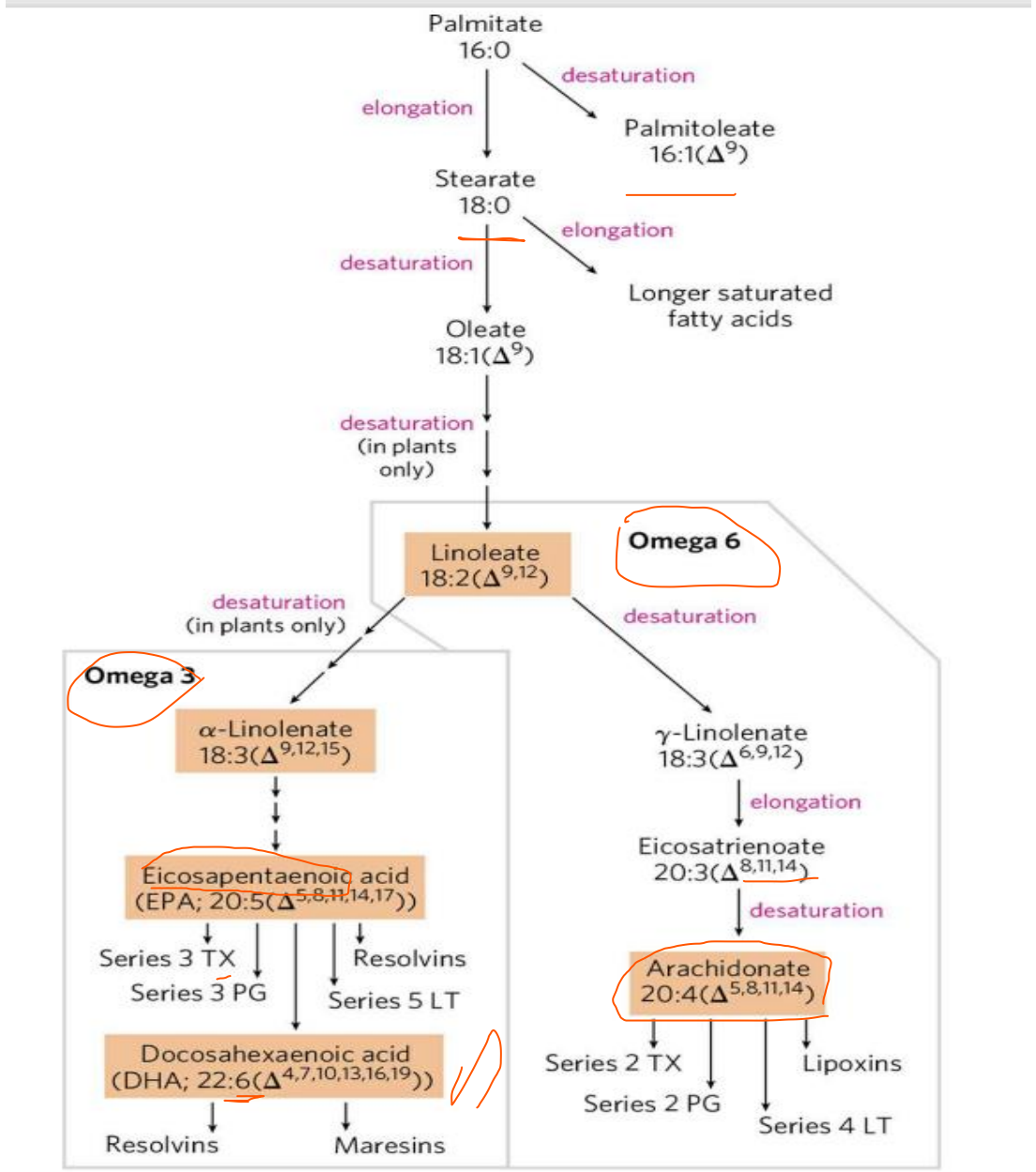


FIGURE 23–9 Microsomal δ9 desaturase.

