Metabolismo del colesterol

Regulación de la ruta del mevalonato

Andrés Trostchansky
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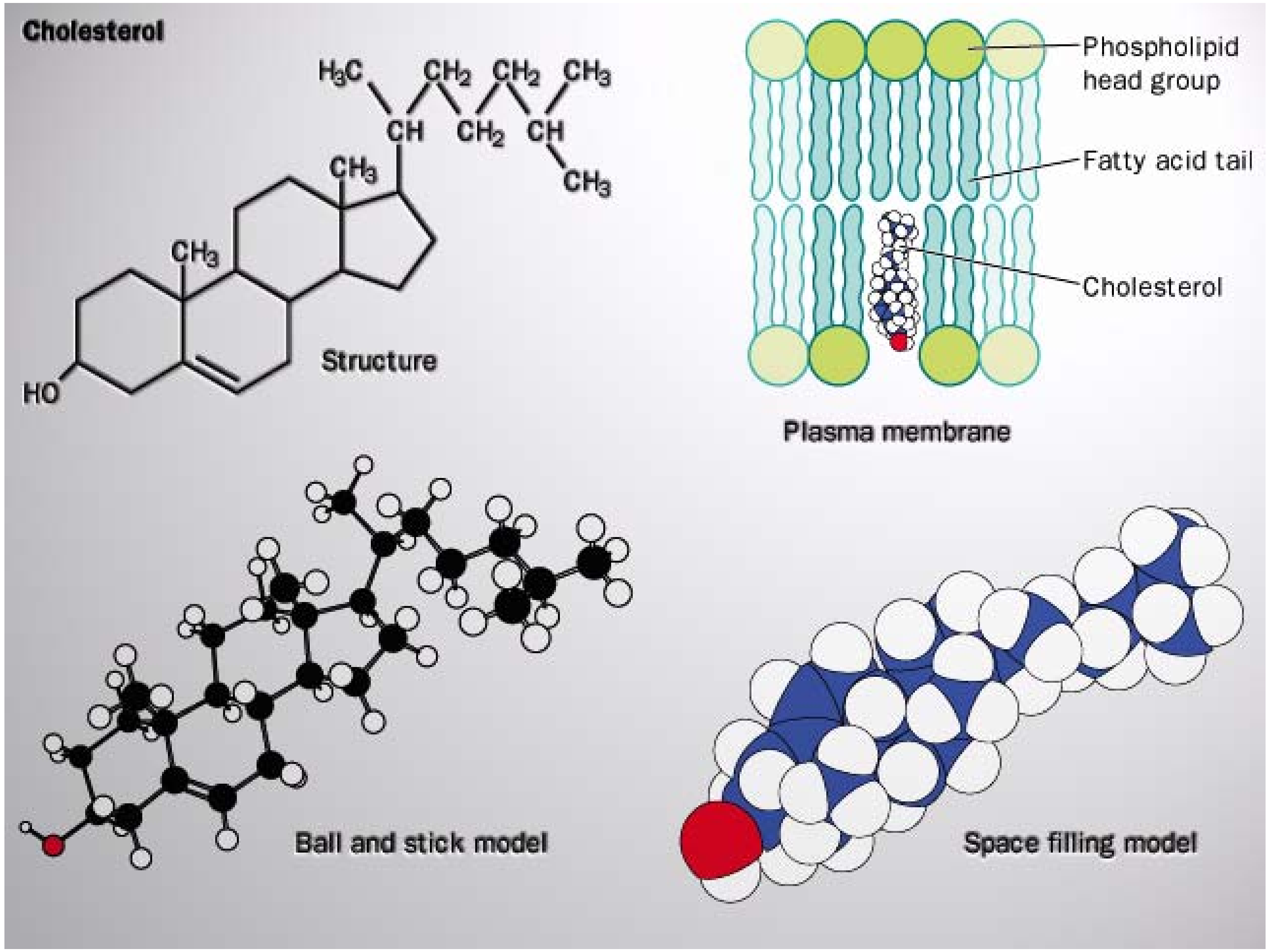
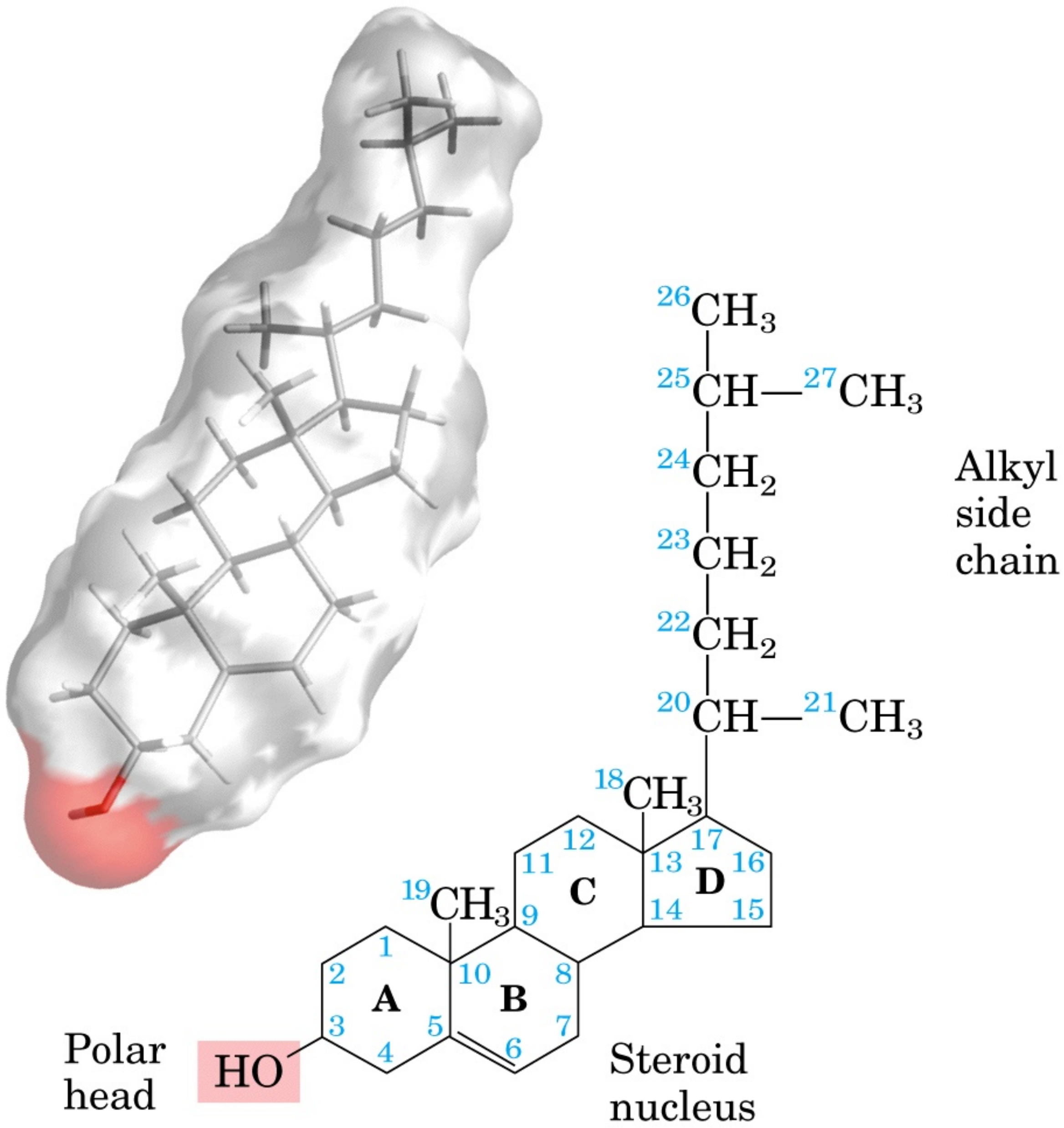
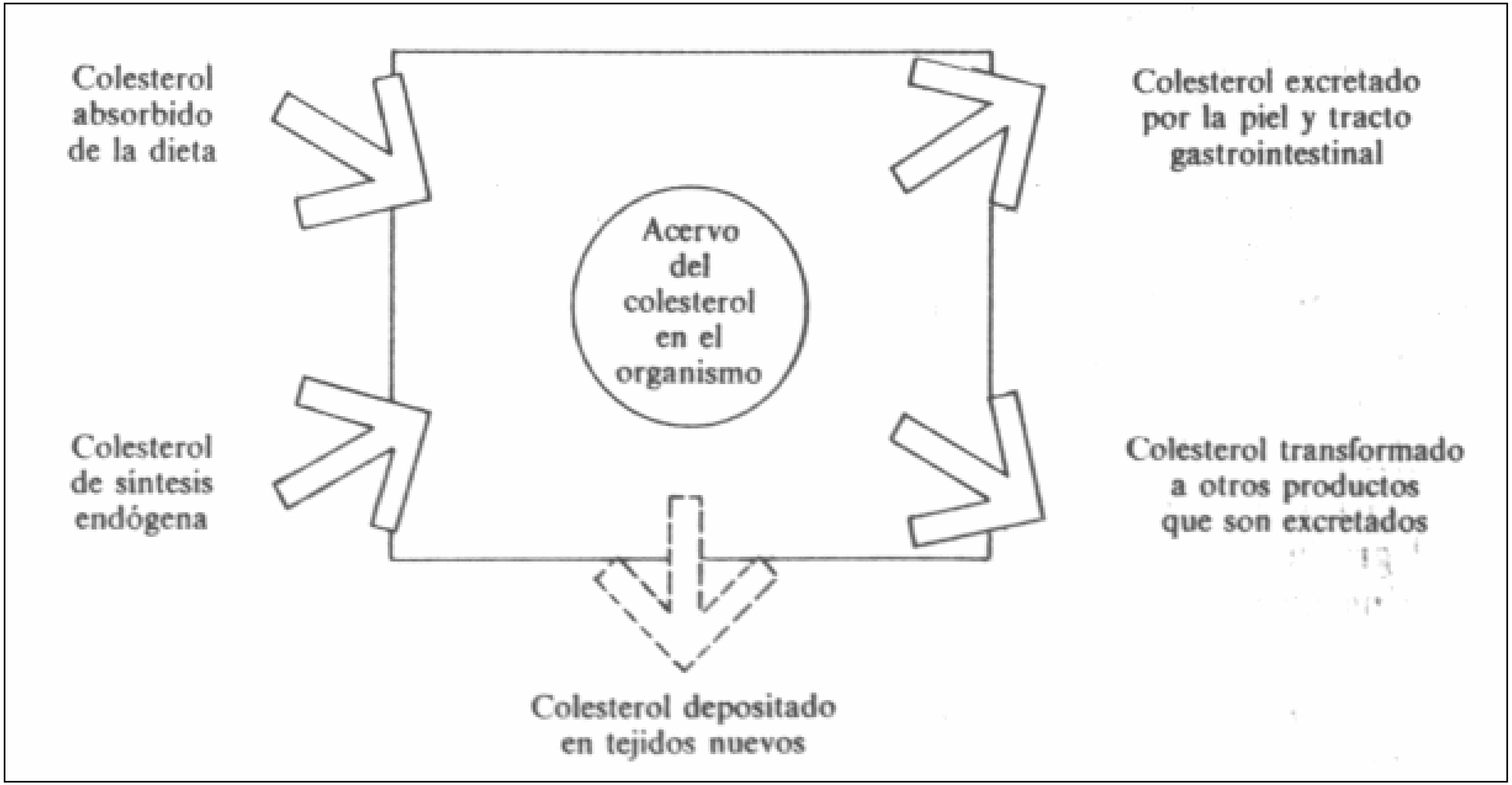


