Metabolism of cholesterol and other isoprenoids (bile acids, steroid hormones, vitamins)

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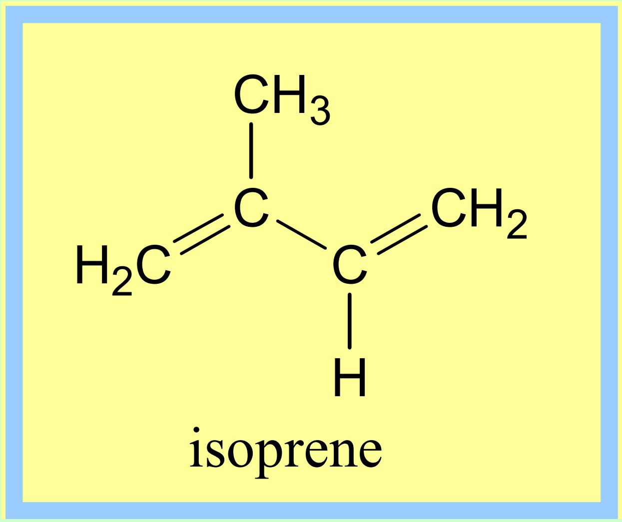
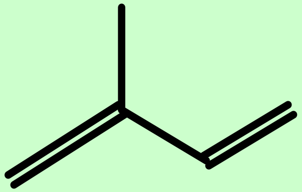
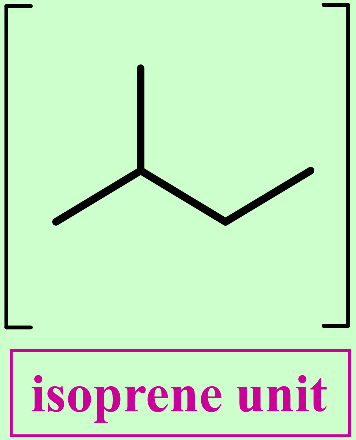
Dienes

(with condensed, <u>conjugated</u>, or isolated double bonds)

<u>isoprene</u> (2-methyl-1,3-butadiene)



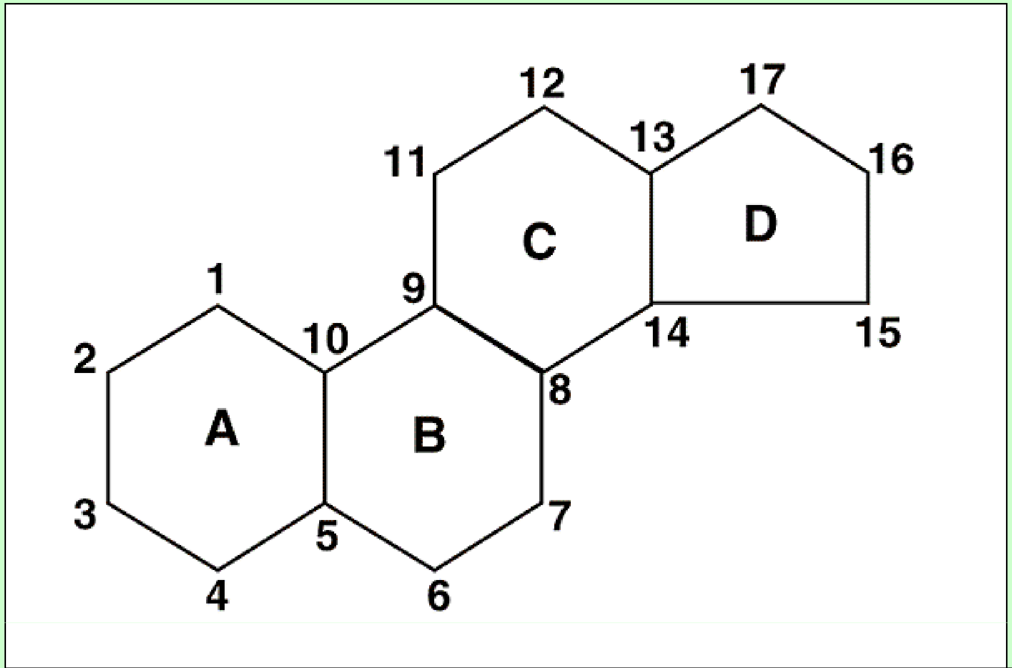




For the synthesis of cholesterol, steroids, some vitamins…

- In biosynthetic reactions, <u>acetyl-CoA</u> is converted to the <u>isoprene units</u>

GONANE



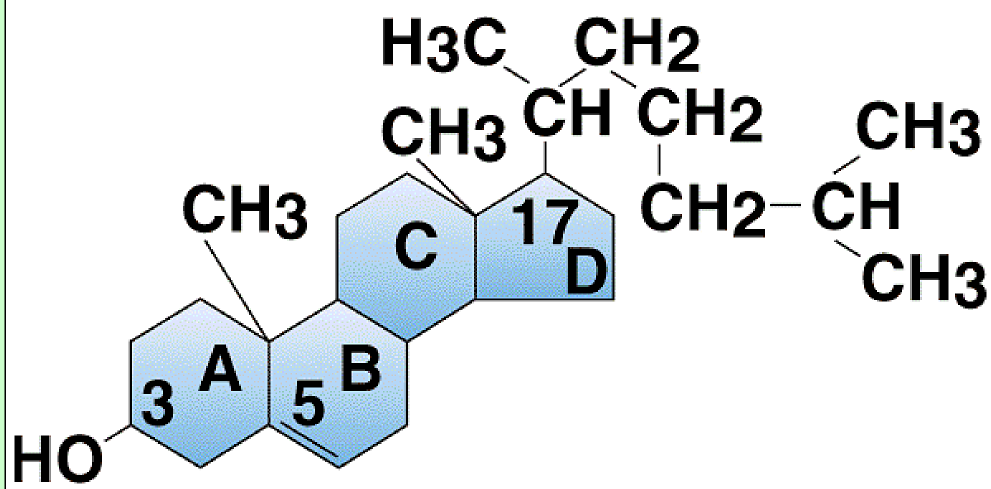
Tetracyclic system:
3 cyclohexane- and
1 cyclopentane ring

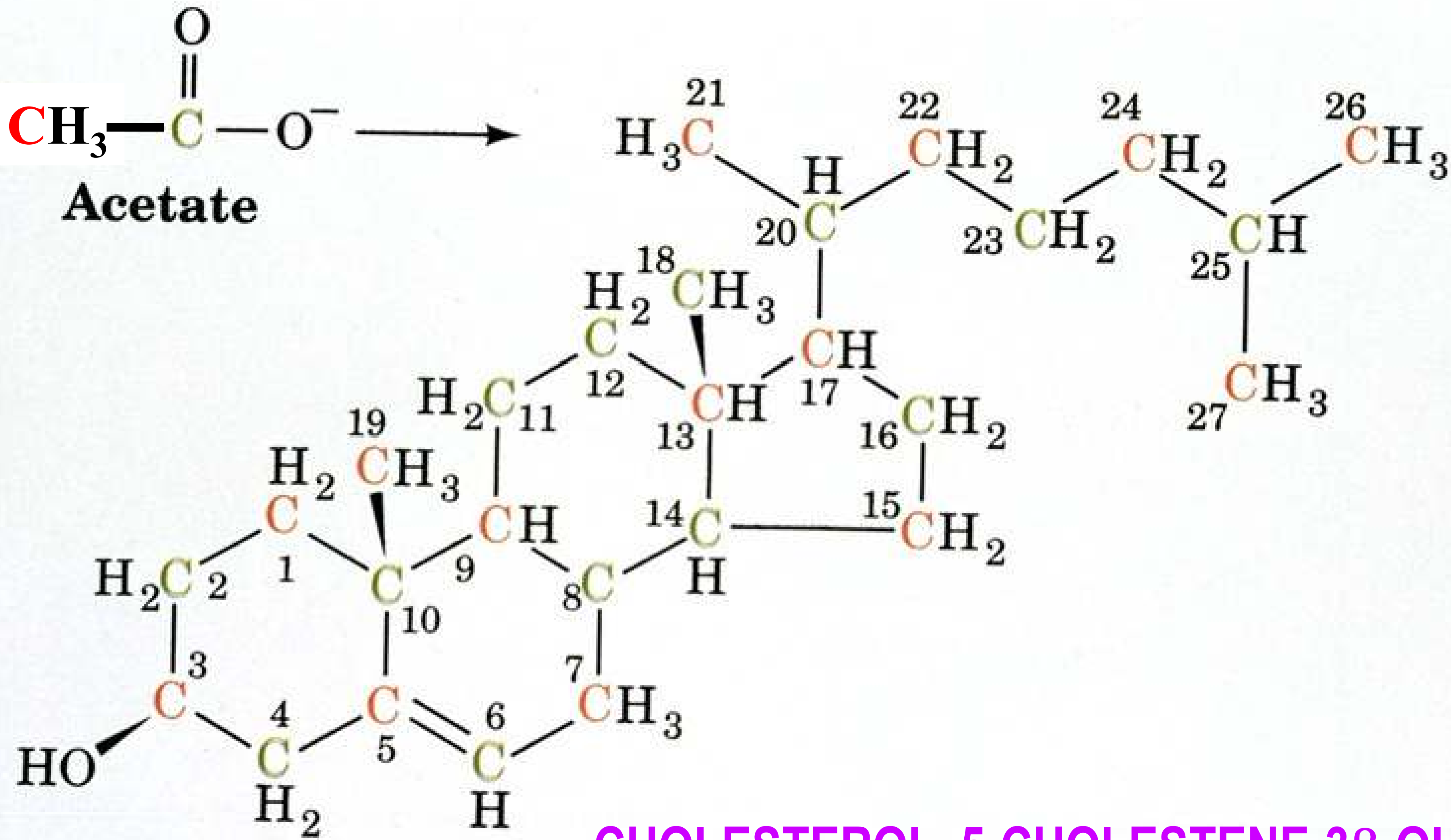
CHOLESTEROL

Steroid skeleton:

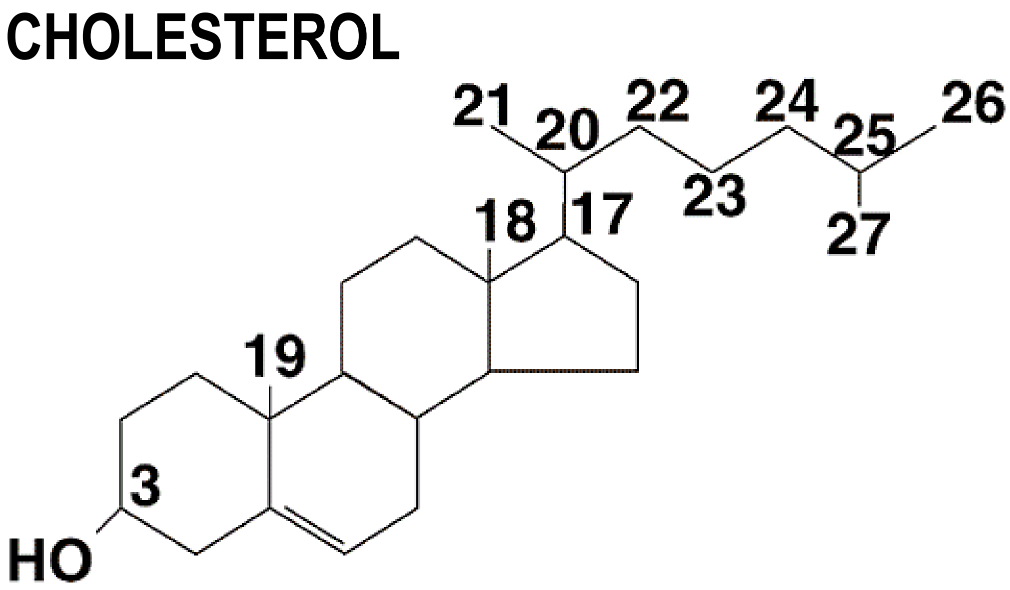
5-α-gonan

(α-configuration at C-5)