SYNTHESIS OF POLYUNSATURATED FATTY ACIDS INVOLVES DESATURASE & ELONGASE ENZYME SYSTEMS



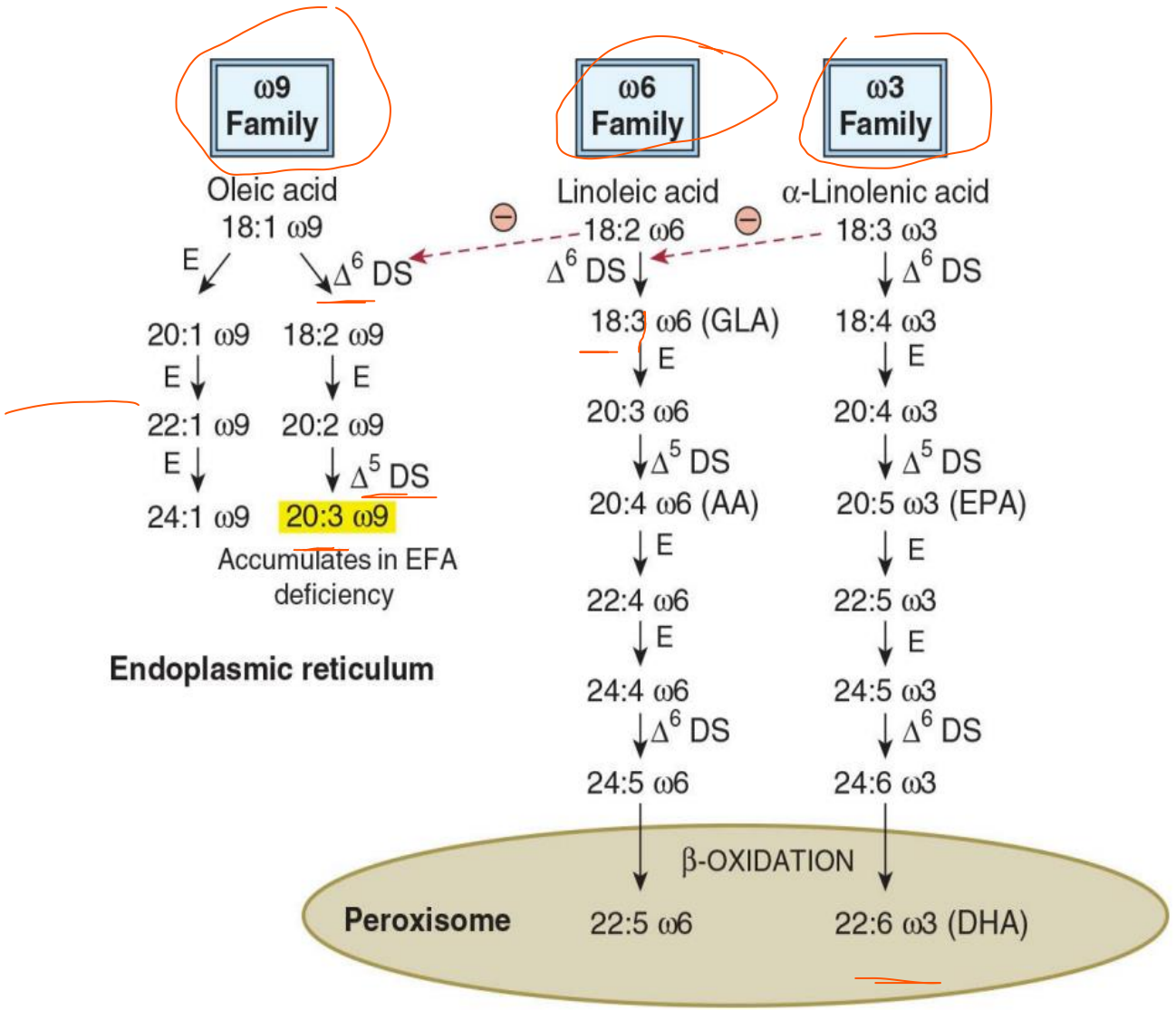


FIGURE 23–10 Biosynthesis of the ω9, ω6, and ω3 families of

DEFICIENCY SYMPTOMS OCCUR WHEN THE ESSENTIAL FATTY ACIDS (EFA) ARE ABSENT FROM THE DIET

Arachidonic acid is present in membranes and accounts for 5 to 15% of the fatty acids in phospholipids. Docosahexaenoic acid (DHA; ω3, 22:6), which is synthesized to a limited extent from α-linolenic acid or obtained directly from fish oils, is present in high concentrations in retina, cerebral cortex, testis, and sperm. DHA is particularly needed for development of the brain and retina and is supplied via the placenta and milk.