Cholesterol
H_3C
CH_2
CH_2
CH_3
CH
CH_2
CH
CH_3
CH_3
CH_3
HO
Structure
Phospholipid head group
Fatty acid tail
Cholesterol
Plasma membrane
Ball and stick model
Space filling model

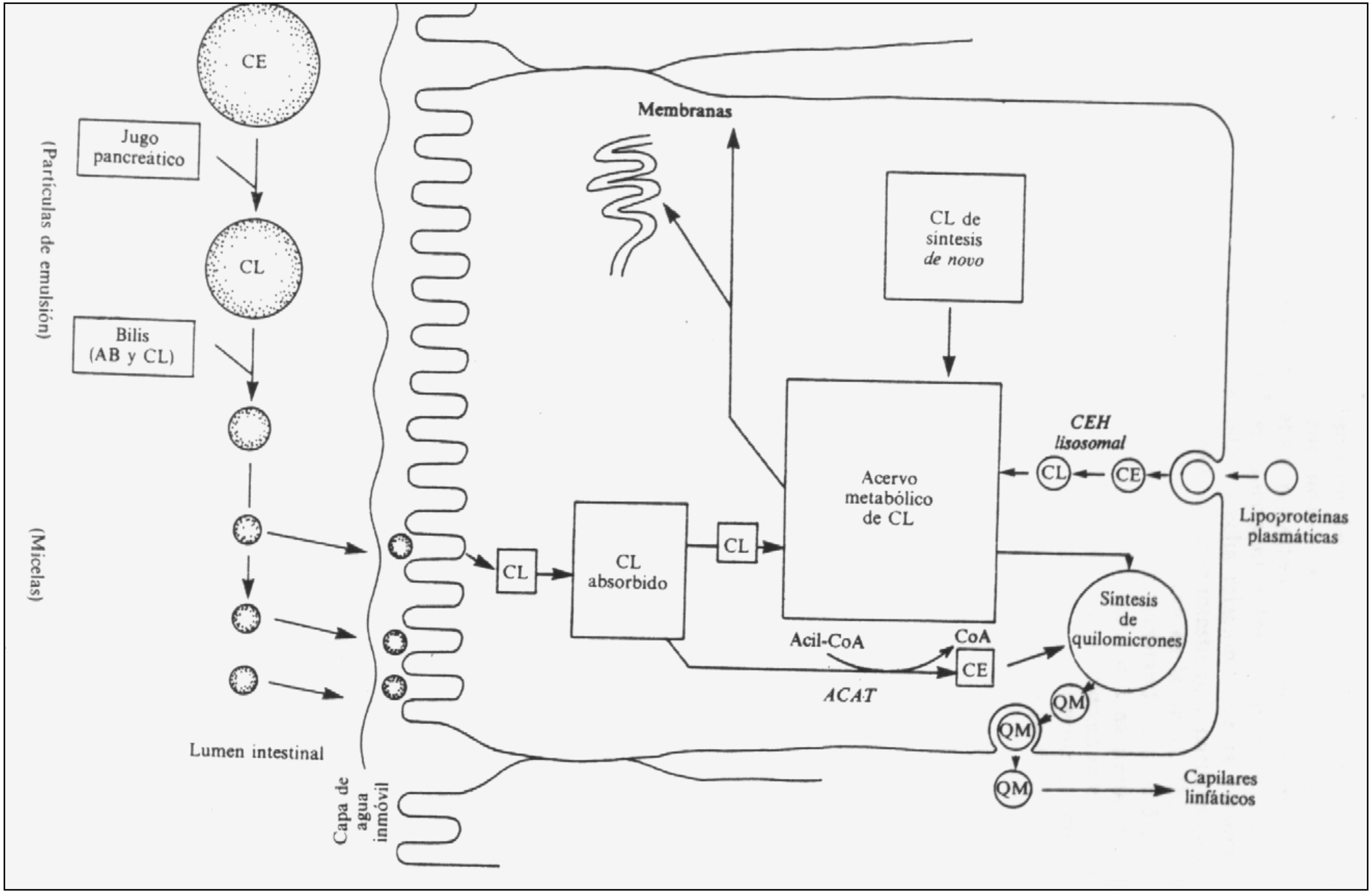


Alkyl
side
chain
Polar
head
HO
Steroid
nucleus
A
B
C
D

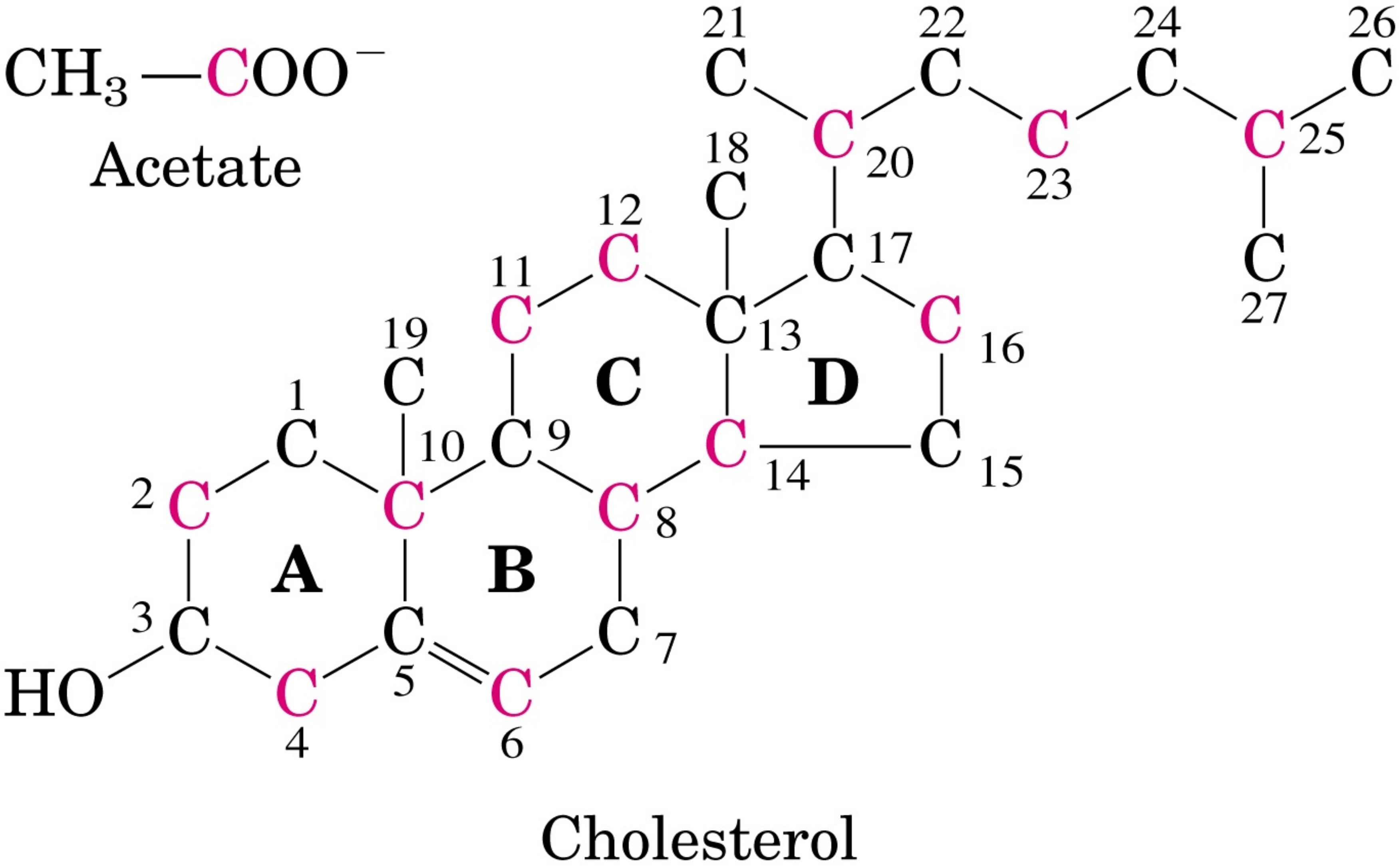
Panorámica del metabolismo del colesterol