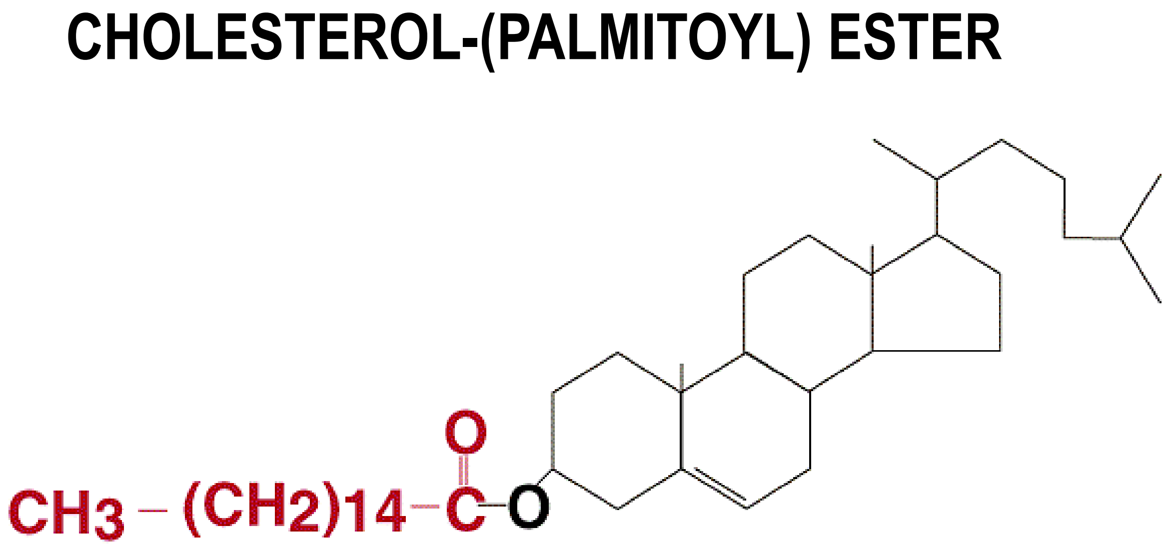




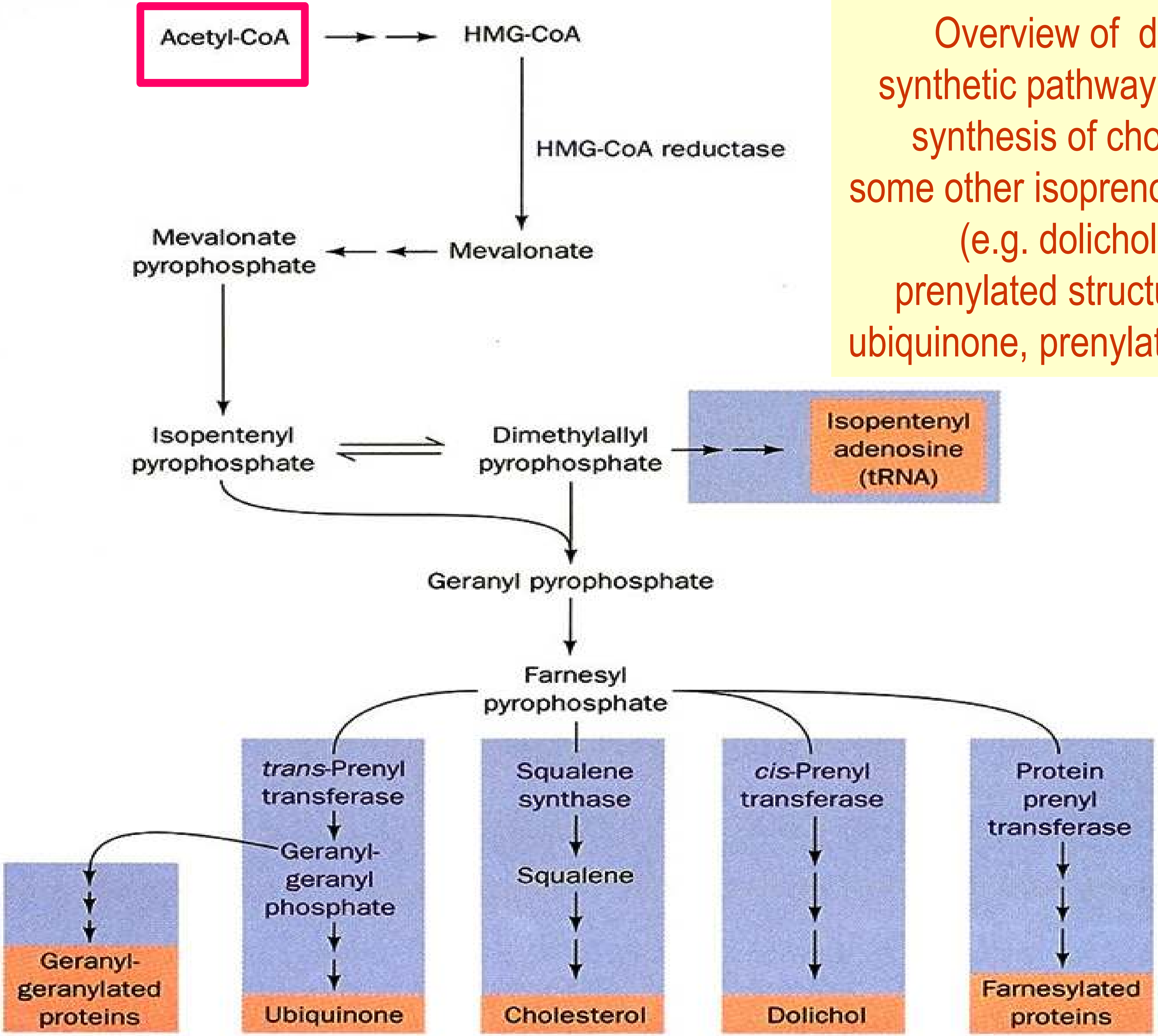
CHOLESTEROL, 5-CHOLESTENE-3β-OL



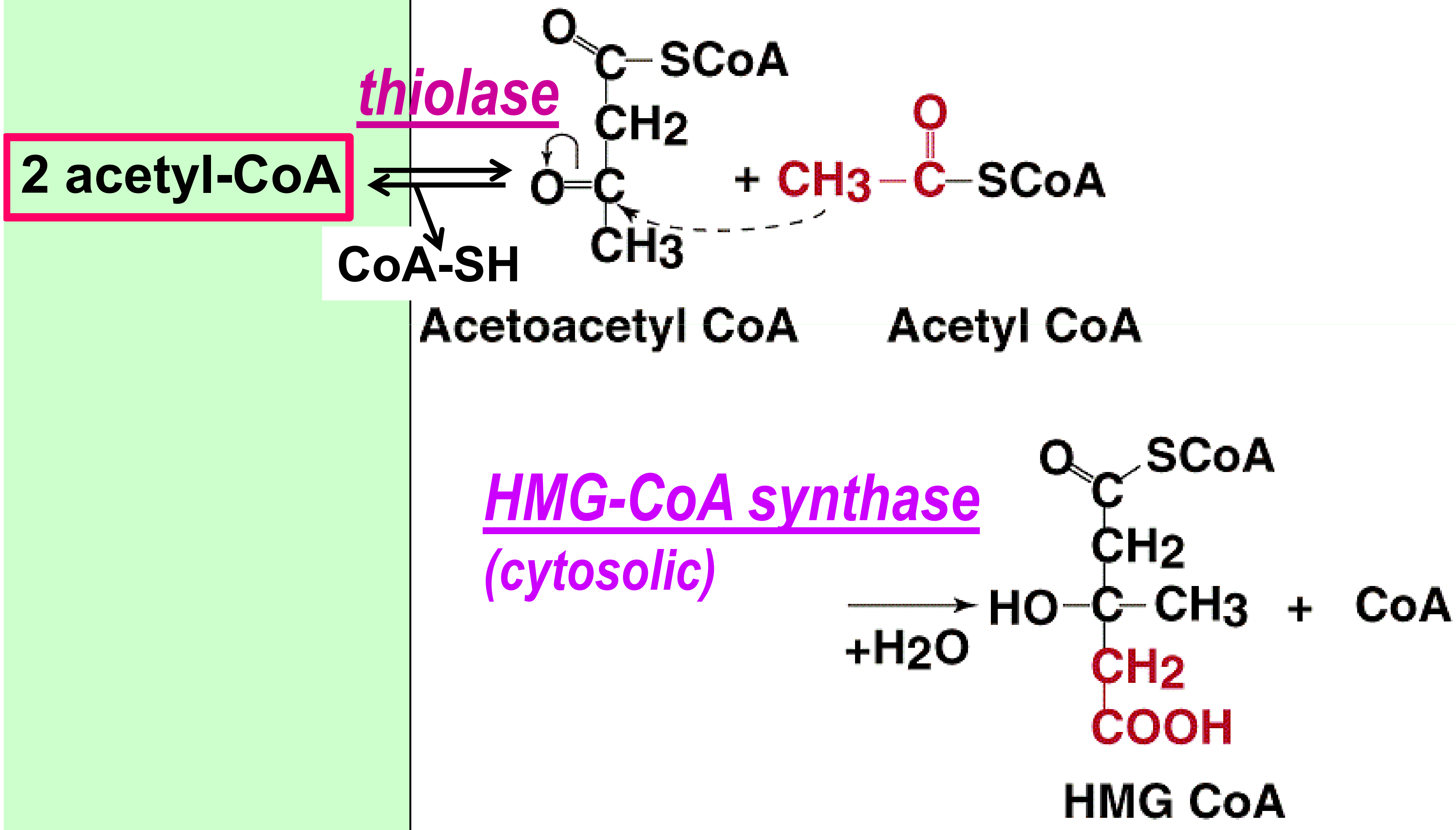
Acyl-CoA-cholesterol-acyl-transferase (ACAT)
- Catalyzes esterification of cholesterol in LIVER

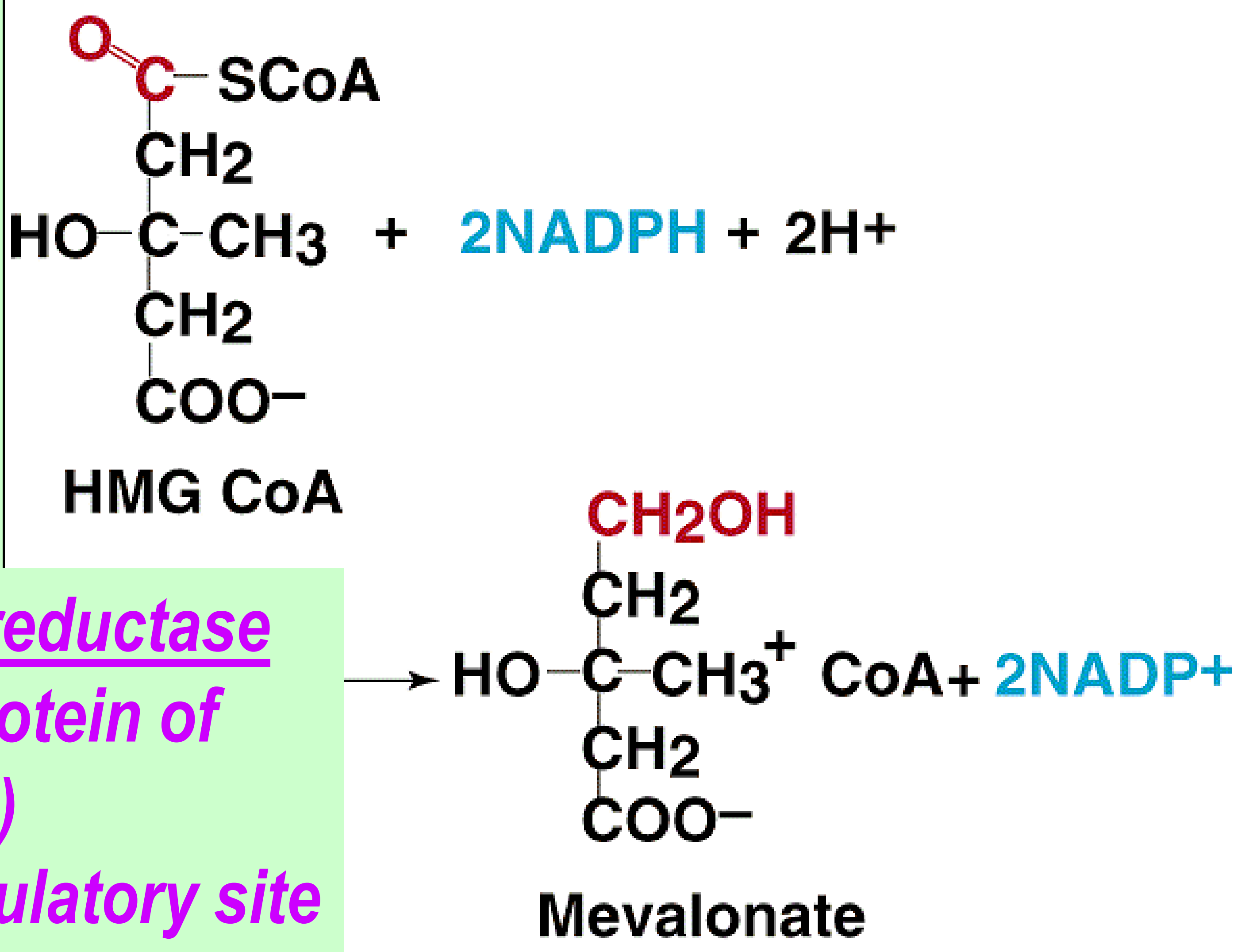


Overview of de novo synthetic pathway leading to synthesis of cholesterol, some other isoprenoid stuctures (e.g. dolichol) and prenylated structures (e.g. ubiquinone, prenylated proteins)

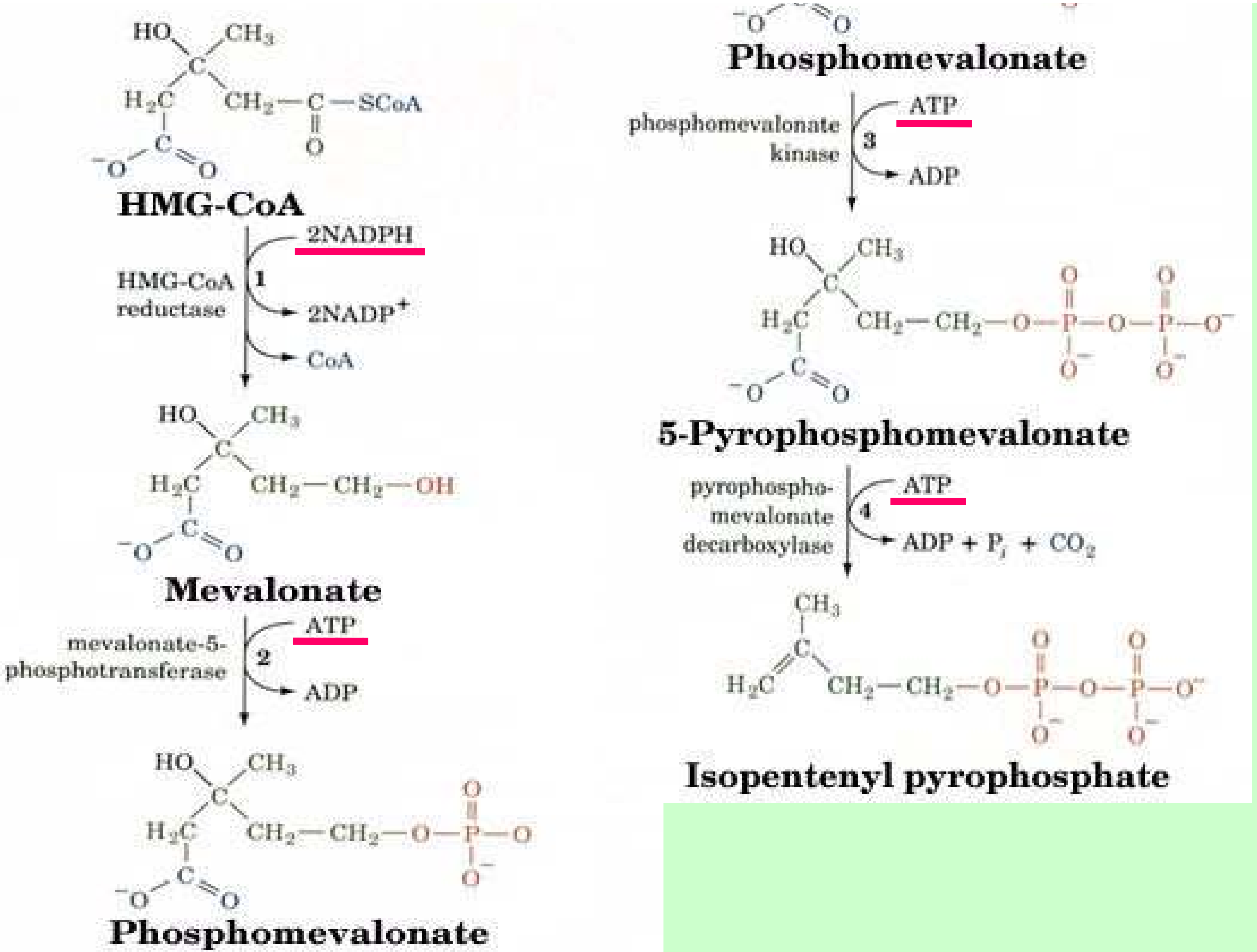


CHOLESTEROL BIOSYNTHESIS

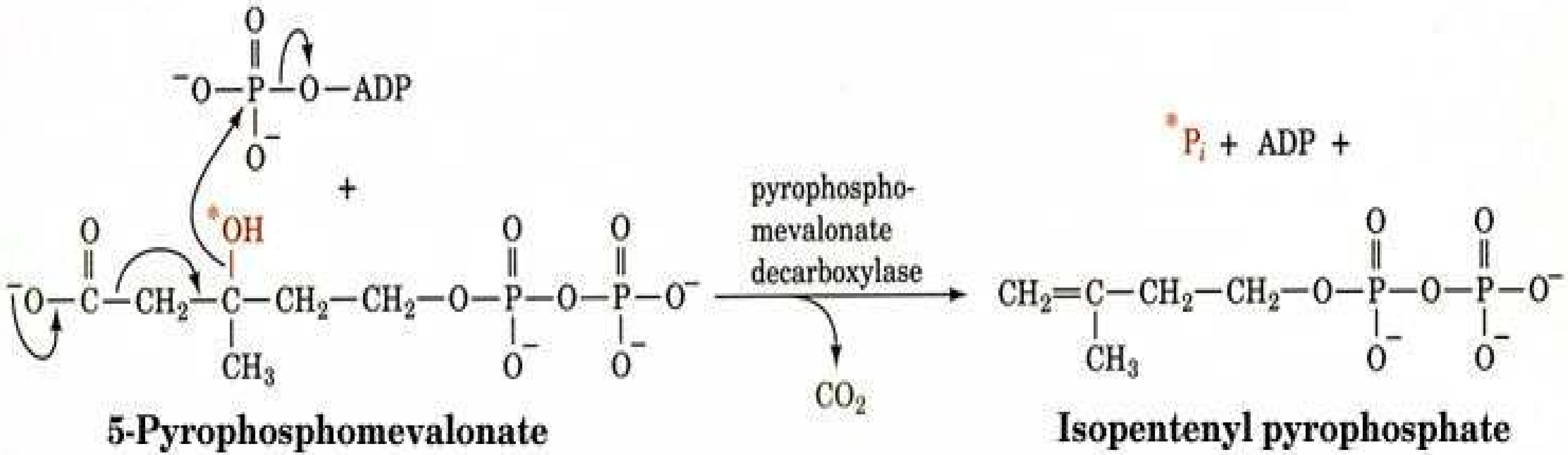




<u>HMG-CoA reductase</u>
(integral protein of smooth ER)
- major regulatory site of the biosynthesis

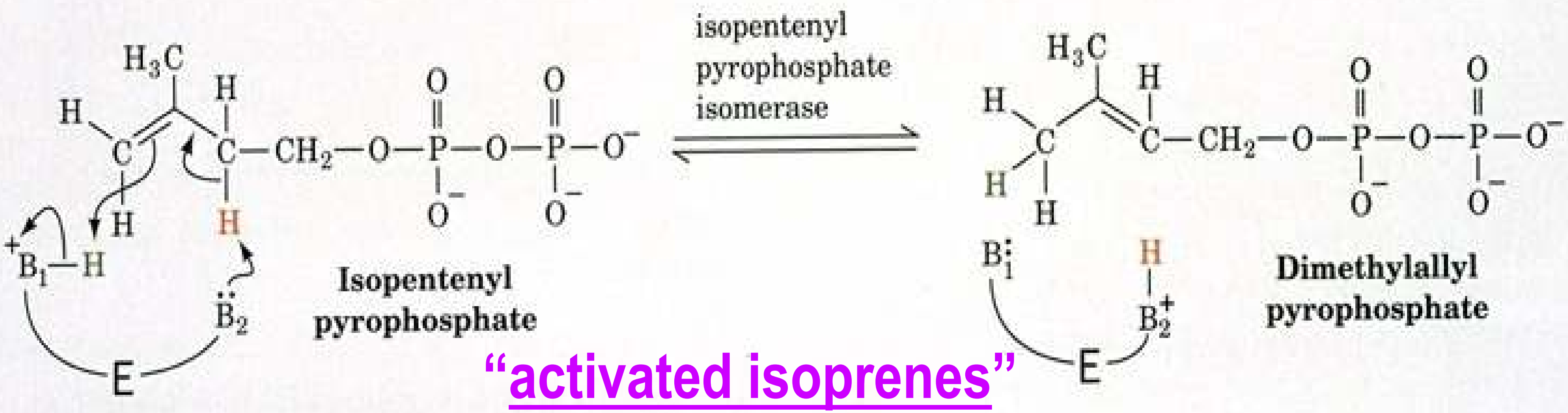

HMG-CoA
2NADPH
HMG-CoA reductase
1
2NADP+
CoA
Mevalonate
ATP
mevalonate-5-phosphotransferase
2
ADP
Phosphomevalonate
Phosphomevalonate
ATP
phosphomevalonate kinase
3
ADP
5-Pyrophosphomevalonate
ATP
pyrophosphomevalonate decarboxylase
4
ADP + Pi + CO2
Isopentenyl pyrophosphate

The enzyme catalyzes the **ATP-dependent** <u>dehydration-decarboxylation</u> reaction:

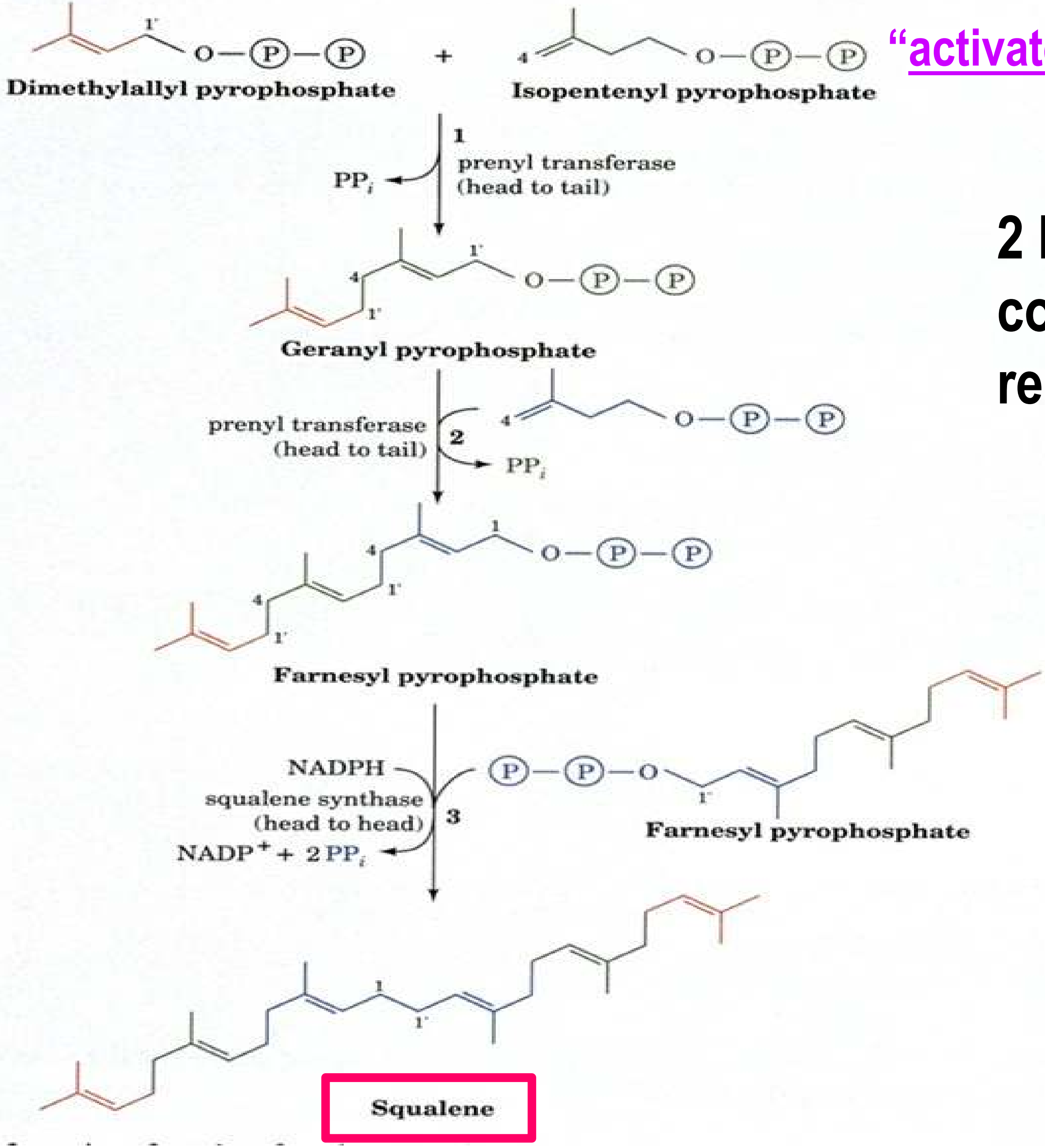


INTERCONVERSION:

The isomerase catalyzes the <u>protonation/deprotonation</u> reaction



"<u>activated isoprenes</u>"

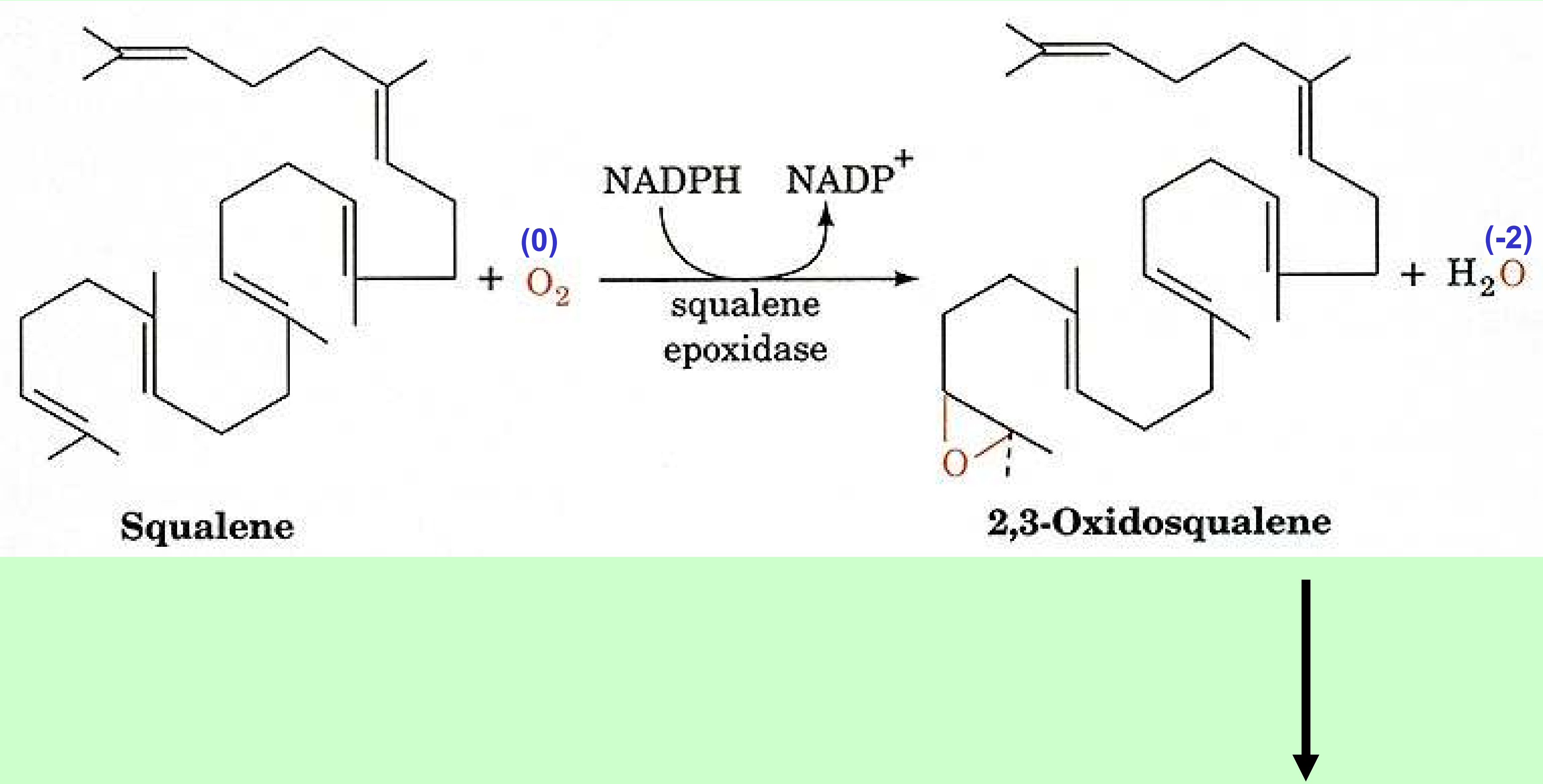


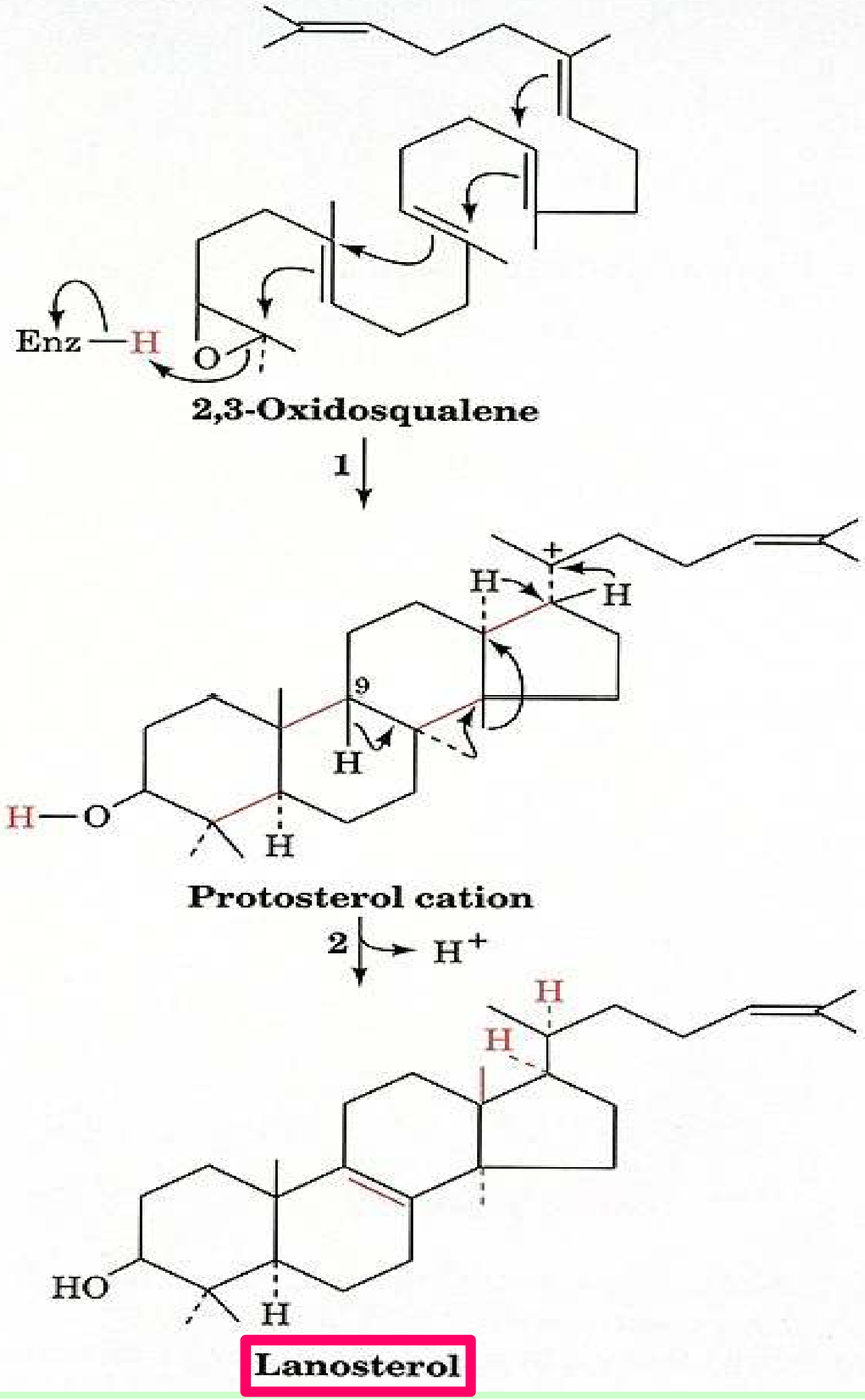
"activated isoprenes"

2 head-to-tail condensation reactions (1,2)

The head-to-head condensation reaction (3)

SQUALENE is converted to LANOSTEROL by CYCLISATION...

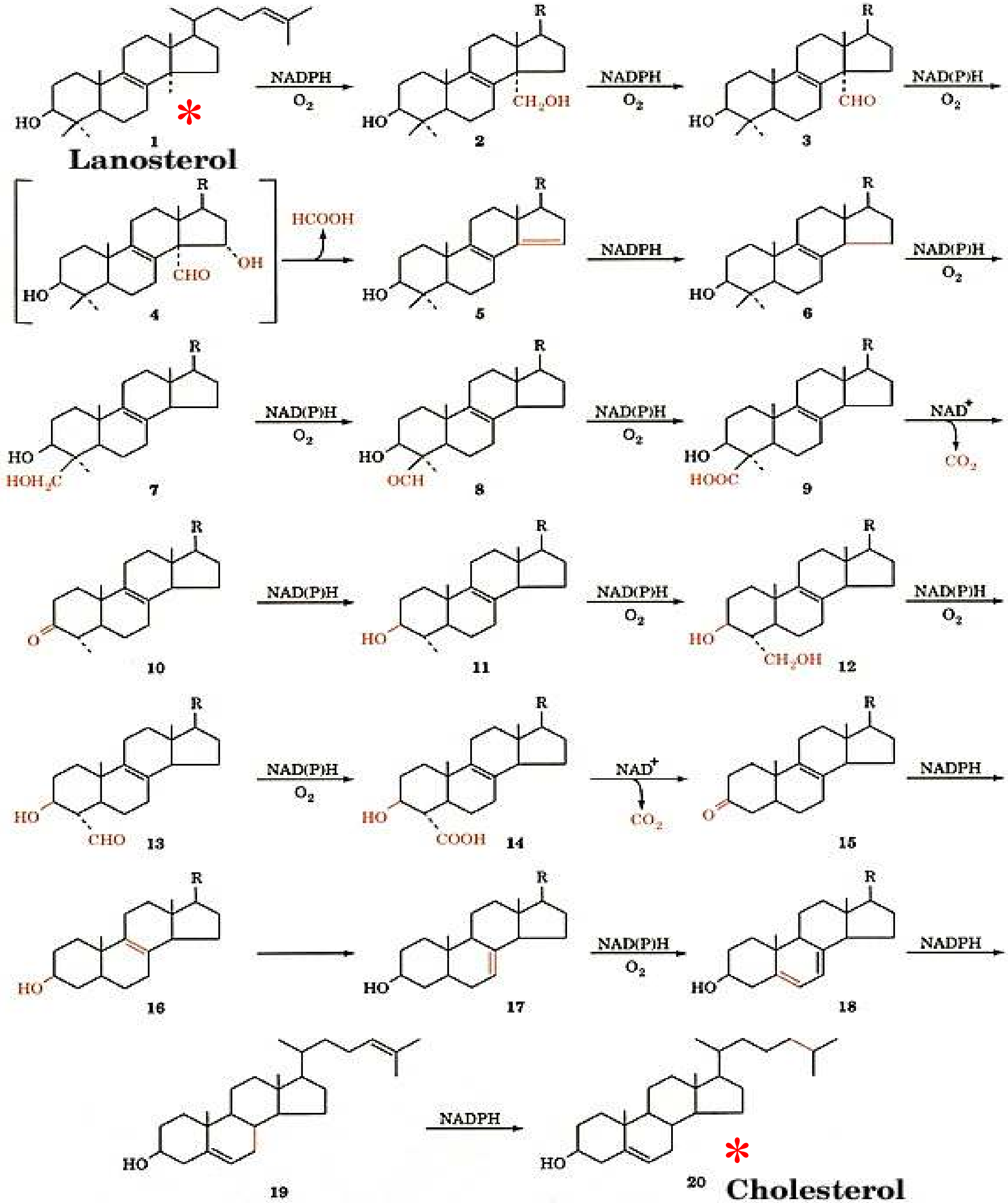




The *SQUALENE-OXIDOCYCLASE* REACTION

1. **PROTONATION of "O"**
   (lack of electrons in the center)

2. **ELIMINATION of PROTON**
   from C9; formation of "=" bond



19 REACTIONS OF LANOSTEROL to CHOLESTEROL CONVERSION.

Enzymes incorporated in the ER membrane

Oxidation and demethylation (3 groups)

For all reactions: NADPH and O_2 required!!!

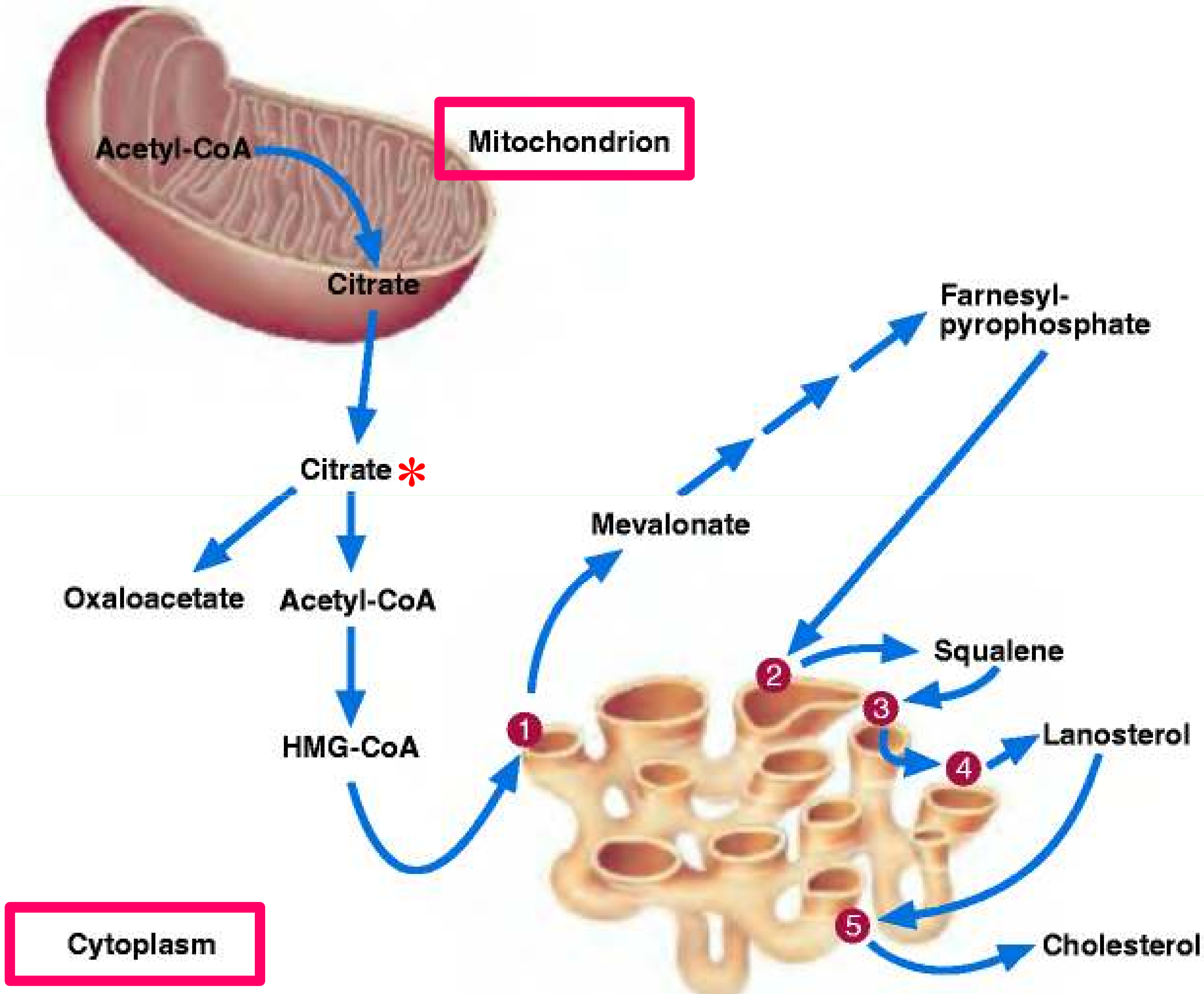
…the reactions are just for information…

Intracellular localization of cholesterol biosyntesis

FIGURE 12.23

Cholesterol Synthesis.