Patients with retinitis pigmentosa are reported to have low blood levels of DHA. In essential fatty acid deficiency, nonessential polyenoic acids of the ω9 family, particularly Δ 5,8,11 - eicosatrienoic acid (ω9 20:3), replace the essential fatty acids in phospholipids, other complex lipids, and membranes. The triene:tetraene ratio in plasma lipids can be used to diagnose the extent of essential fatty acid deficiency.

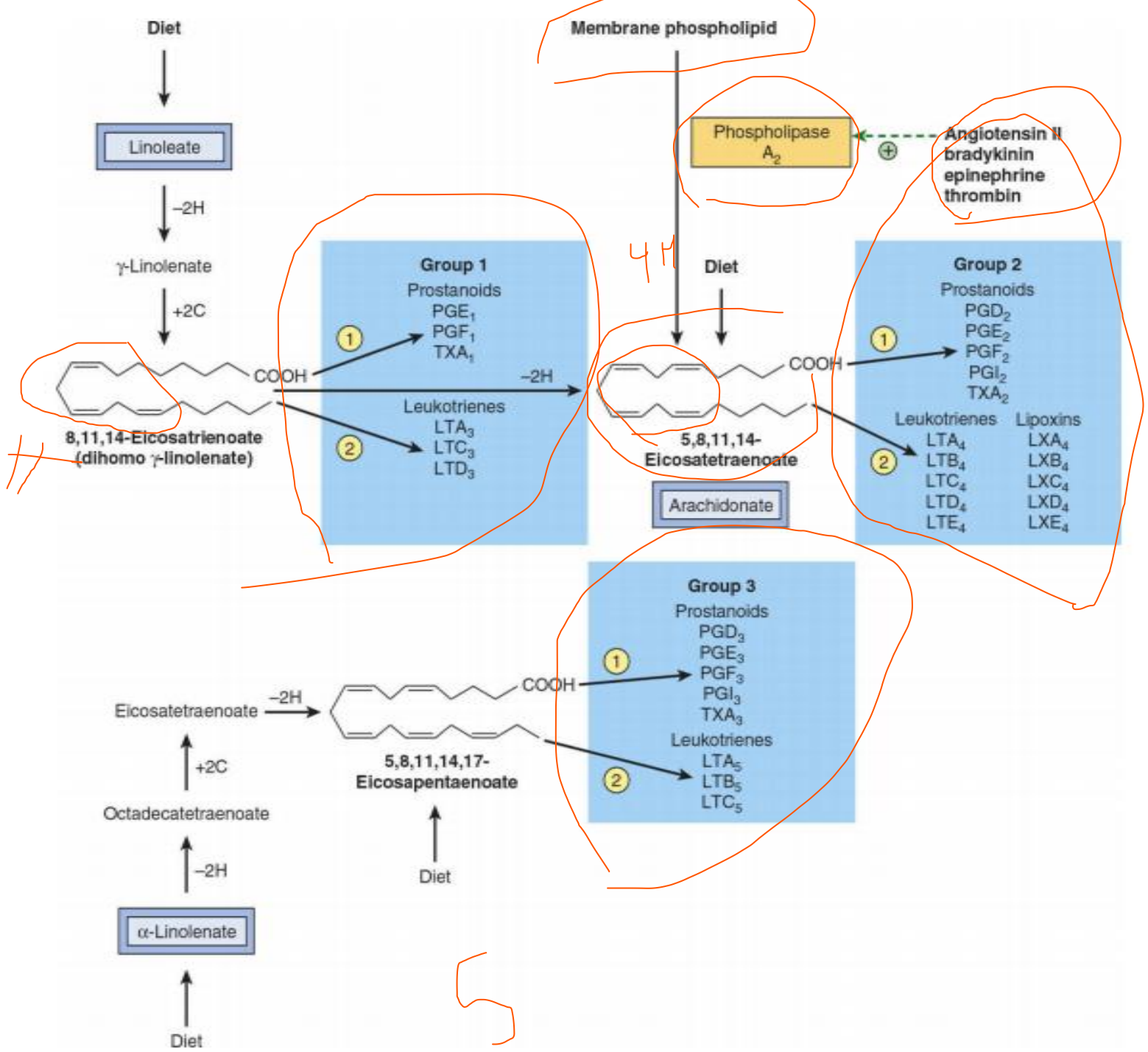
EICOSANOIDS ARE FORMED FROM C20 POLYUNSATURATED FATTY ACIDS

Arachidonate and some other C20 polyunsaturated fatty acids give increase to eicosanoids, physiologically and pharmacologically active compounds known as prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), and lipoxins (LX). Physiologically, they are considered to act as local hormones functioning

There are three groups of eicosanoids that are synthesized from C20 eicosanoic acids derived from the essential fatty acids linoleate and αlinolenate, or directly from dietary arachidonate and eicosapentaenoate.

- PG2 , TX2 series (prostanoids) by the cyclooxygenase pathway
- LT4 and LX4 series by the lipoxygenase pathway

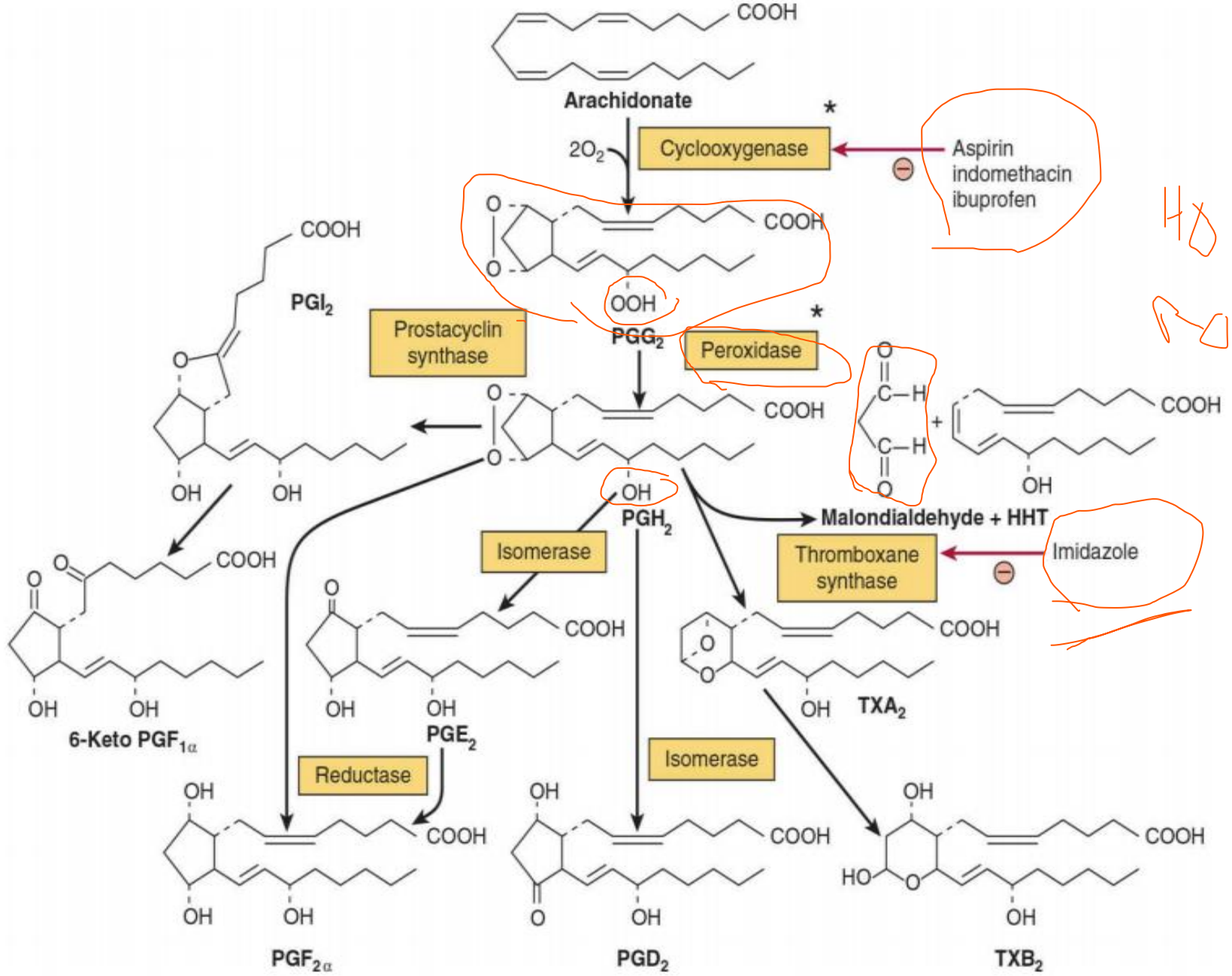
The three groups of eicosanoids and their biosynthetic origins. (, cyclooxygenase pathway; , lipoxygenase pathway; LT, leukotriene; LX, lipoxin; PG, prostaglandin; PGI, prostacyclin; TX, thromboxane.)