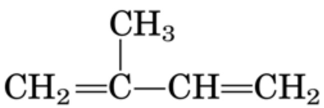
Absorción del colesterol de la dieta



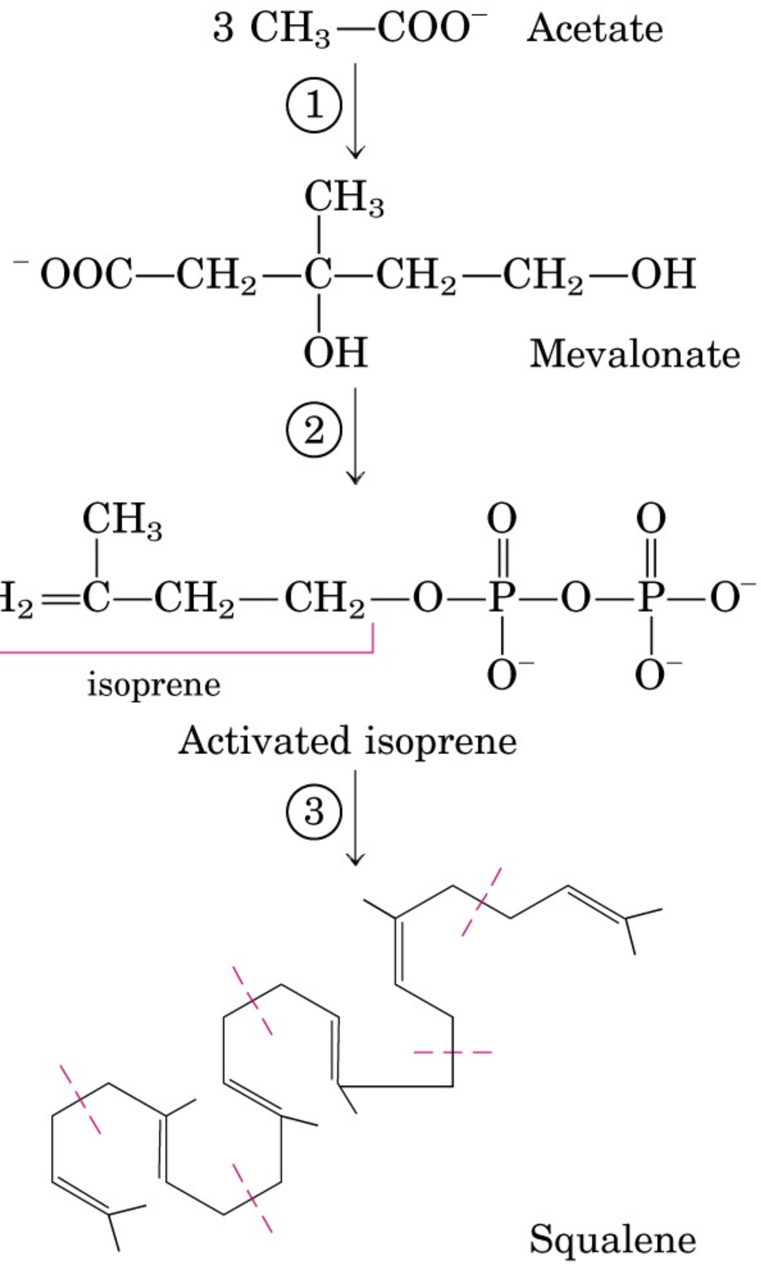
Biosíntesis de colesterol