Several reactions occur in the cytoplasm, but most enzymes involved in cholesterol biosynthesis occur within ER membrane. The enzymes are indicated by the following numbers: 1 = HMG-CoA reductase, 2 = Squalene synthase, 3 = Squalene monooxygenase, 4 = 2,3-Oxidosqualene lanosterol cyclase, 5 = Enzymes catalyzing 20 separate reactions. Note that squalene and lanosterol are acted upon by ER membrane enzymes while they are bound to carrier proteins in the cytoplasm.



* Citrate carries acetyl units from mitochondrion into cytosol!!!

ER membrane

REGULATION of cholesterol biosynthesis

Regulation of cholesterol formation balances synthesis with dietary uptake and energy state!!!

Short-term regulation by:

1) AMP-Dependent Protein Kinase

2) Hormones (insulin, glucagon)

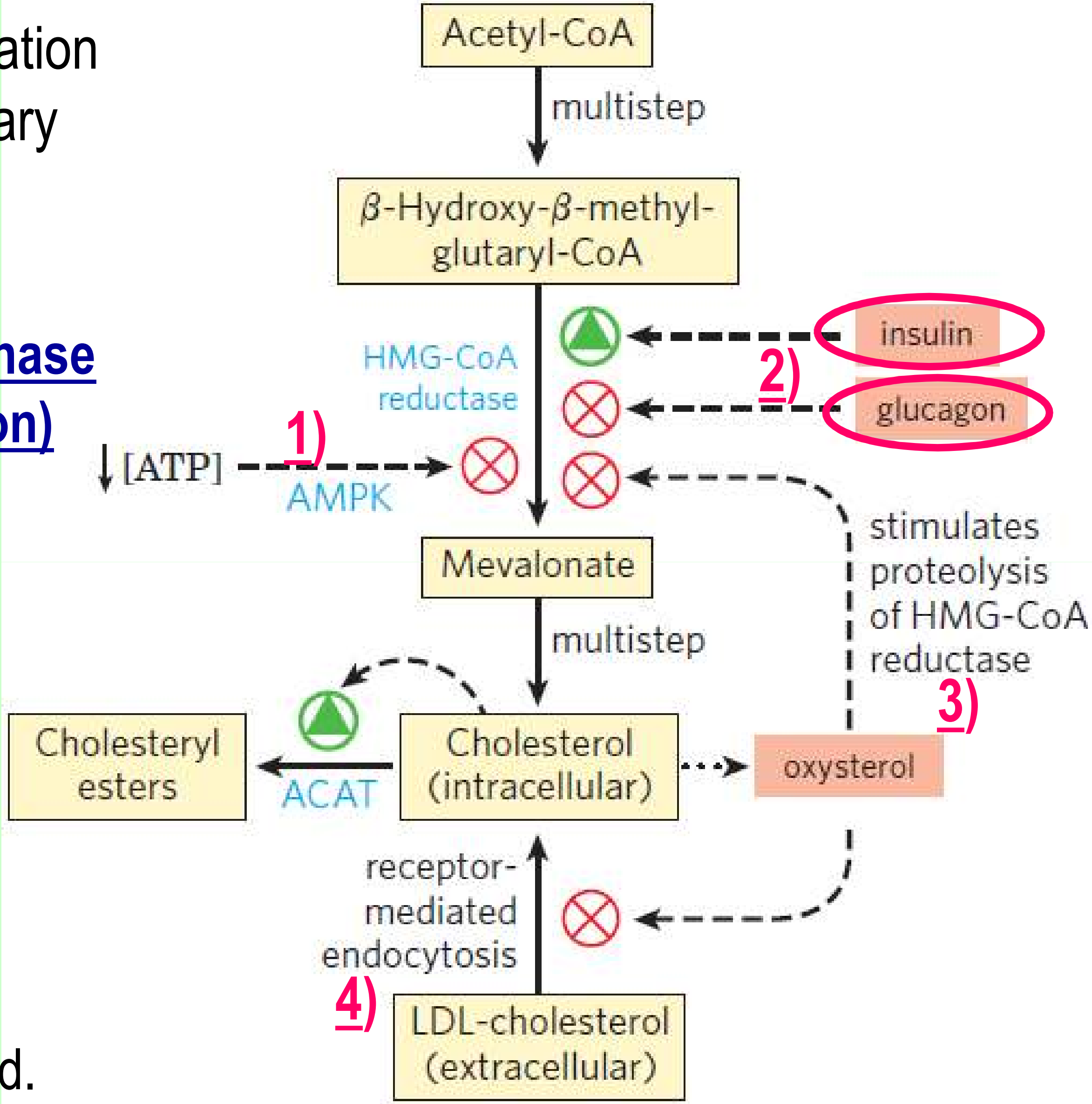
Long-term regulation:

3) by proteolysis

(the enzyme degradation)

4) at the transcription level

(when extracellular cholesterol is taken up by the cells, the genes for synthesis of HMG-Co A reductase and of LDL-receptor are not activated.



Short-term regulation:

1) HMG-CoA Reductase is **inactivated** (**inhibited)** by **phosphorylation**, catalyzed by **AMP-Dependent Protein Kinase, AMPK,**
(also regulates fatty acid synthesis and catabolism).
This **kinase** is active when cellular [AMP] is high, corresponding to when ATP is low.
Thus, when cellular [ATP] is low, energy is not expended in synthesizing cholesterol.

2) **Insulin** promotes **dephosphorylation (activation)** of HMG-CoA reductase; **glucagon** promotes its **phosphorylation (inactivation);**

Long-term regulation is by varied **formation** and **degradation** of HMG-CoA reductase and other enzymes of the pathway for synthesis of cholesterol.

3) Regulated proteolysis of HMG-CoA Reductase:

Degradation of HMG-CoA Reductase is **stimulated by** cholesterol, oxidized derivatives of cholesterol (oxysterol or 24(S)-hydroxycholesterol), mevalonate, farnesol (dephosphorylated farnesyl pyrophosphate), and probably bile acids.

HMG-CoA Reductase includes a transmembrane **sterol-sensing domain** that has a role in activating degradation of the enzyme *via* the proteasome.

4) Regulated transcription:

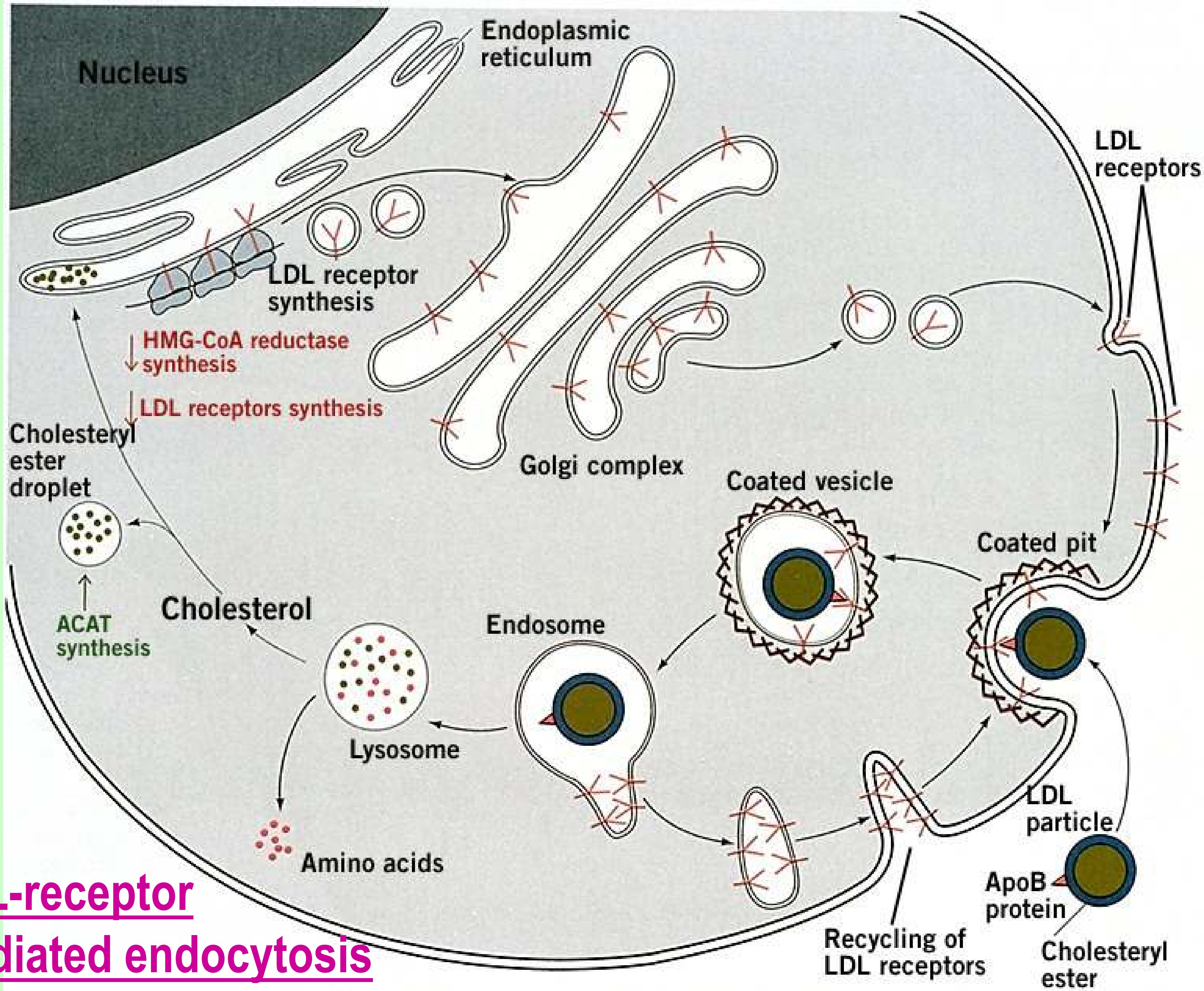
A family of transcription factors designated **SREBP** (sterol regulatory element binding proteins) regulate synthesis of cholesterol and fatty acids.

Of these, **SREBP-2** mainly **regulates cholesterol synthesis**.

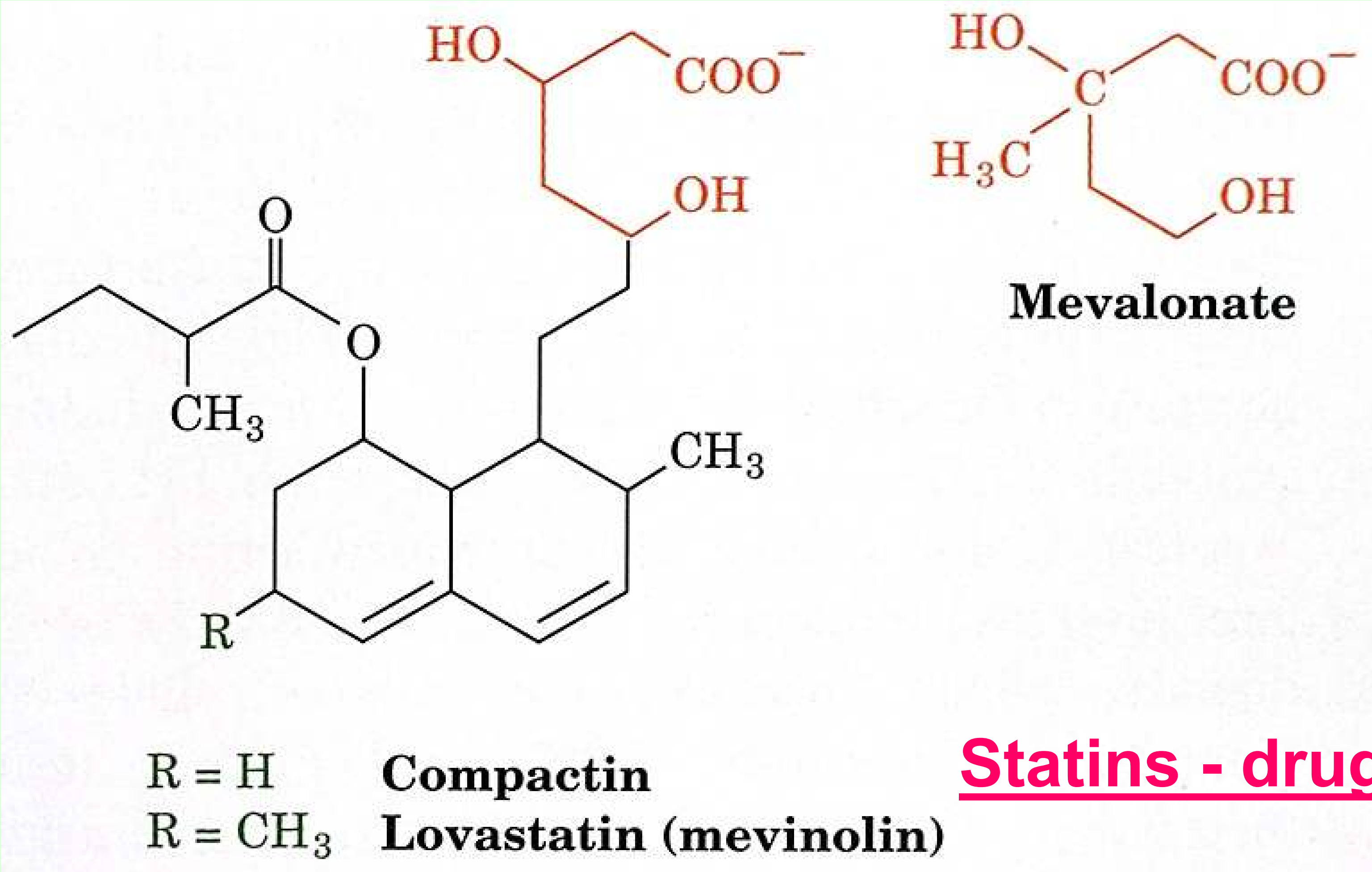
(SREBP-1c mainly regulates fatty acid synthesis.)

When sterol concentrations are **low**, **SREBP-2** is **released** by cleavage of a membrane-bound precursor protein.

SREBP-2 **activates transcription** of genes for **HMG-CoA Reductase** and other enzymes of the pathway for cholesterol synthesis.