THE CYCLOOXYGENASE PATHWAY IS RESPONSIBLE FOR

Conversion of arachidonic acid to prostaglandins and thromboxanes of series 2

Note Aspirin is a nonsteroidal anti-inflammatory drug (NSAID) that inhibits COX-1 and COX-2. Other NSAIDs include indomethacin and ibuprofen, and these usually inhibit cyclooxygenases by competing with arachidonate



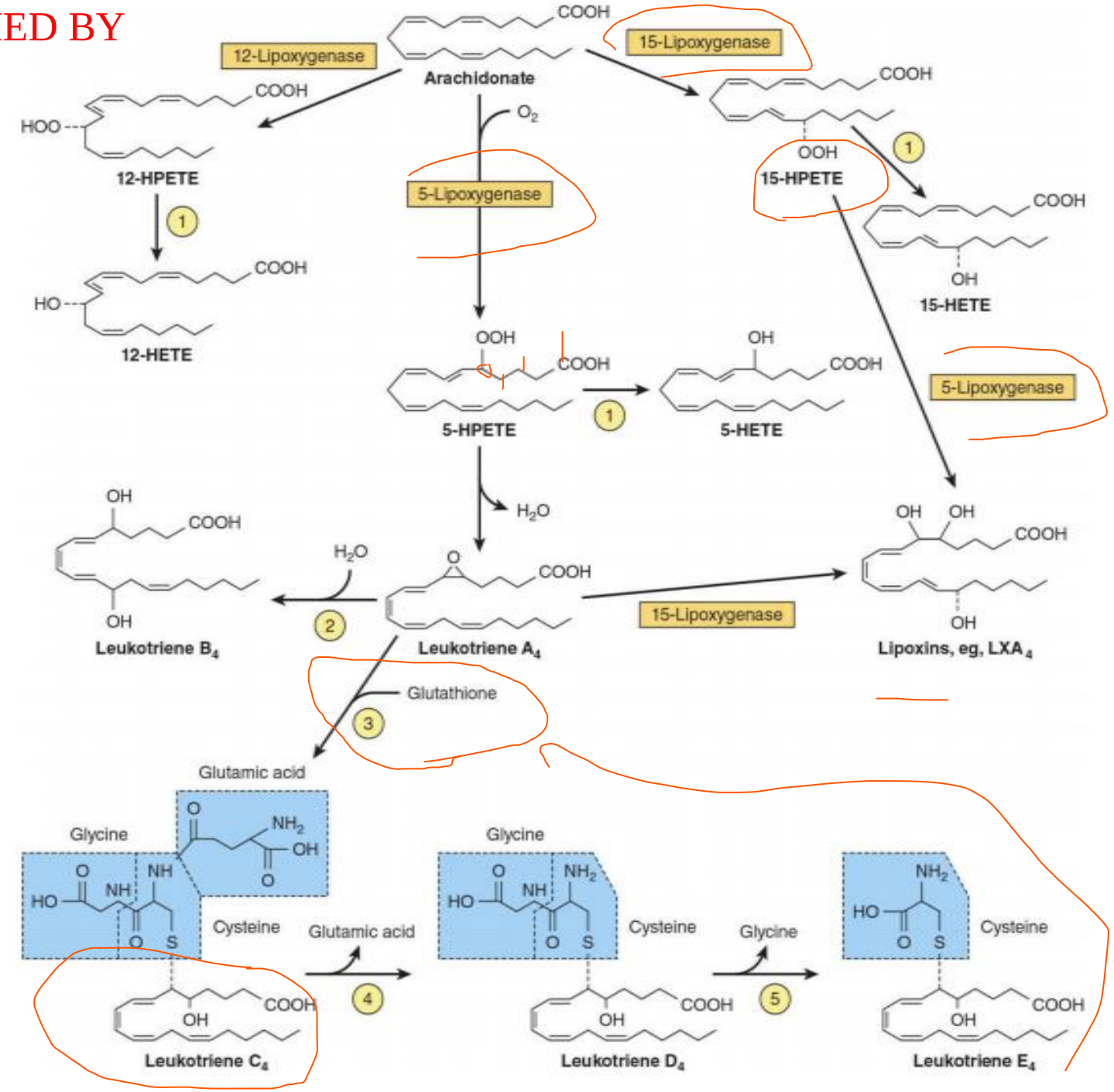
Prostanoids Are Potent, Biologically Active Substances