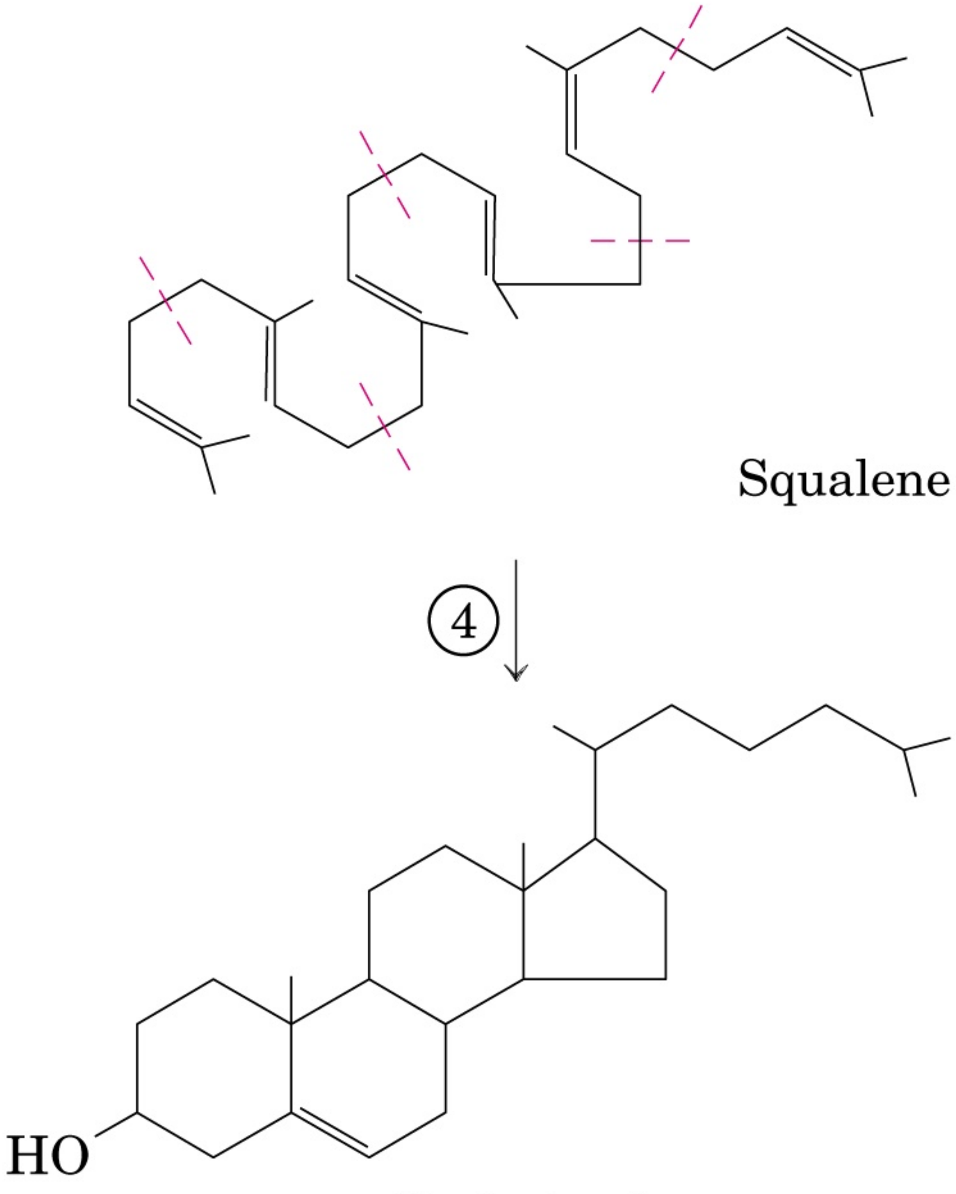


Etapas claves en la síntesis de colesterol



Isoprene

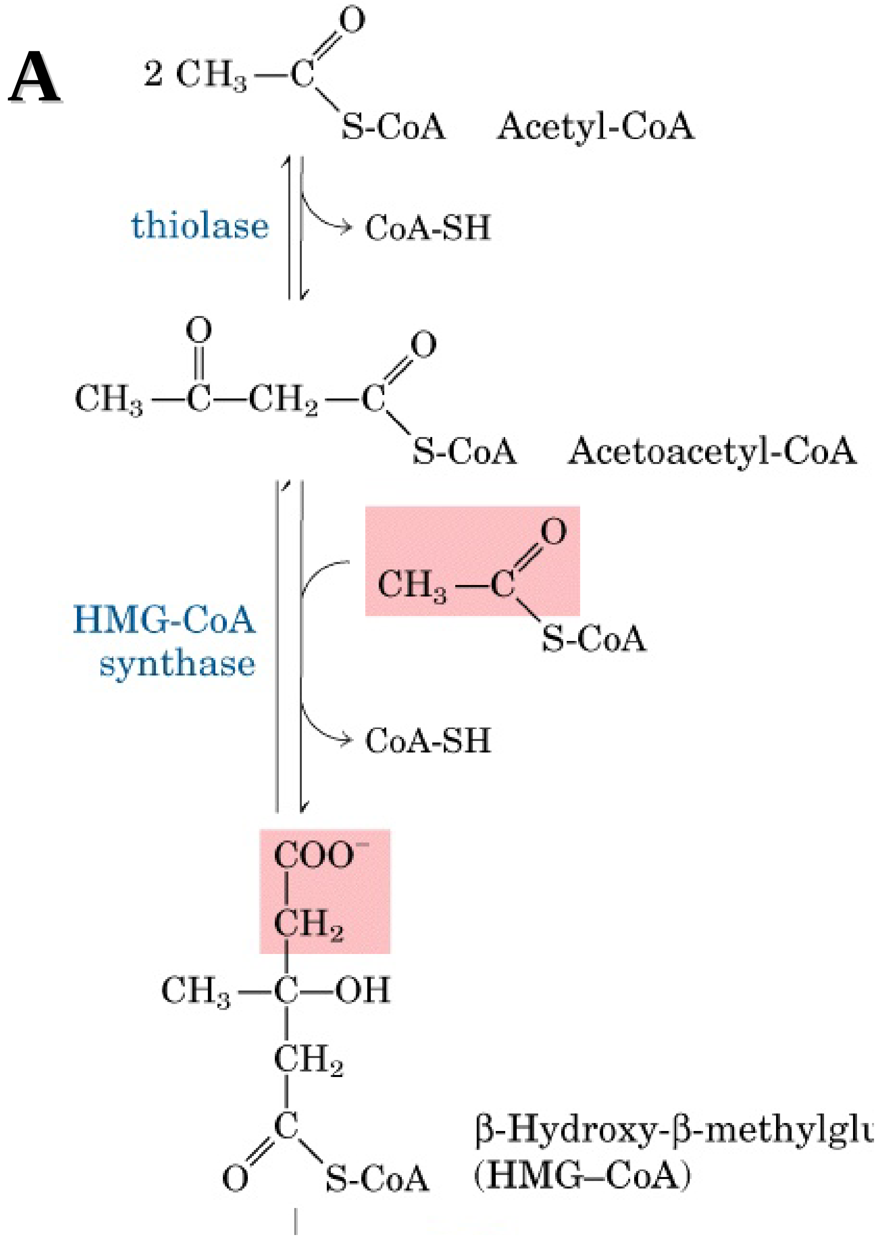