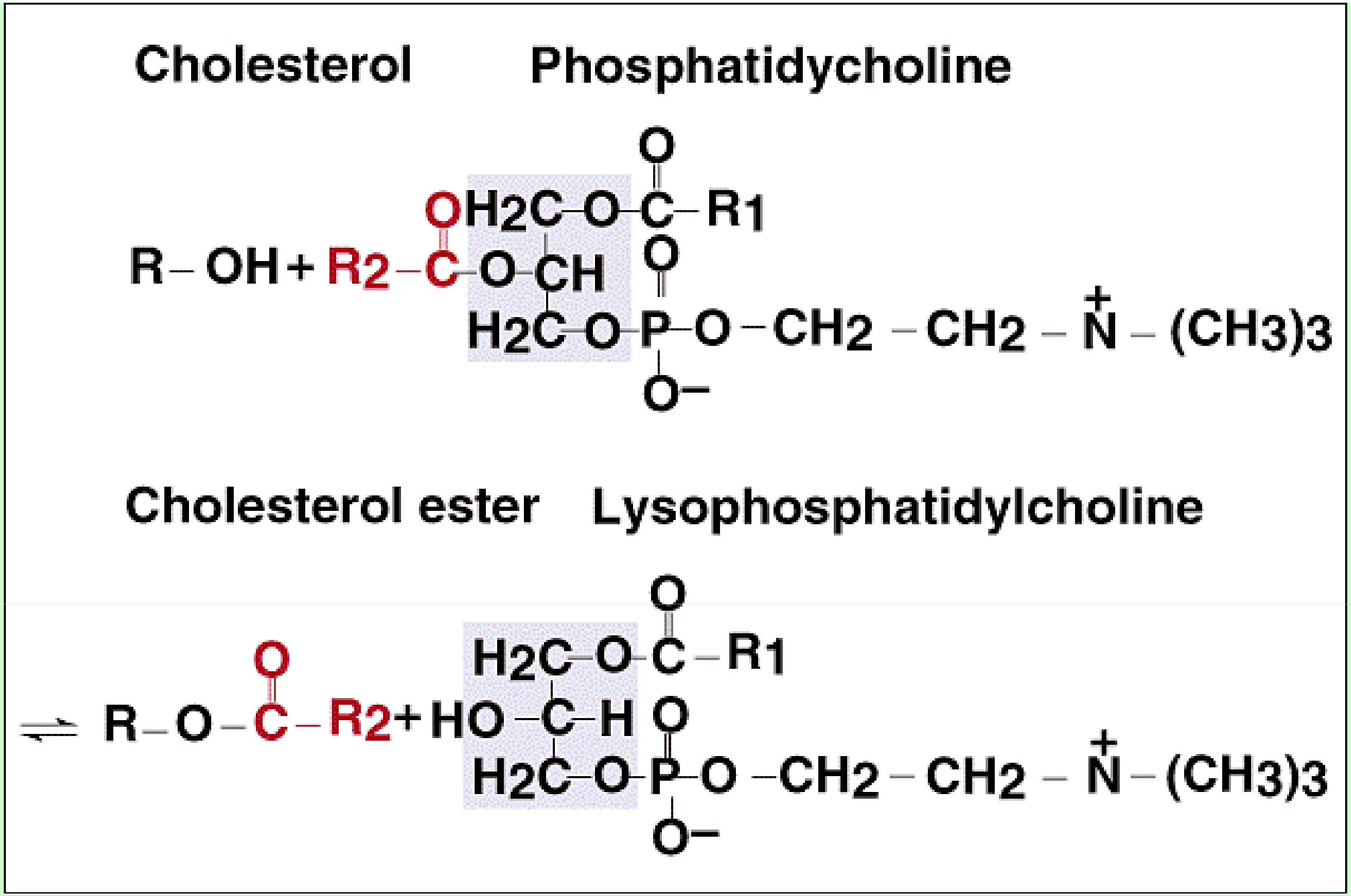


LDL-receptor mediated endocytosis

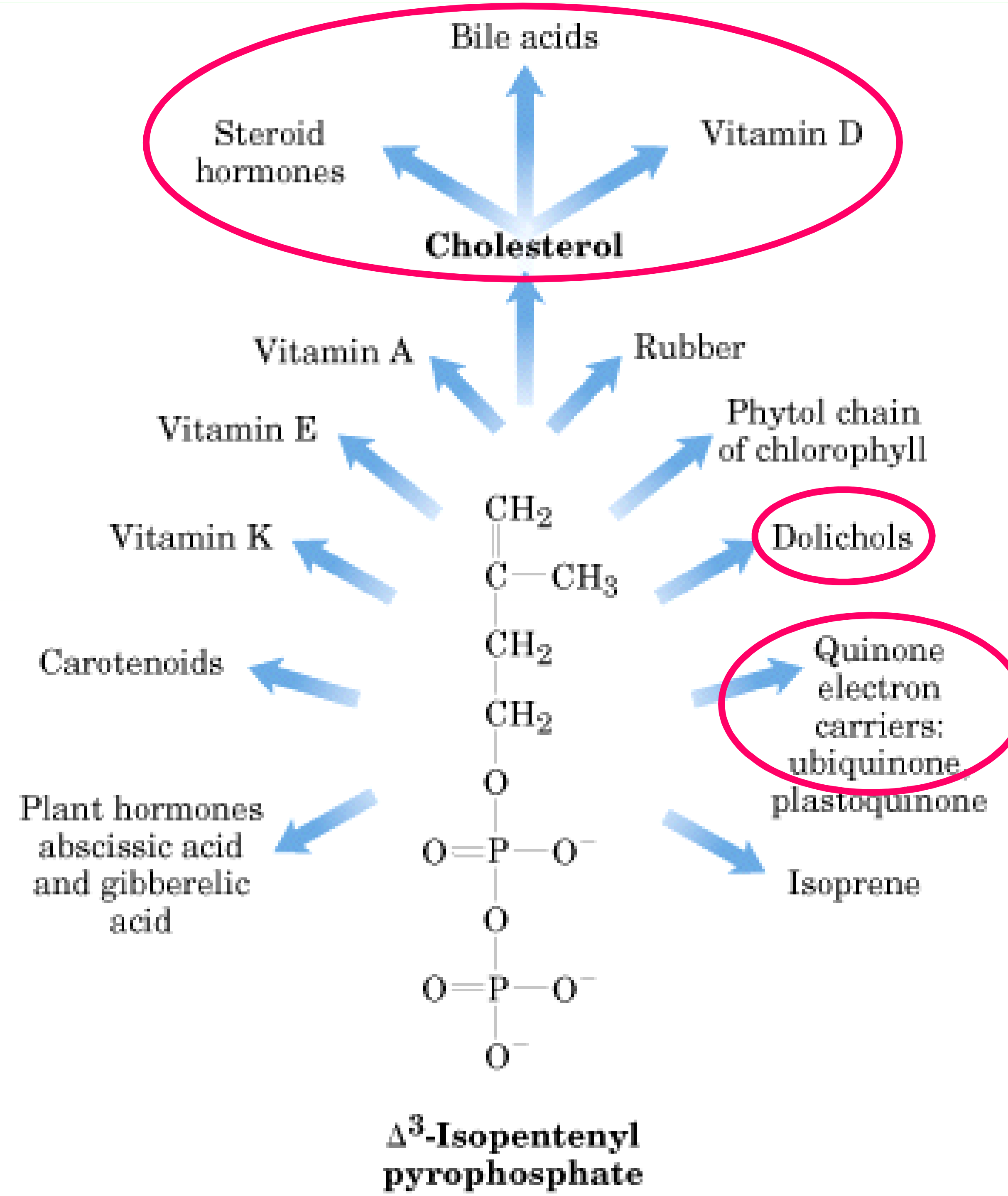


Statins - drugs

INHIBITORS of HMG-CoA reductase (competitive inhibitors due to similarity with mevalonate).

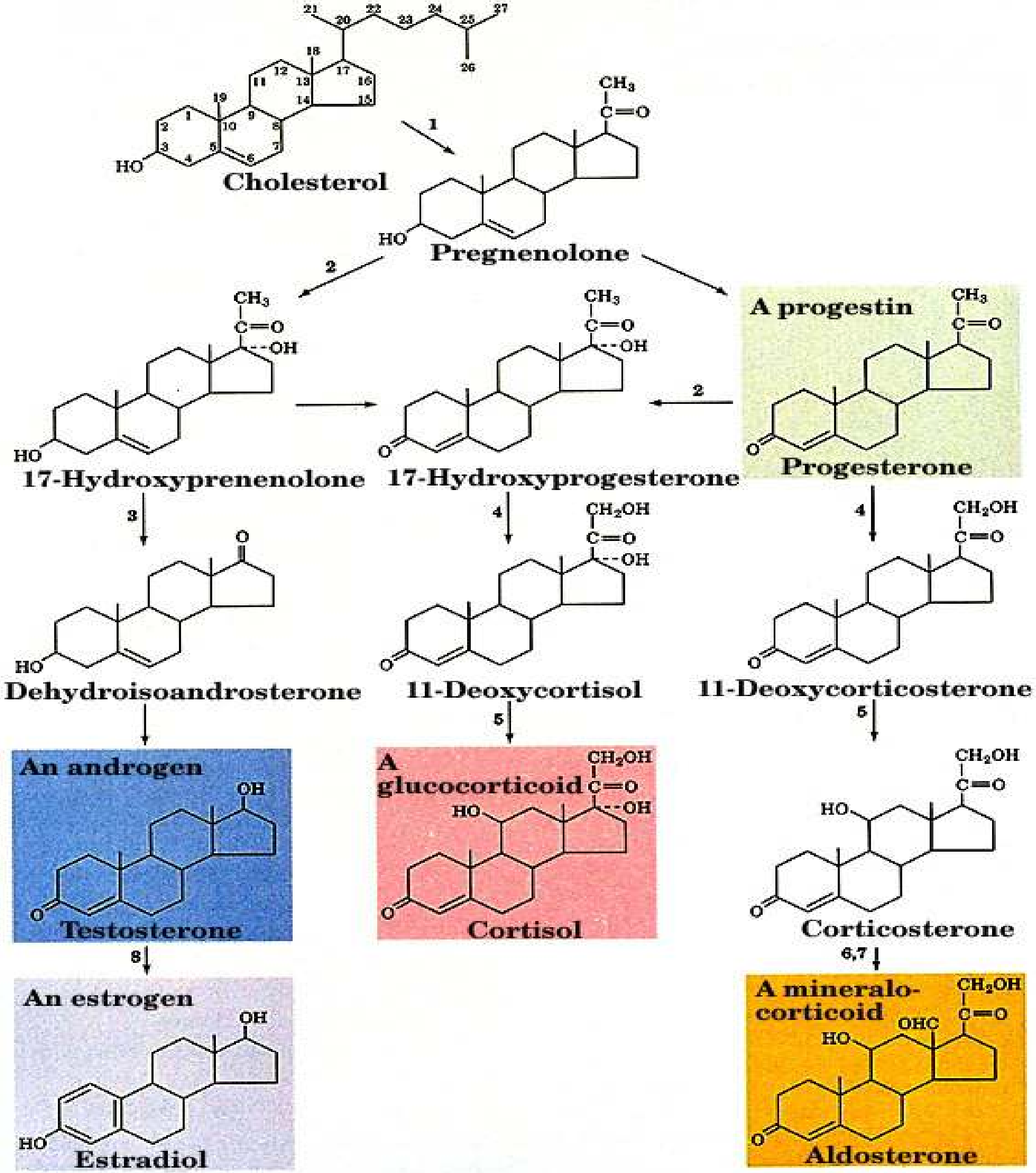


Conversion of cholesterol into cholesterol-esters by the
<u>LECITHIN:CHOLESTEROL ACYL TRANSFERASE (LCAT),</u>
enzyme in <u>plasma</u> bound to HDL (synthesized by the liver, mostly)
- the cholestrol-esters diffuse into the core of HDL.

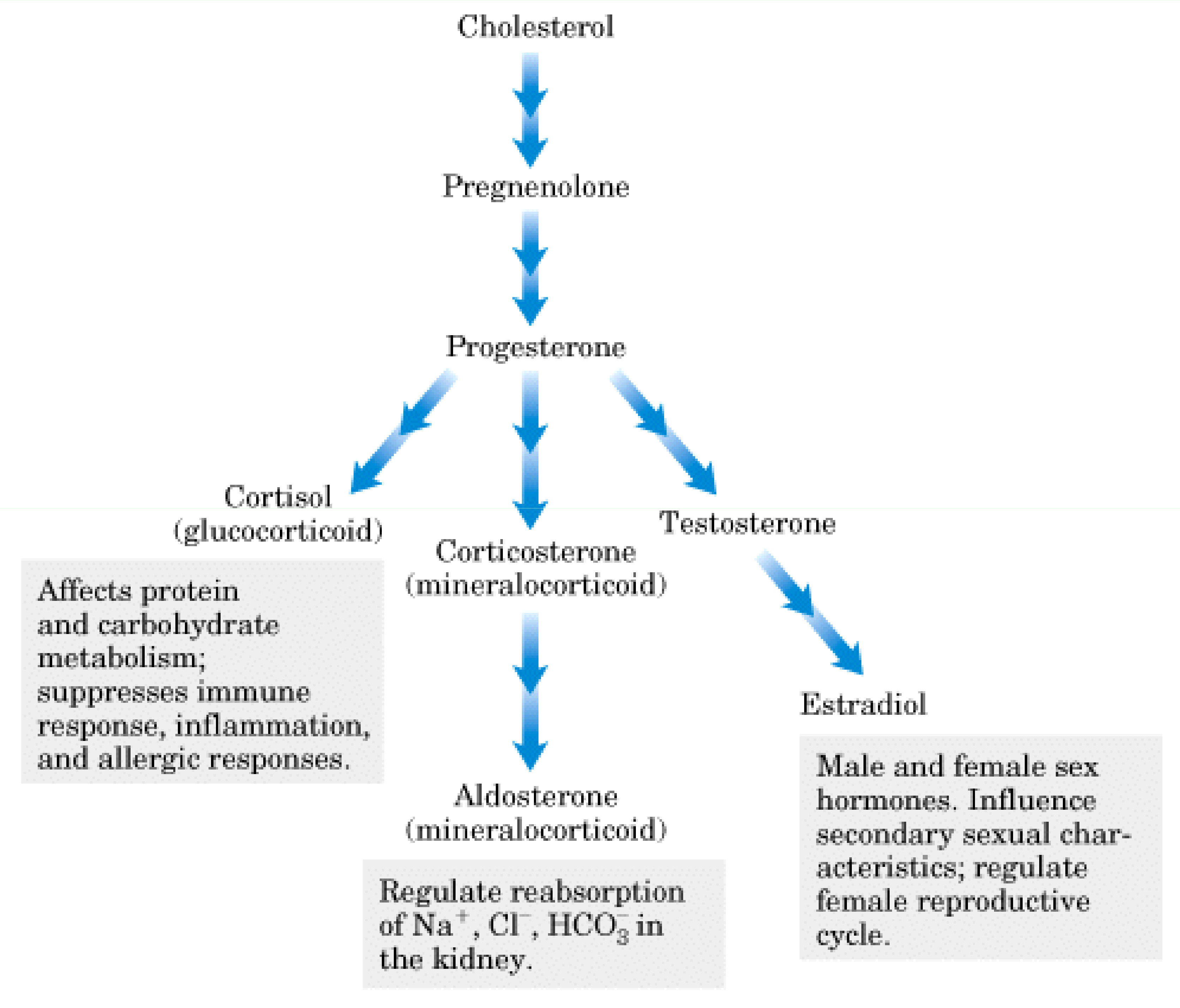

Bile acids
Steroid hormones
Vitamin D
Cholesterol
Vitamin A
Rubber
Vitamin E
Phytol chain of chlorophyll
Vitamin K
Dolichols
Carotenoids
Quinone electron carriers: ubiquinone, plastoquinone
Plant hormones abscissic acid and gibberelic acid
Isoprene
CH_2
C
CH_3
CH_2
CH_2
O
O=P—O⁻
O
O=P—O⁻
O⁻
Δ^3-Isopentenyl pyrophosphate

In humans…

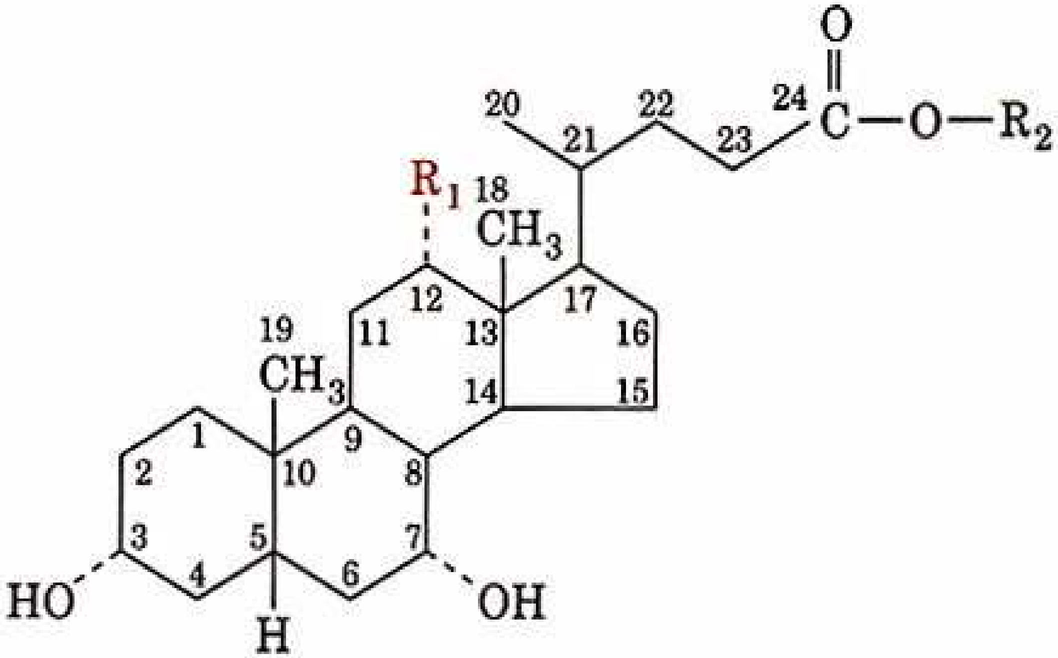
HORMONES, synthesis



1. Enzyme for the side chain cleavage
2. Steroid-C17-hydroxylase
3. Steroid-C17,C20-lyase
4. Steroid-C21-hydroxylase
5. Steroid-11β-hydroxylase
6. Steroid-C18-hydroxylase
7. 18-hydroxysteroid-oxidase
8. aromathase


Cholesterol
Pregnenolone
Progesterone
Cortisol
(glucocorticoid)
Affects protein
and carbohydrate
metabolism;
suppresses immune
response, inflammation,
and allergic responses.
Corticosterone
(mineralocorticoid)
Aldosterone
(mineralocorticoid)
Regulate reabsorption
of Na^+, Cl^-, HCO_3^- in
the kidney.
Testosterone
Estradiol
Male and female sex
hormones. Influence
secondary sexual char-
acteristics; regulate
female reproductive
cycle.