Thromboxanes are synthesized in platelets and upon release cause vasoconstriction and platelet aggregation. Their synthesis is specifically inhibited by low-dose aspirin. Prostacyclins (PGI2) are produced by blood vessel walls and are potent inhibitors of platelet aggregation.

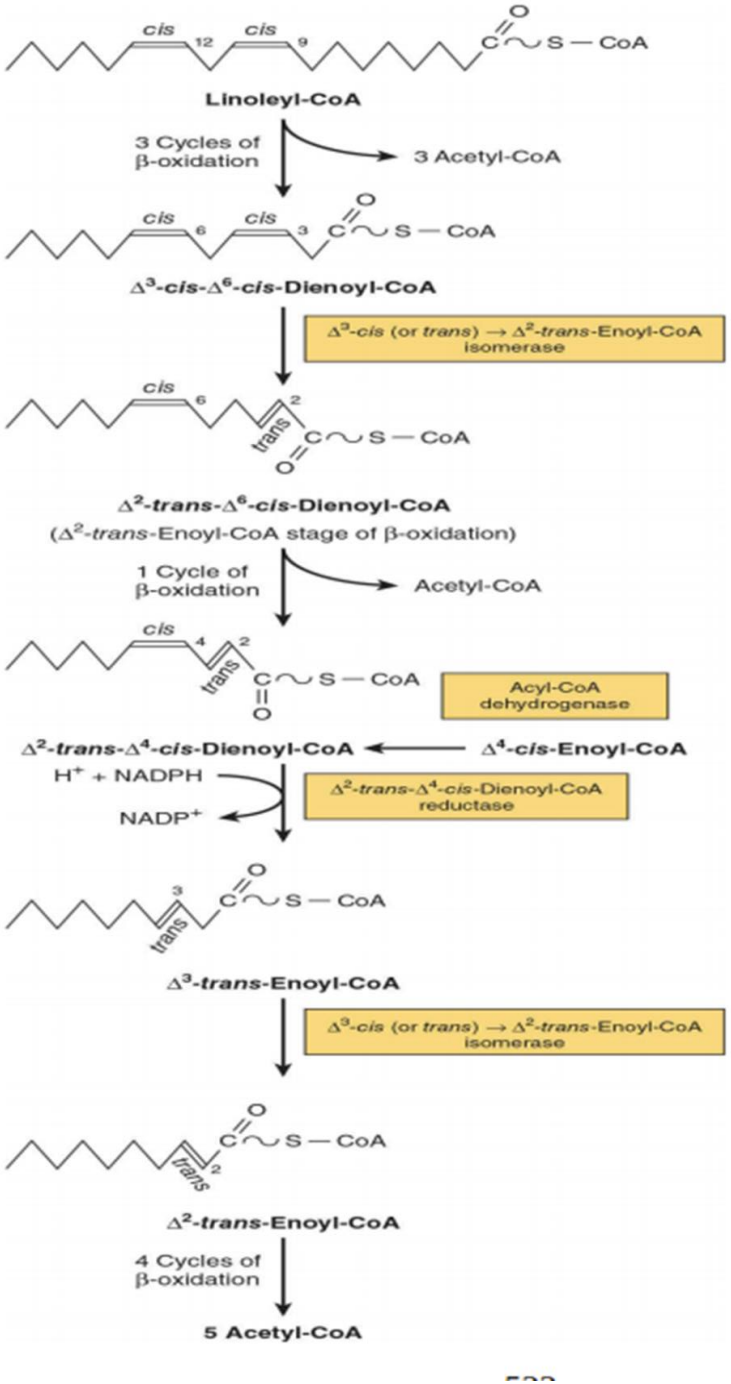
LEUKOTRIENES & LIPOXINS ARE FORMED BY THE LIPOXYGENASE PATHWAY

The leukotrienes are a family of conjugated trienes formed from eicosanoic acids in leukocytes, mastocytoma cells, platelets, and macrophages by the lipoxygenase pathway in response to both immunologic and nonimmunologic stimuli.

Conversion of arachidonic acid to leukotrienes and lipoxins of series 4 via the lipoxygenase pathway. Some similar conversions occur in series 3 and 5 leukotrienes. (1 peroxidase; 2 leukotriene A4 epoxide hydrolase; 3 glutathione S-transferase; 4 γglutamyltranspeptidase; 5 cysteinyl-glycine dipeptidase; HETE, hydroxyeicosatetraenoate; HPETE, hydroperoxyeicosatetraenoate.)



Oxidation of Unsaturated Fatty Acids Occurs by a Modified β-Oxidation Pathway / lecture 3