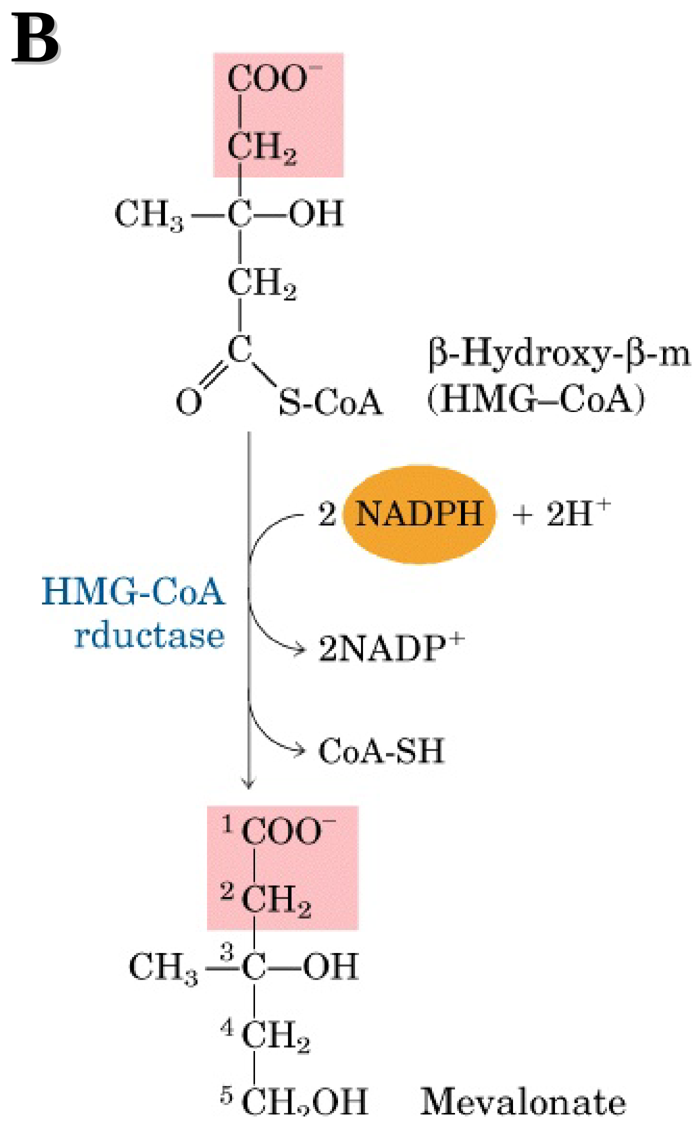


Squalene

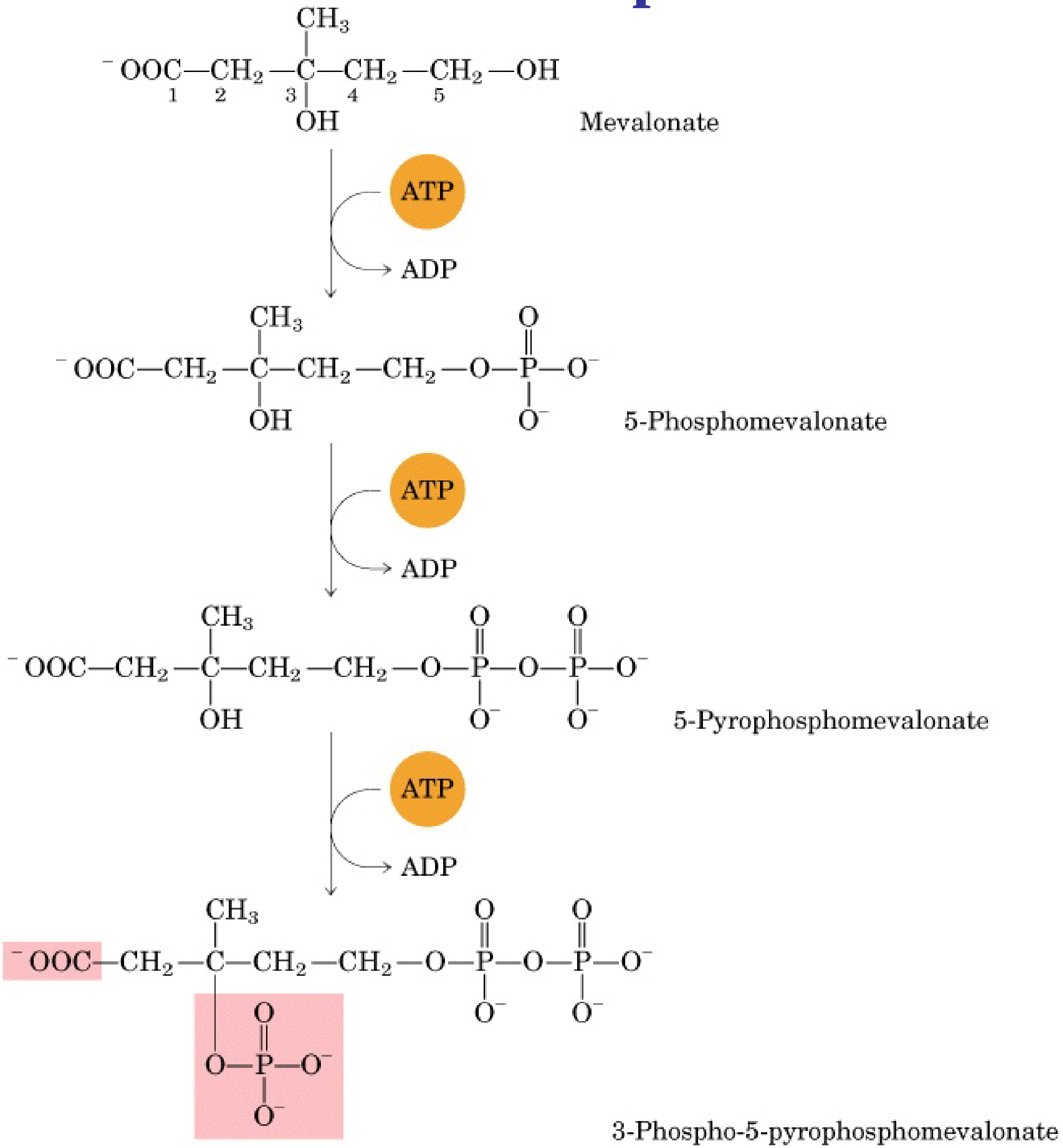
Cholesterol

1. Síntesis de mevalonato a partir de acetato