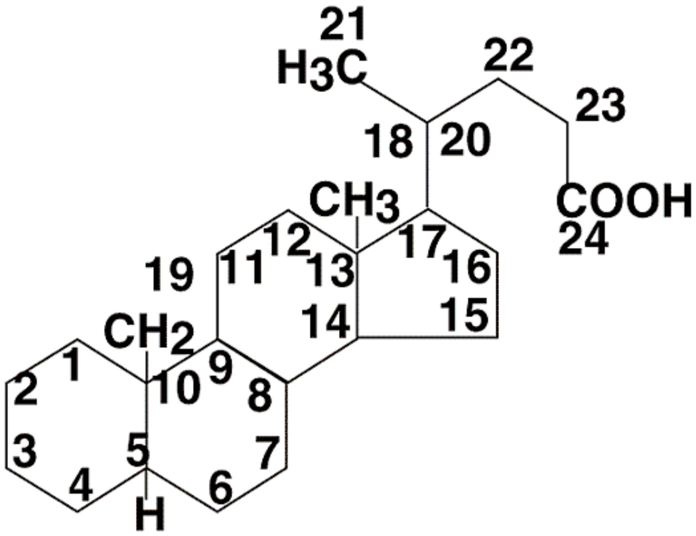
Bile acids

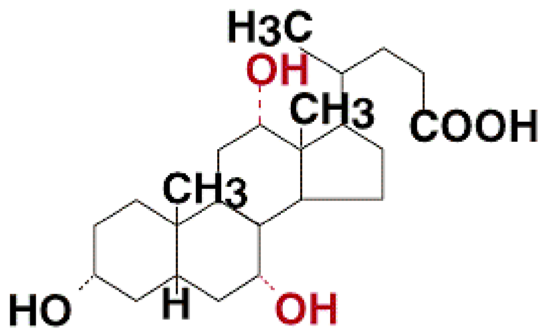


	$R_1 = OH$	$R_1 = H$
$R_2 = H$	**Cholic acid**	**Chenodeoxycholic acid**
$R_2 = NH—CH_2—COOH$	**Glycocholic acid**	**Glycochenodeoxycholic acid**
$R_2 = NH—CH_2—CH_2—SO_3H$	**Taurocholic acid**	**Taurochenodeoxycholic acid**

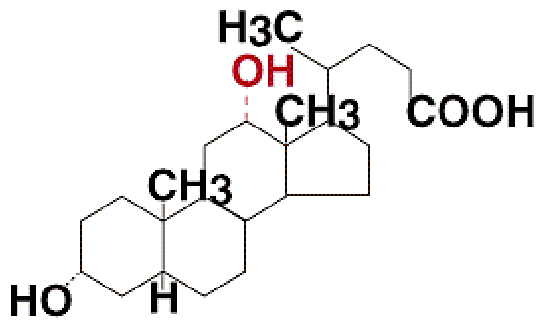
Bile acids

Cholanic acid

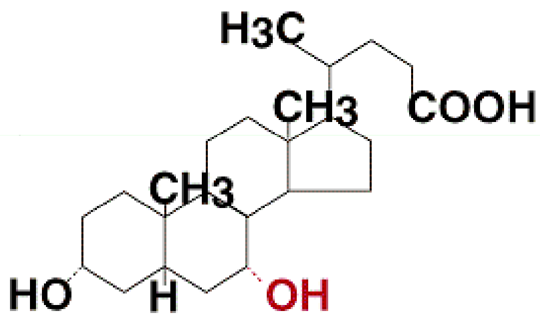




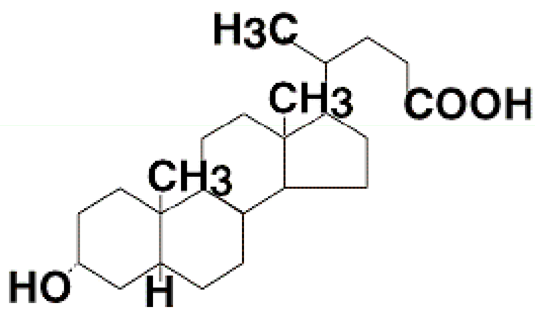
Cholic acid



Deoxycholic acid



Chenodeoxycholic acid



Lithocholic acid

CHOLANIC ACID DERIVATIVES

The primary bile acids are synthesized in the LIVER from cholesterol. The first synthesized are cholic acid (found in the largest amount) and chenodeoxycholic acid.

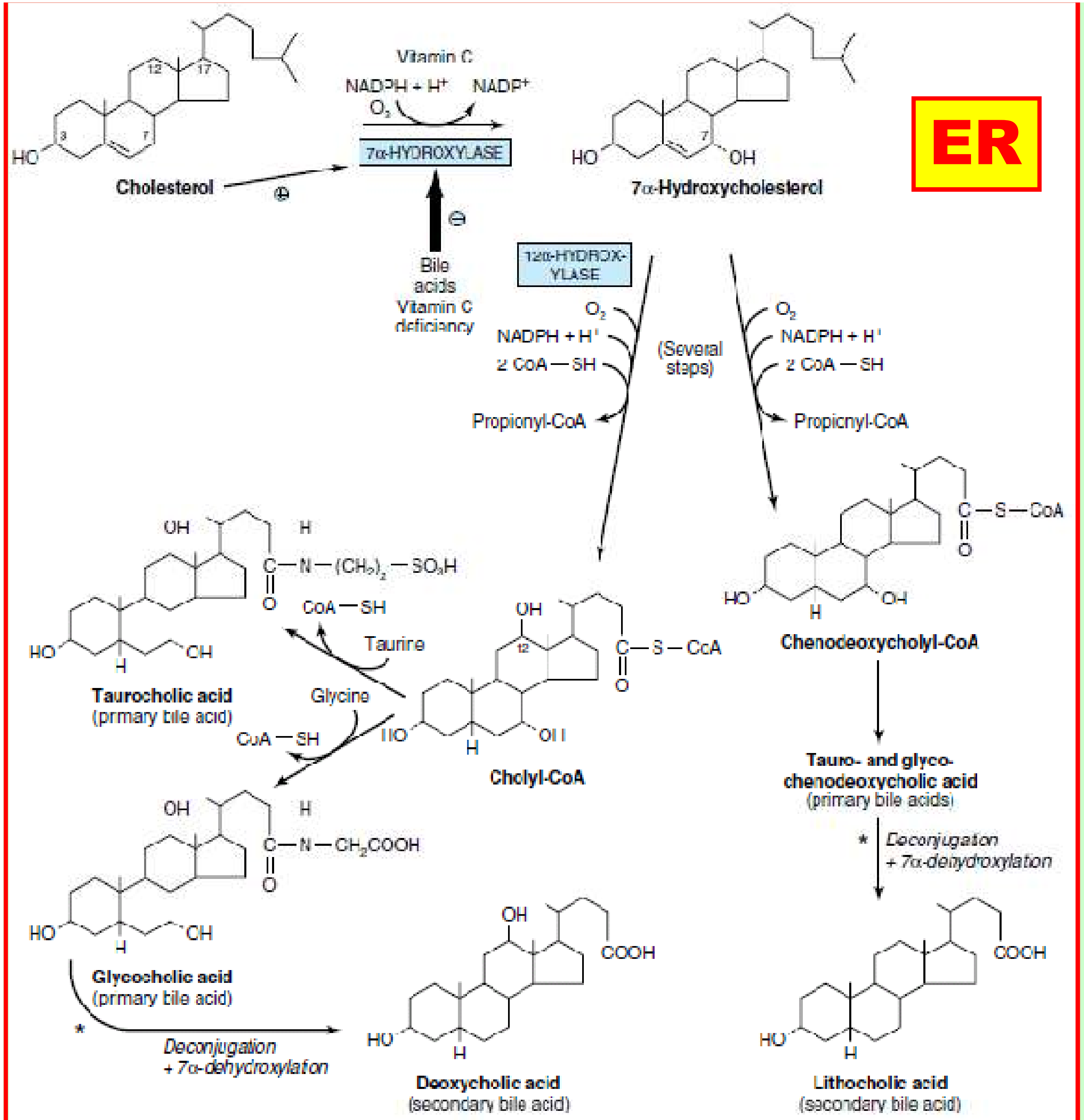


Figure 26-7. Biosynthesis and degradation of bile acids. A second pathway in mitochondria involves hydroxylation of cholesterol by sterol 27-hydroxylase. Asterisk: Catalyzed by microbial enzymes.

The 7α-hydroxylation of cholesterol is the first and principal **regulatory step** in the biosynthesis of bile acids catalyzed by **7-hydroxylase, a microsomal enzyme**. (A typical monooxygenase, it requires oxygen, NADPH, and cytochrome P450.)

Subsequent **hydroxylation steps** are also catalyzed by monooxygenases.

The pathway of bile acid biosynthesis divides early into one subpathway leading to **cholyl-CoA,** characterized by an extra *α*-OH group on position 12, and another pathway leading to **chenodeoxycholyl-CoA.**

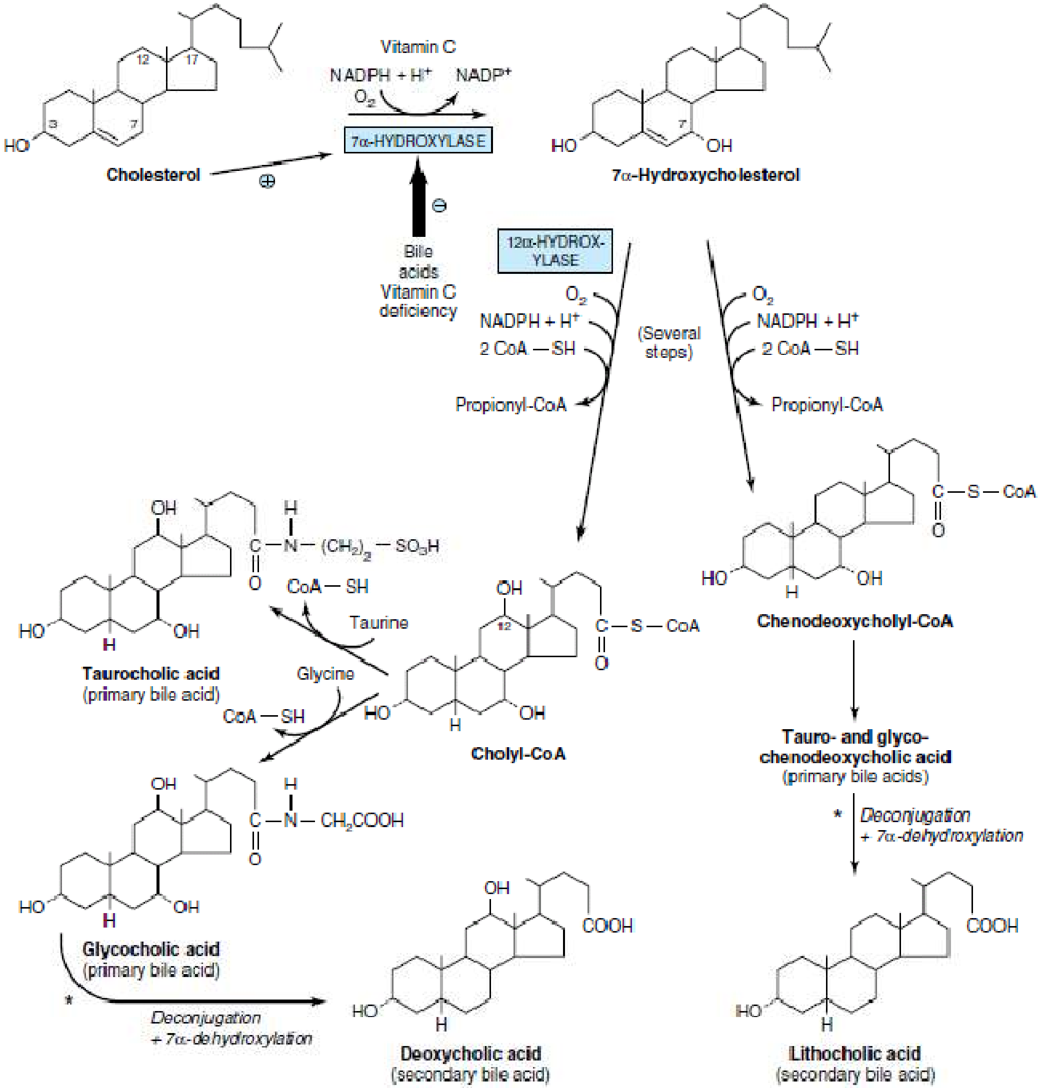


Figure 26–7. Biosynthesis and degradation of bile acids. A second pathway in mitochondria involves hydroxylation of cholesterol by sterol 27-hydroxylase. Asterisk: Catalyzed by microbial enzymes.

A **second pathway in mitochondria** involving the 27-hydroxylation of cholesterol by **sterol 27-hydroxylase** as the first step is responsible for a significant proportion of the primary bile acids synthesized.

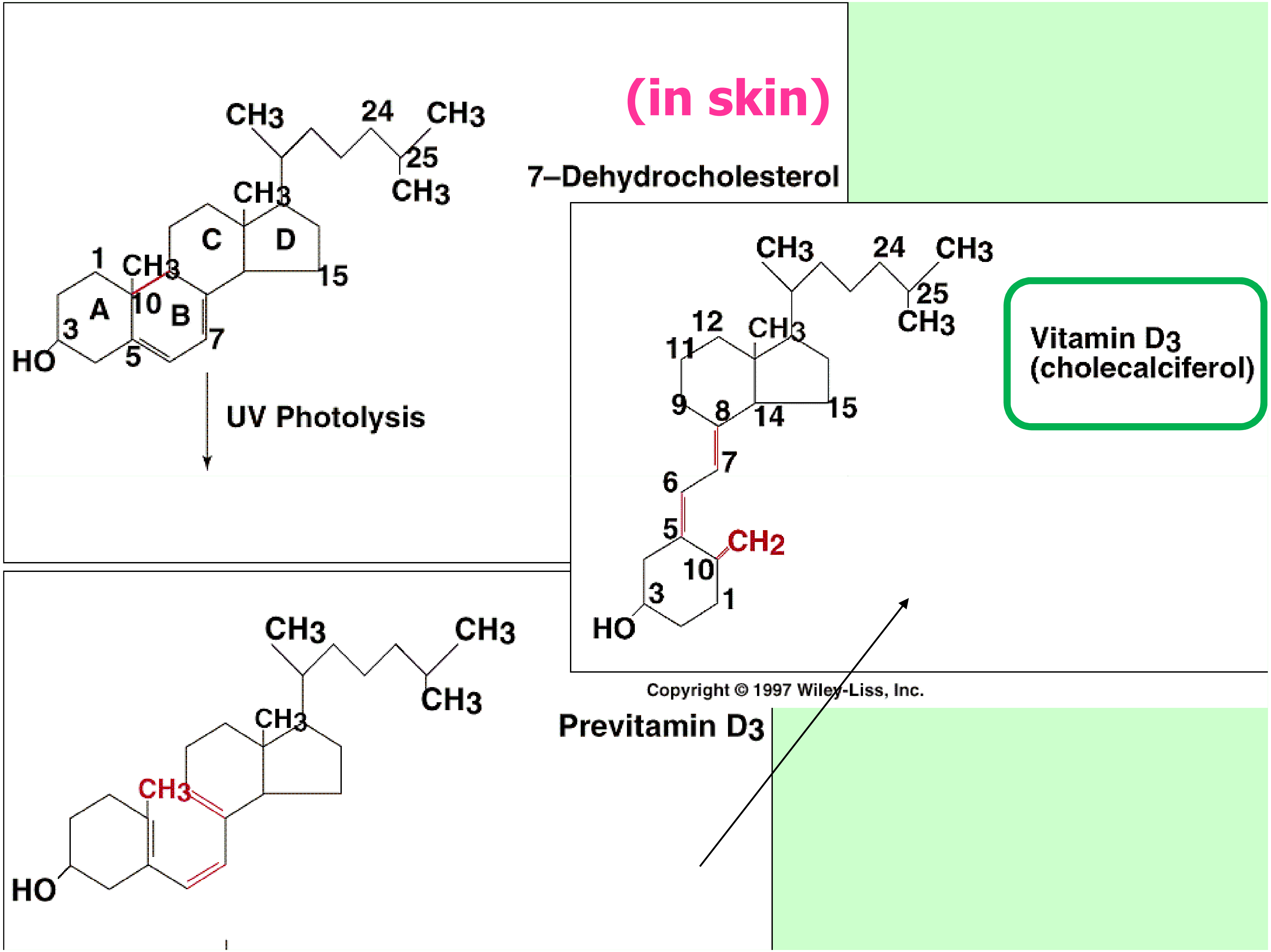
The primary bile acids **enter the bile as glycine or taurine conjugates**.

Conjugation takes place in peroxisomes.

In humans, the ratio of the glycine to the taurine conjugates is normally 3:1.

(In the alkaline bile, the bile acids and their conjugates are assumed to be in a salt form — hence the term “bile salts.”)

A portion of the primary bile acids **in the intestine** is subjected to further changes by **the activity of the intestinal bacteria**. These include deconjugation and 7α-dehydroxylation, which produce the **secondary bile acids**, deoxycholic acid and lithocholic acid.


(in skin)
7–Dehydrocholesterol
UV Photolysis
Previtamin D3
Vitamin D3
(cholecalciferol)
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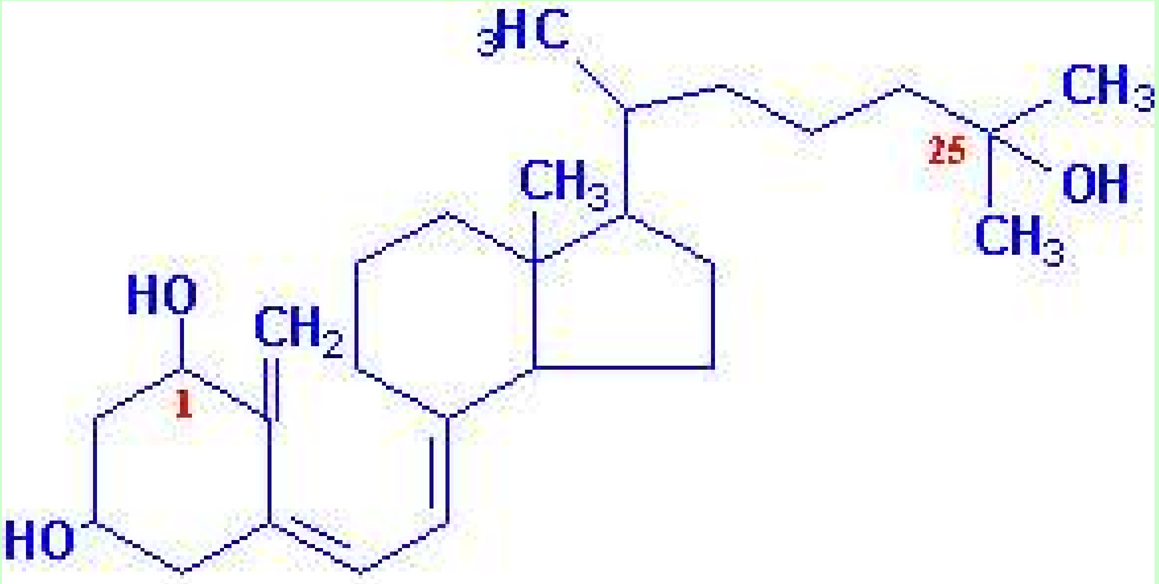
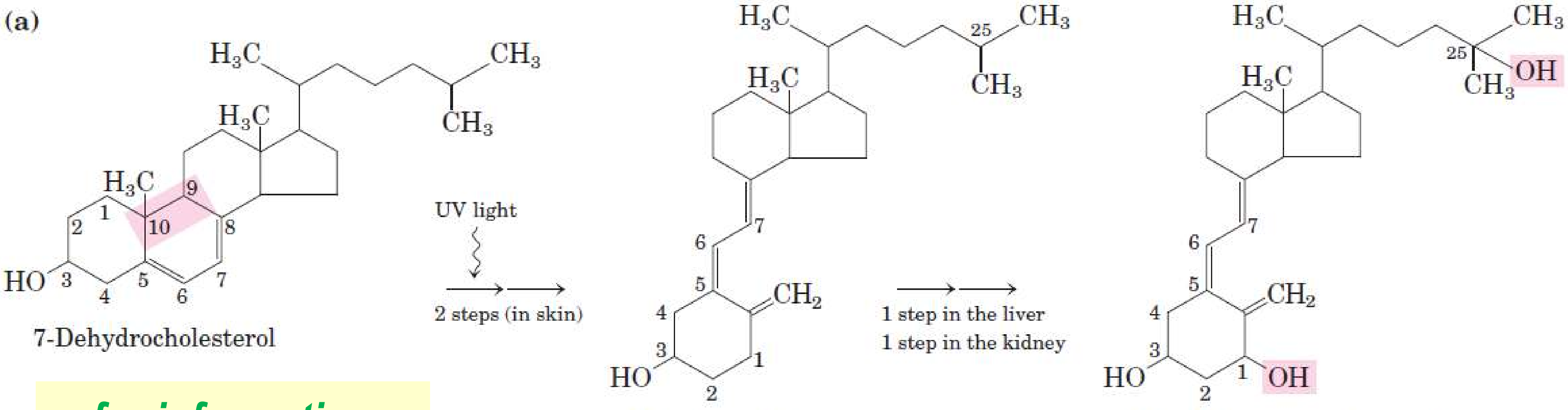
..... Hydroxylation at C25 and C1:



1,25-dihydroxyvitamin D_3 (calcitriol; hormon)

- Hydroxylation at C25: in liver
- Hydroxylation at C1: in kidney, bones and placenta

(a)



7-Dehydrocholesterol

Cholecalciferol (vitamin D_3)

1,25-Dihydroxycholecalciferol
(1,25-dihydroxyvitamin D_3)

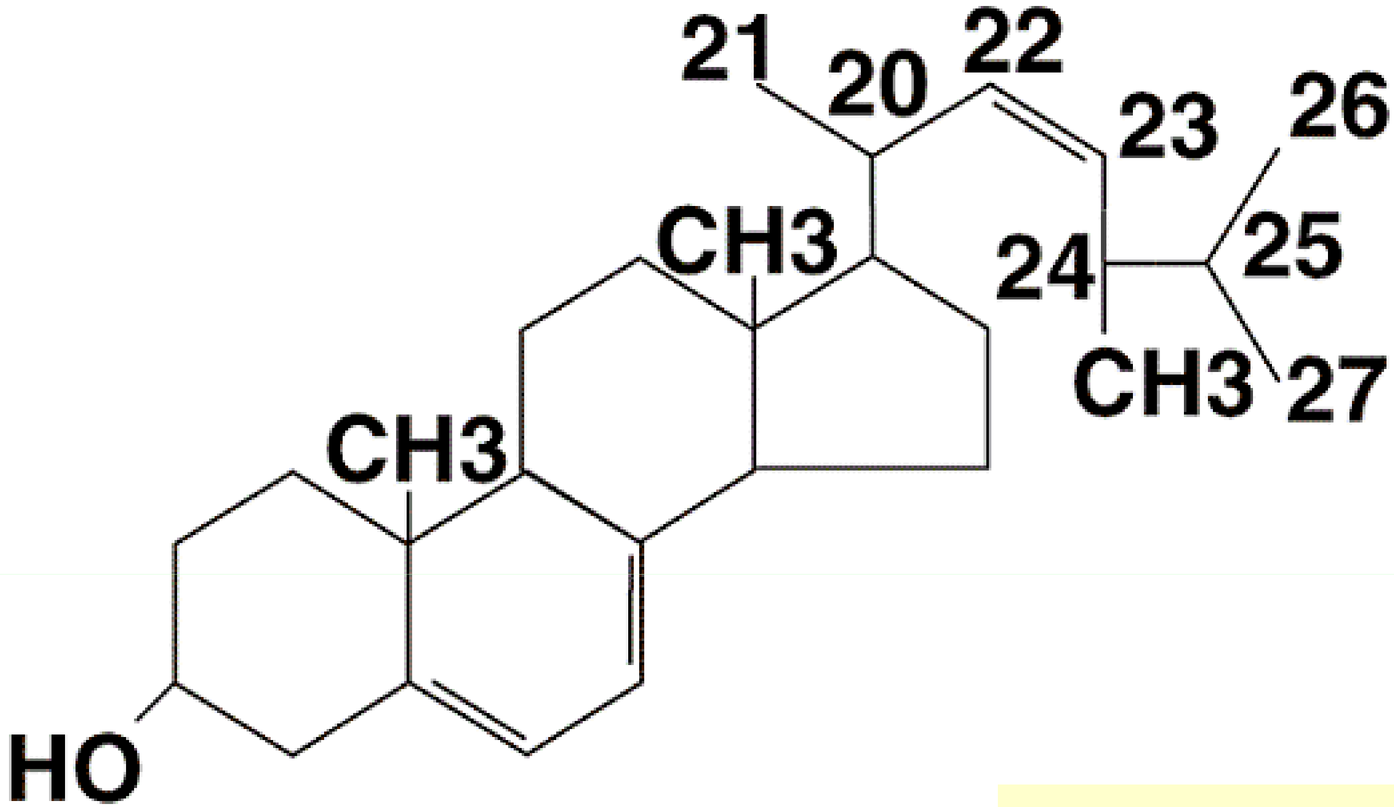
…for information…

FIGURE 10–20 Vitamin D_3 production and metabolism. (a) Cholecalciferol (vitamin D_3) is produced in the skin by UV irradiation of 7-dehydrocholesterol, which breaks the bond shaded pink. In the liver, a hydroxyl group is added at C-25 (pink); in the kidney, a second hydroxylation at C-1 (pink) produces the active hormone, 1,25-dihydroxycholecalciferol. This hormone regulates the metabolism of Ca^{2+} in kidney, intestine, and bone. **(b)** Dietary vitamin D prevents rickets, a disease once common in cold climates where heavy clothing blocks the UV component of sunlight necessary for the production of vitamin D_3 in skin. On the left is a 2½-year-old boy with severe rickets; on the right, the same boy at age 5, after 14 months of vitamin D therapy.

Before vitamin D treatment

After 14 months of vitamin D treatment

(b)

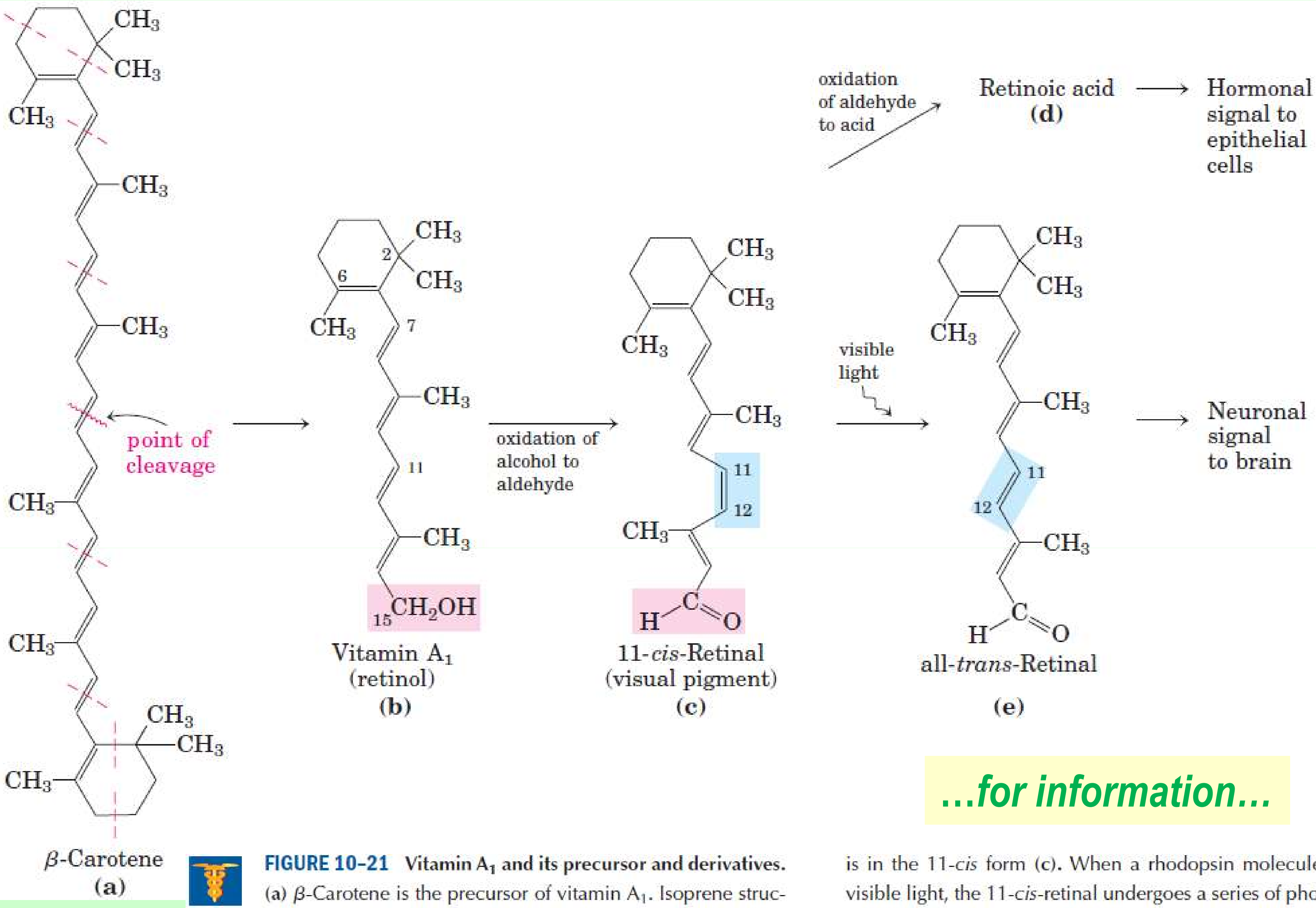


…for information…

ERGOSTEROL (PLANT STEROL)

Precursor of VITAMIN D_2 (ergocalciferol):

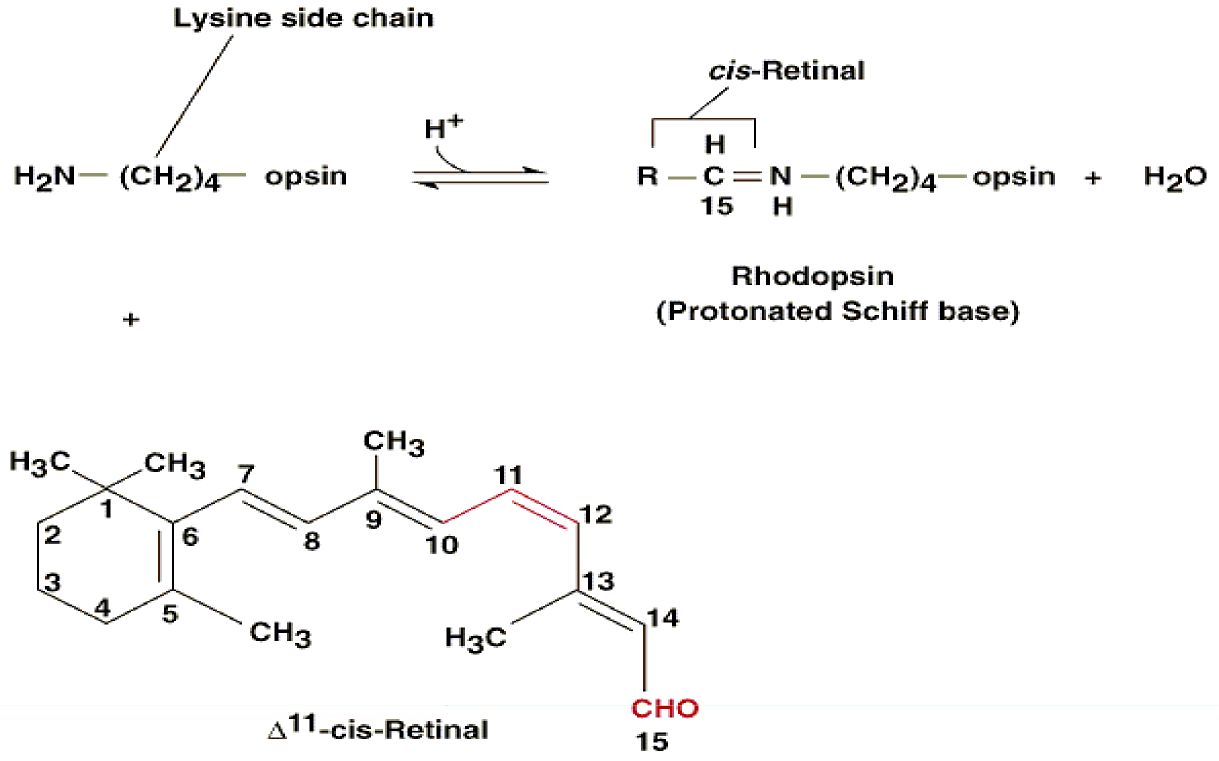
- transformation occurs in skin by UV-LIGHT

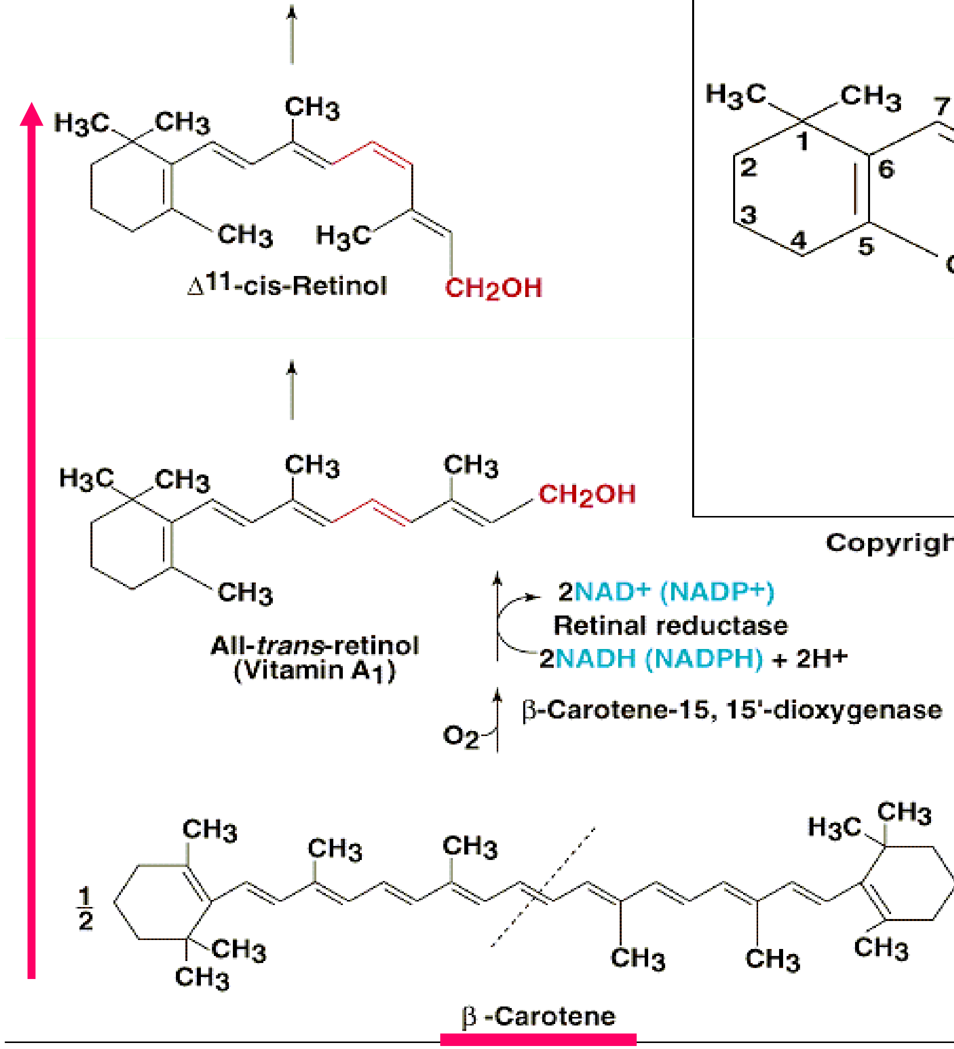


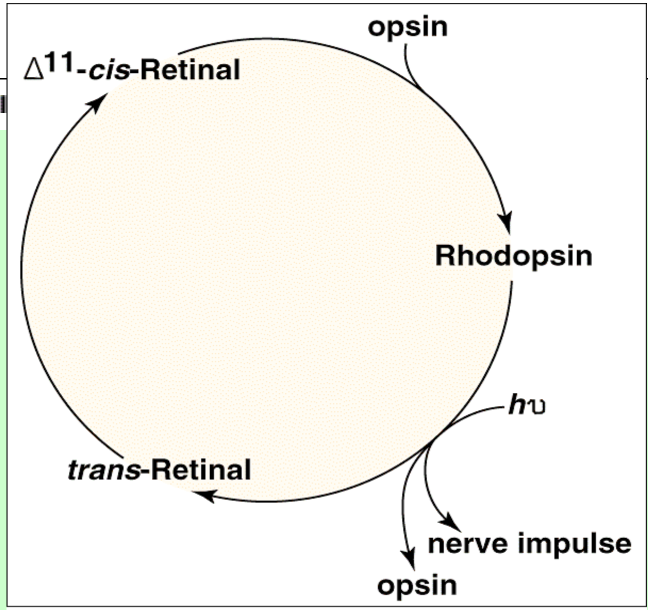
…for information…

FIGURE 10–21 Vitamin A_1 and its precursor and derivatives. (a) β-Carotene is the precursor of vitamin A_1. Isoprene structural units are set off by dashed red lines. Cleavage of β-carotene yields two molecules of vitamin A_1 (retinol) (**b**). Oxidation at C-15 converts retinol to the aldehyde, retinal (**c**), and further oxidation produces retinoic acid (**d**), a hormone that regulates gene expression. Retinal combines with the protein opsin to form rhodopsin (not shown), a visual pigment widespread in nature. In the dark, retinal of rhodopsin is in the 11-*cis* form (**c**). When a rhodopsin molecule is excited by visible light, the 11-*cis*-retinal undergoes a series of photochemical reactions that convert it to all-*trans*-retinal (**e**), forcing a change in the shape of the entire rhodopsin molecule. This transformation in the rod cell of the vertebrate retina sends an electrical signal to the brain that is the basis of visual transduction, a topic we address in more detail in Chapter 12.