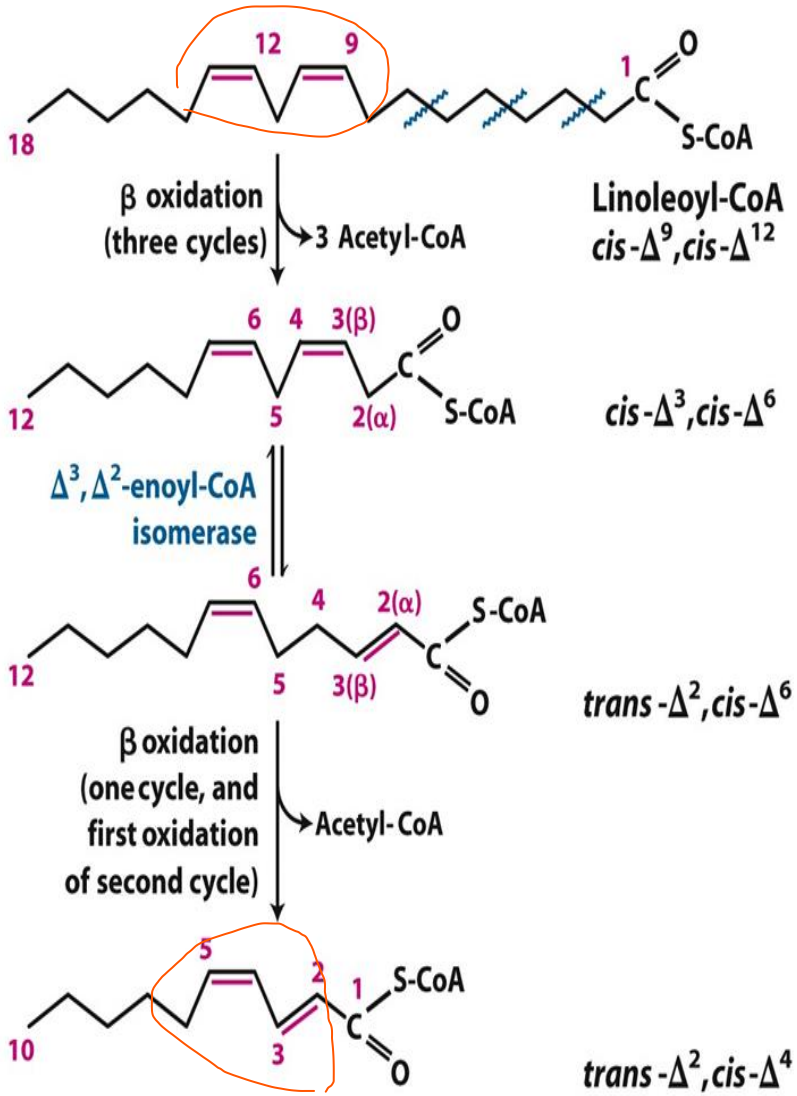


Figure 17-10 part 1
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