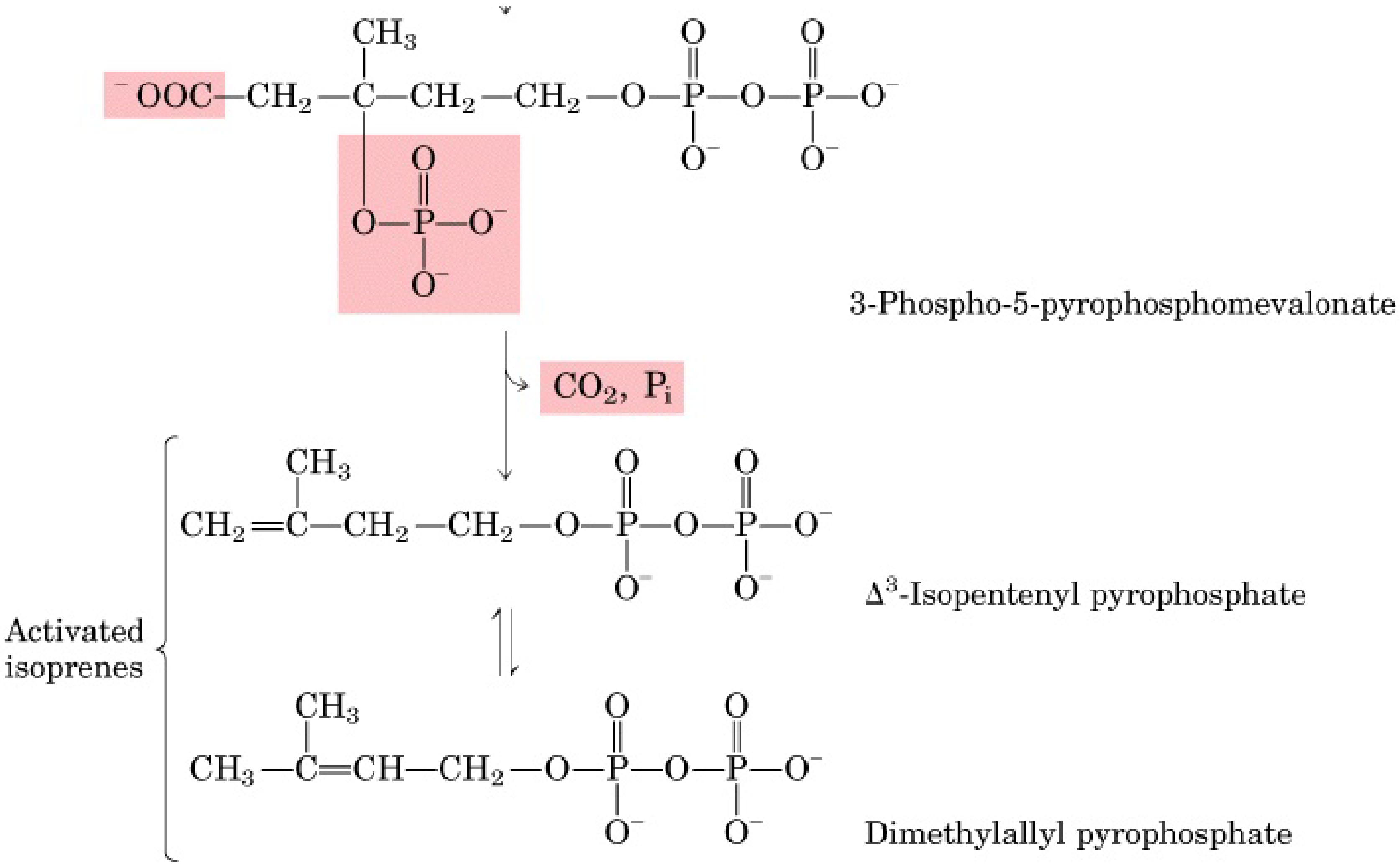




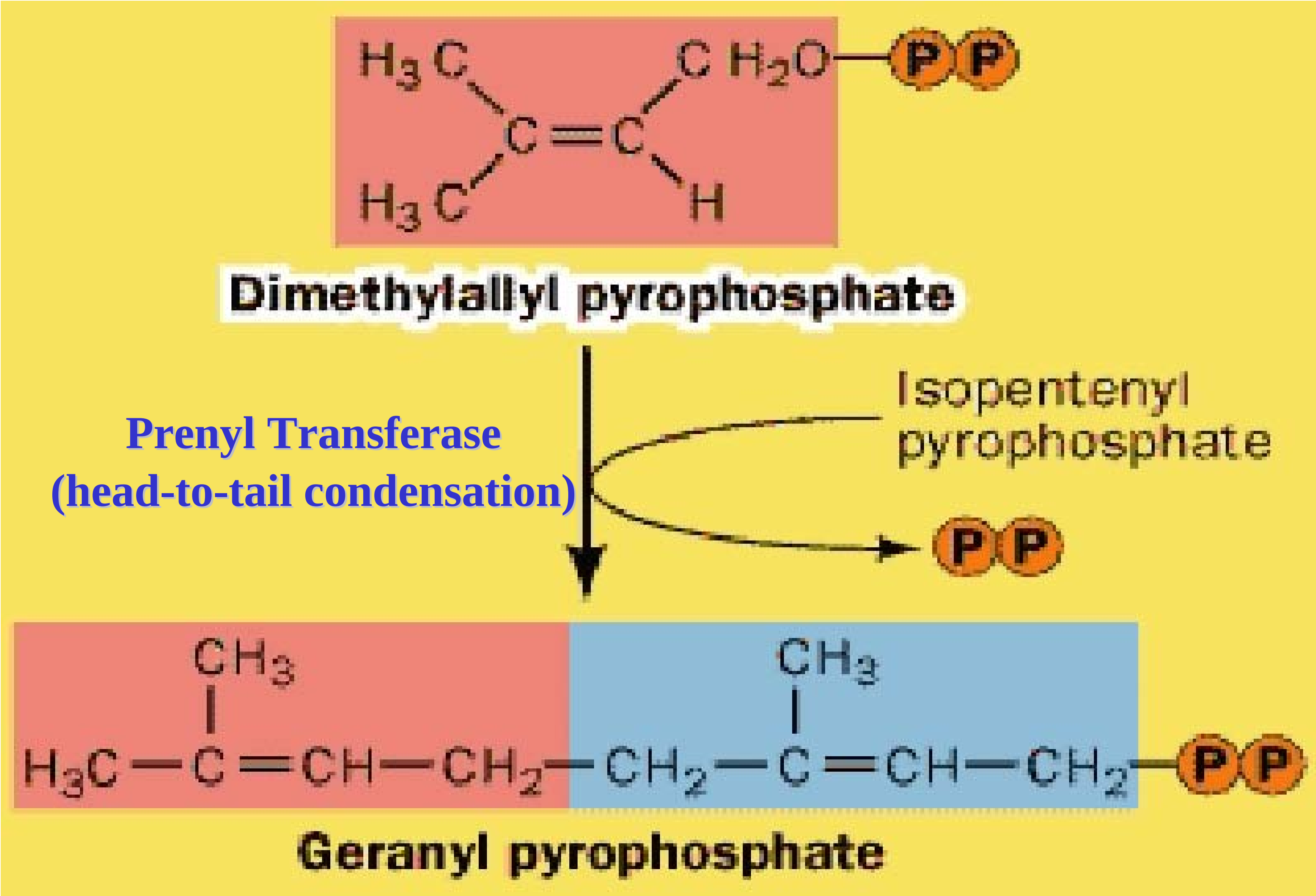
2. Conversión de mevalonato en 2 unidades activadas de isopreno