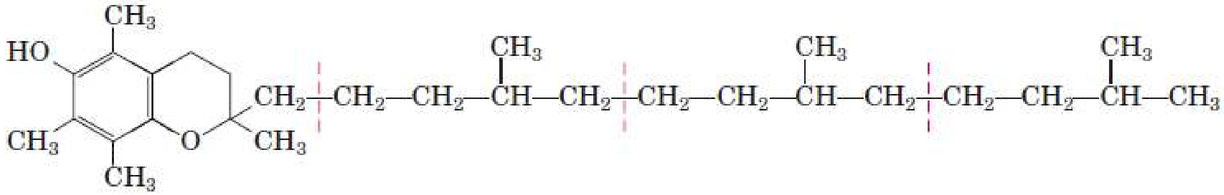
...for information...



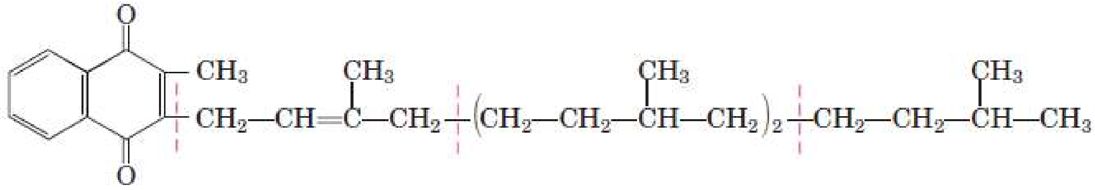




(a)
Vitamin E: an antioxidant



(b)
Vitamin K_1: a blood-clotting cofactor (phylloquinone)



(c)
Warfarin: a blood anticoagulant

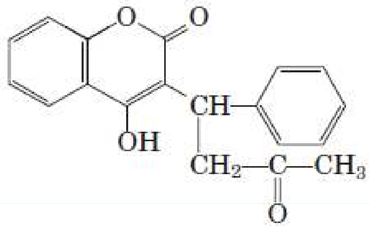
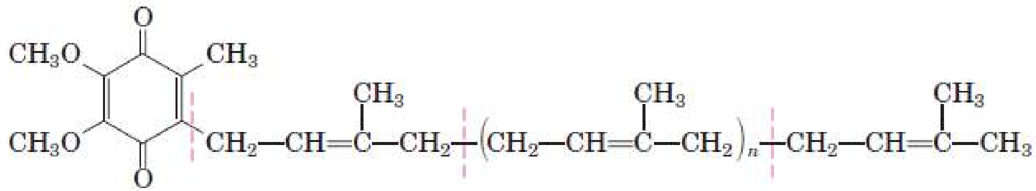
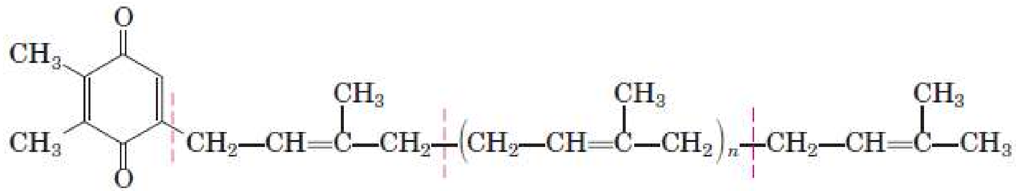


FIGURE 10–22 Some other biologically active isoprenoid compounds or derivatives. Isoprene structural units are set off by dashed red lines. In most mammalian tissues, ubiquinone (also called coenzyme Q) has 10 isoprene units. Dolichols of animals have 17 to 21 isoprene units (85 to 105 carbon atoms), bacterial dolichols have 11, and those of plants and fungi have 14 to 24.

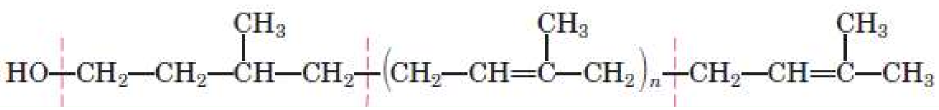
(d)
Ubiquinone: a mitochondrial electron carrier (coenzyme Q)
(n = 4 to 8)



(e)
Plastoquinone: a chloroplast electron carrier (n = 4 to 8)

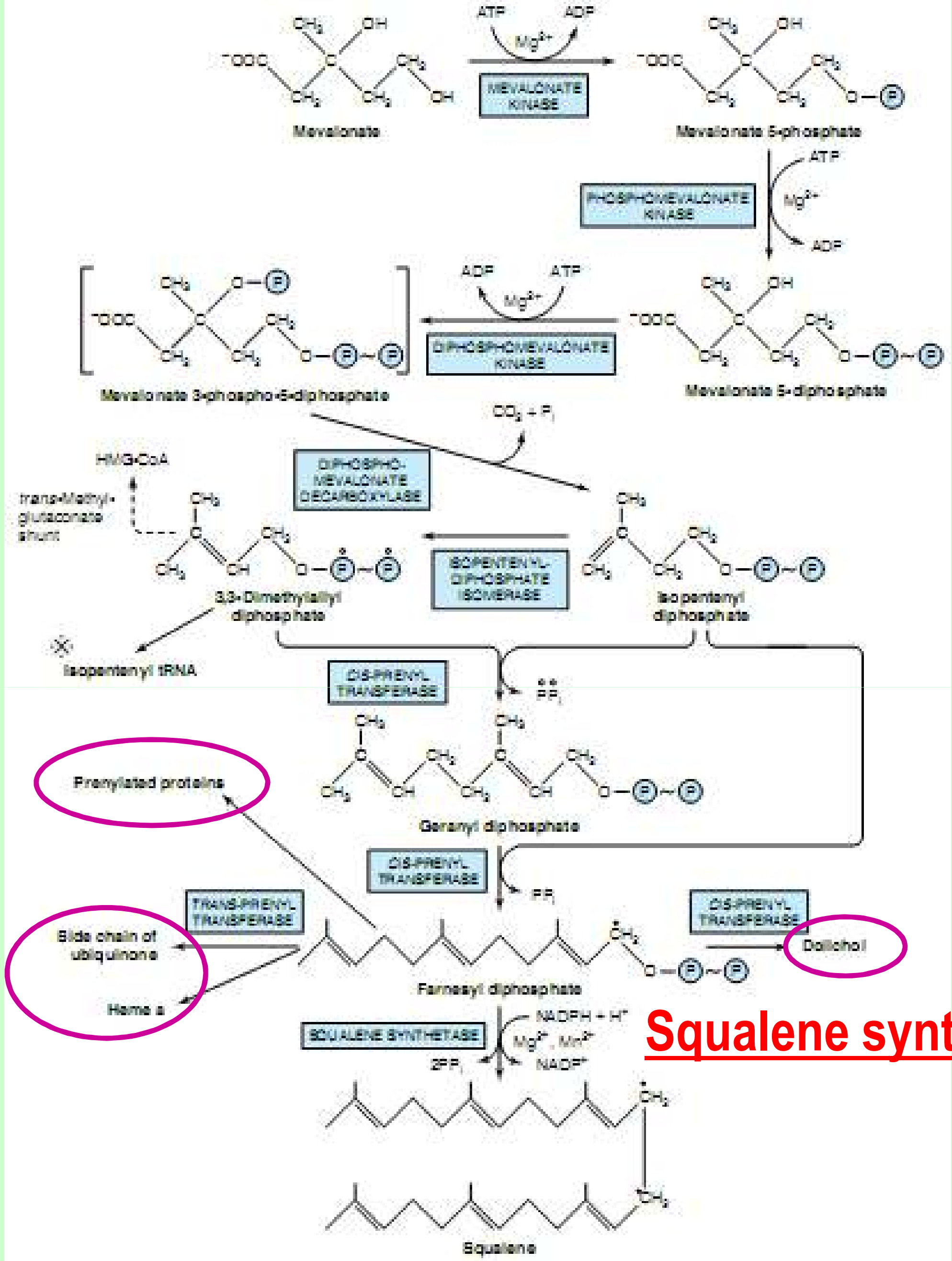


(f)
Dolichol: a sugar carrier
(n = 9 to 22)



…for information…

Farnesyl diphosphate: **precursor** for synthesis of some other isoprenoid stuctures (e.g. dolichol) and prenylated structures (e.g. ubiquinone, prenylated proteins)



Squalene synthase

Figure 26–2. Biosynthesis of squalene, ubiquinone, dolichol, and other polyisoprene derivatives. (HMG, 3-hydroxy-3-methylglutaryl; ※, cytokinin.) A farnesyl residue is present in heme a of cytochrome oxidase. The carbon marked with asterisk becomes C_{11} or C_{12} in squalene. Squalene synthetase is a microsomal enzyme; all other enzymes indicated are soluble cytosolic proteins, and some are found in peroxisomes.

…for information…

Outline of Cholestrol Synthesis

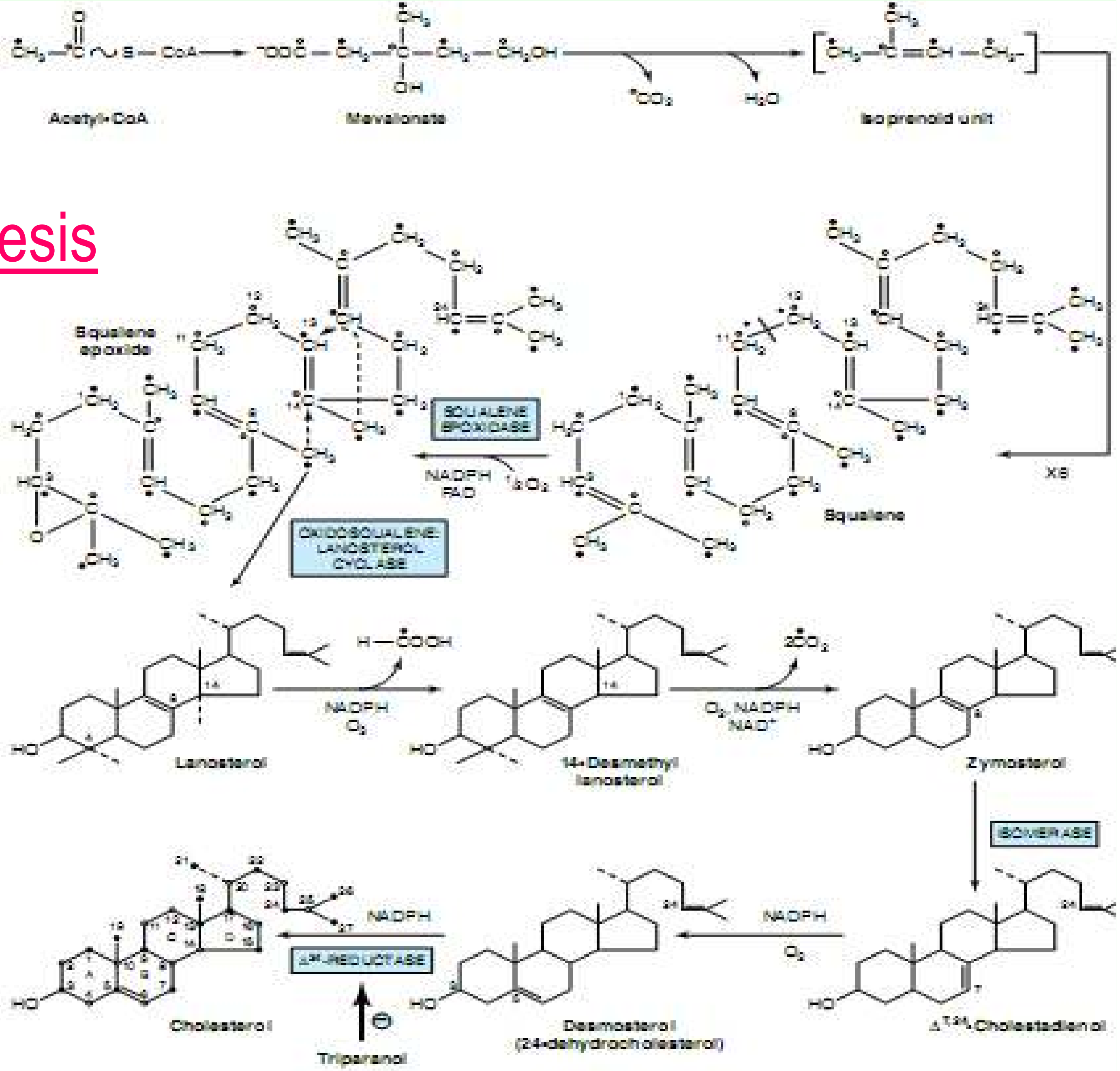


Figure 26–3. Biosynthesis of cholesterol. The numbered positions are those of the steroid nucleus and the open and solid circles indicate the fate of each of the carbons in the acetyl moiety of acetyl-CoA. Asterisks: Refer to labeling of squalene in Figure 26–2.

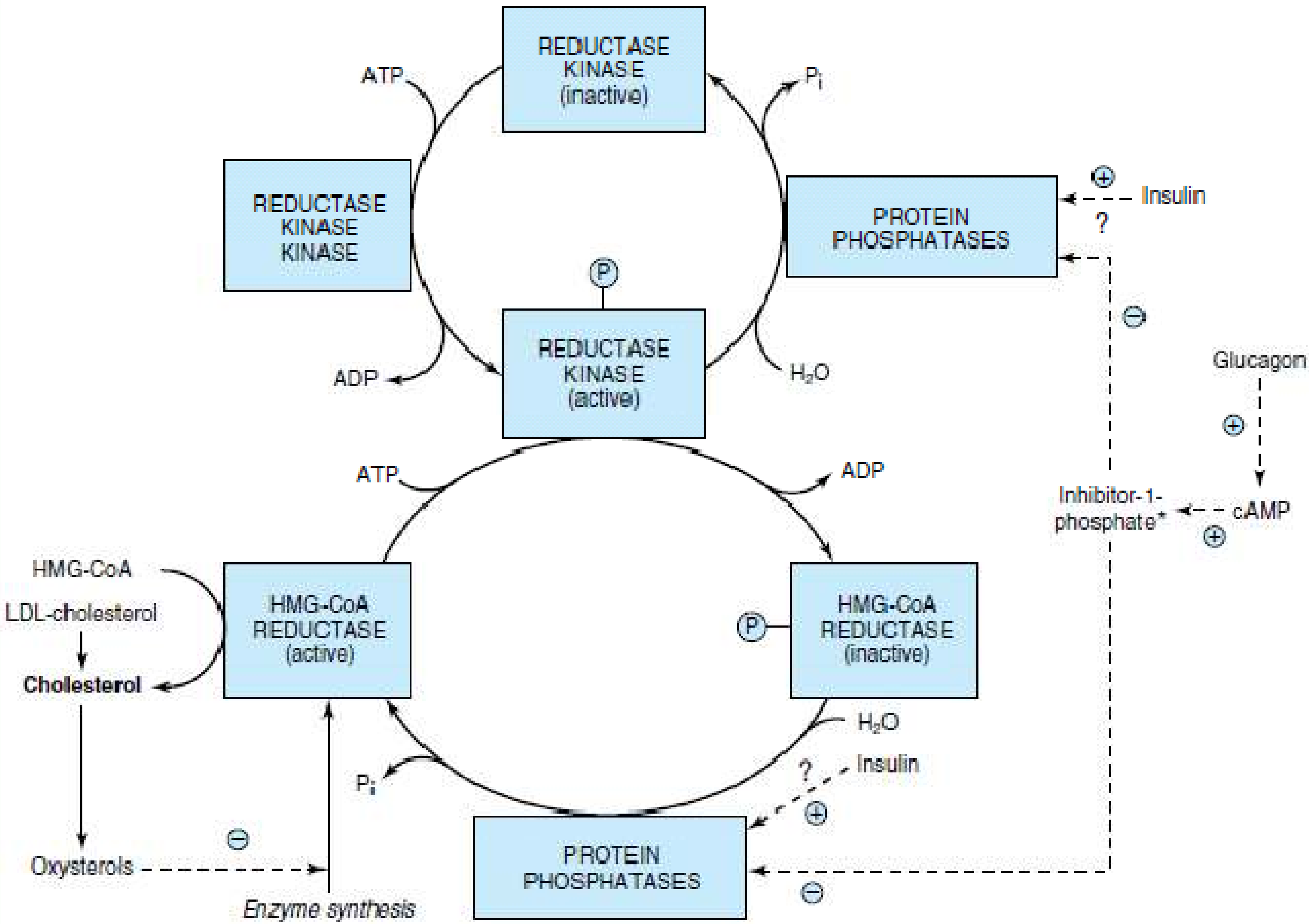


Figure 26–4. Possible mechanisms in the regulation of cholesterol synthesis by HMG-CoA reductase. Insulin has a dominant role compared with glucagon. Asterisk: See Figure 18–6.

HOMEWORK: Questions to be answered

1. Represent by structural formulas the reaction step of cholesterol biosynthesis catalyzed by the key regulatory enzyme of the pathway! Name the key enzyme!

2. Briefly explain the levels and ways of regulation of the key regulatory enzyme of cholesterol biosynthesis!

3. Which metabolically important biomolecules are synthesized from the cholesterol as precursor molecule?