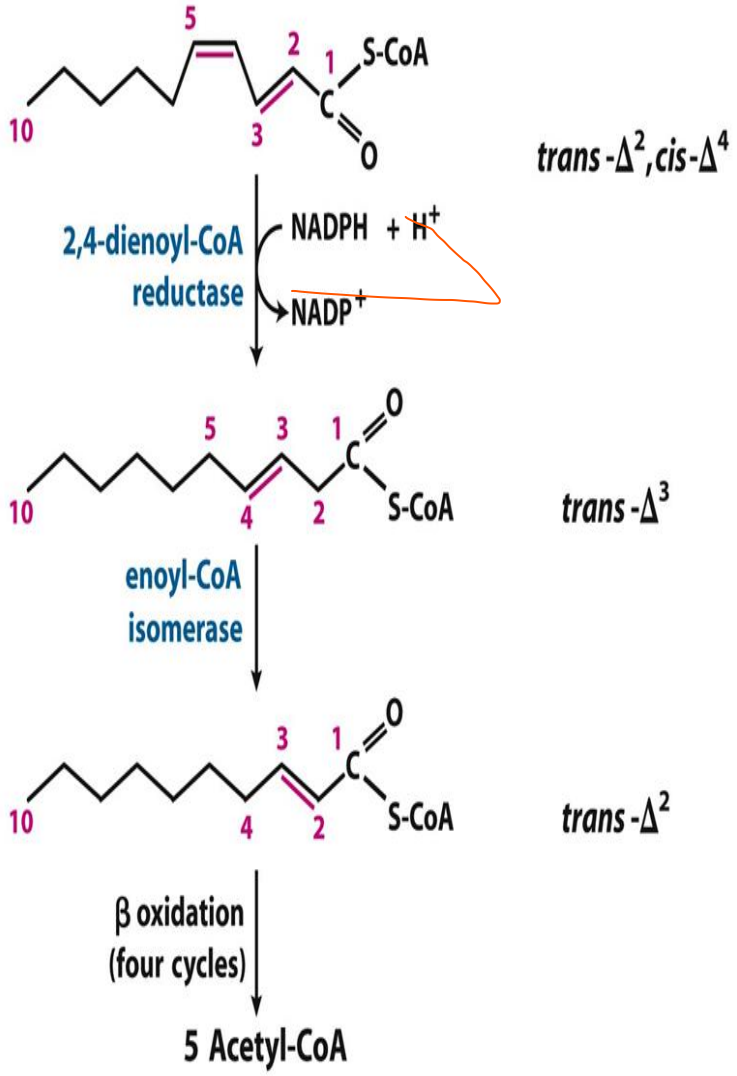

Figure 17-10 part 2
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THANK YOU