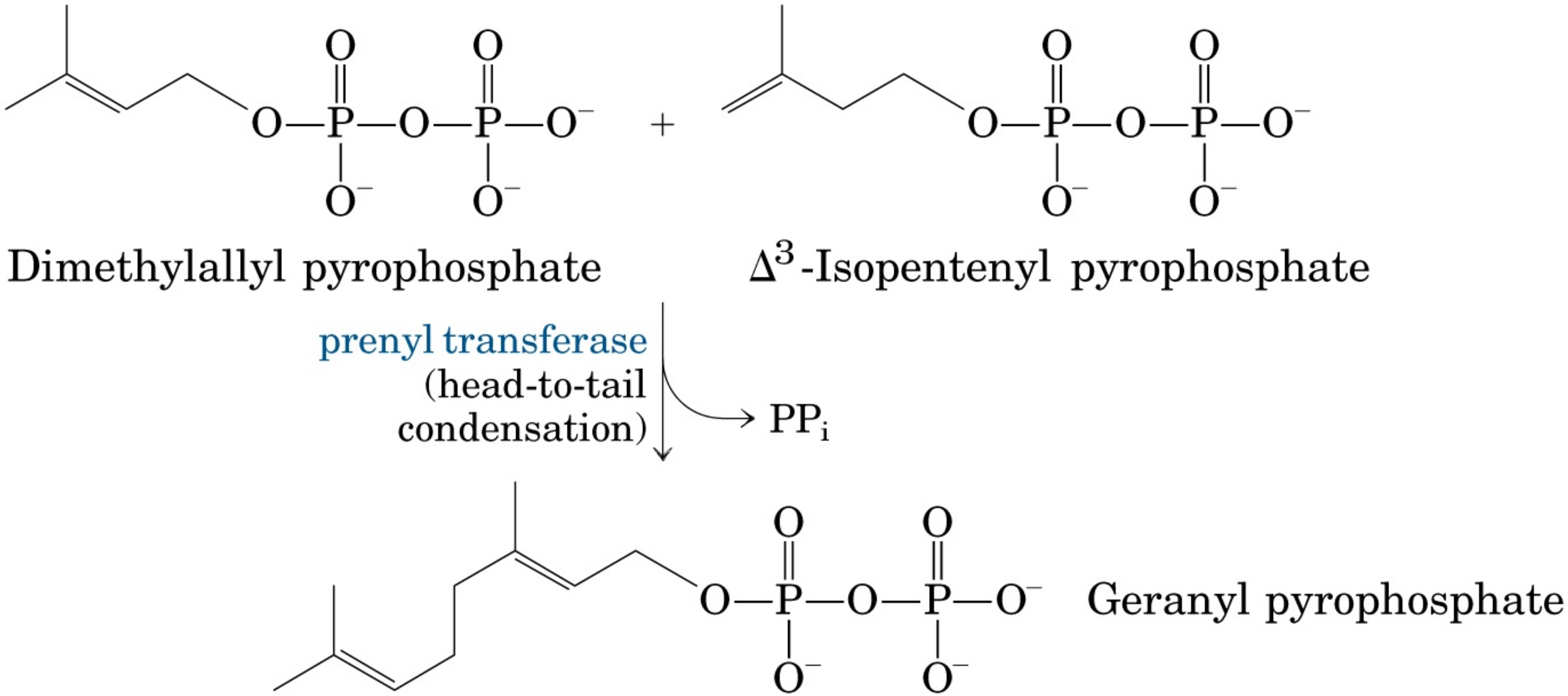


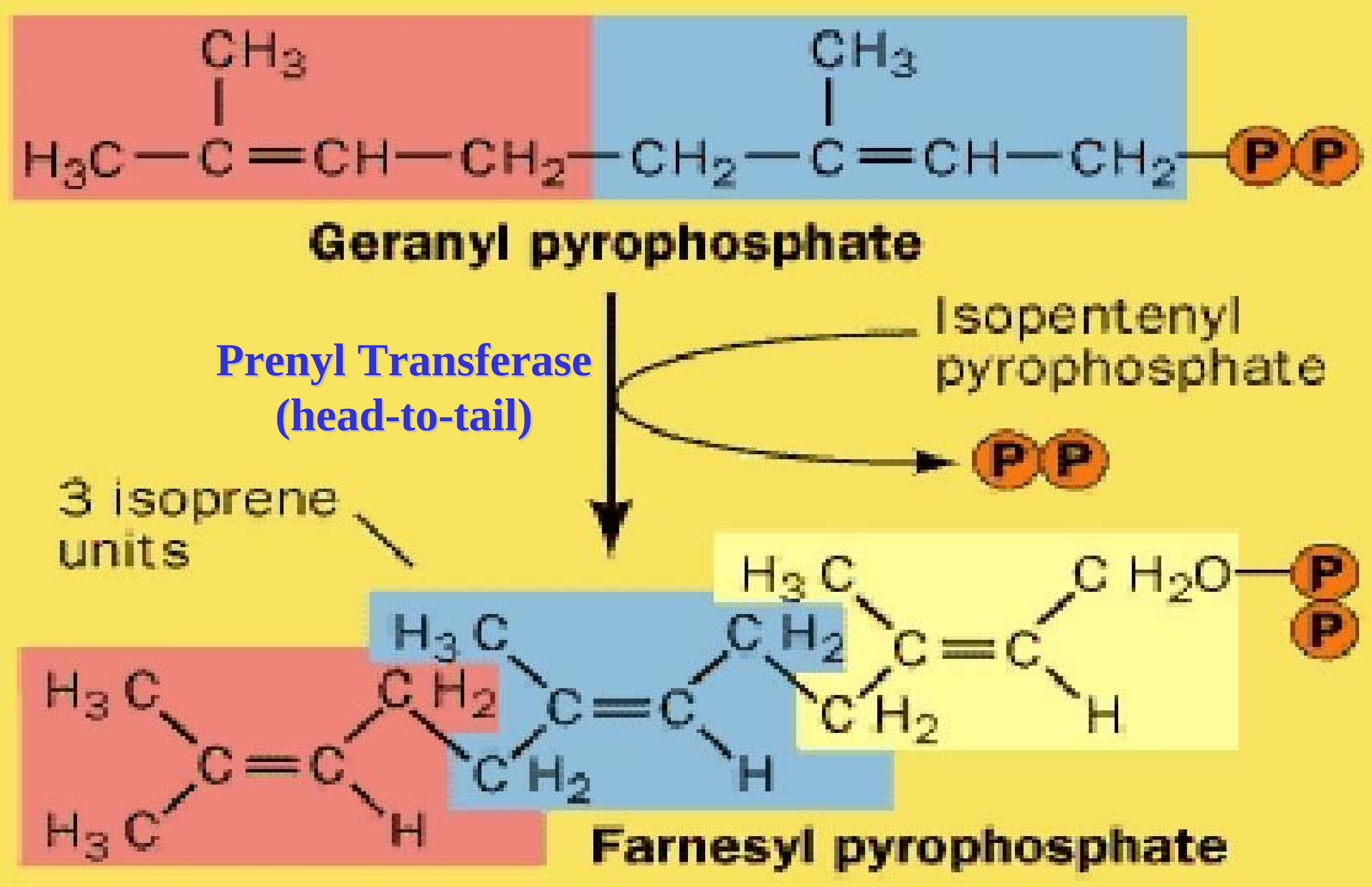
2. Conversión de mevalonato en 2 unidades activadas de isopreno



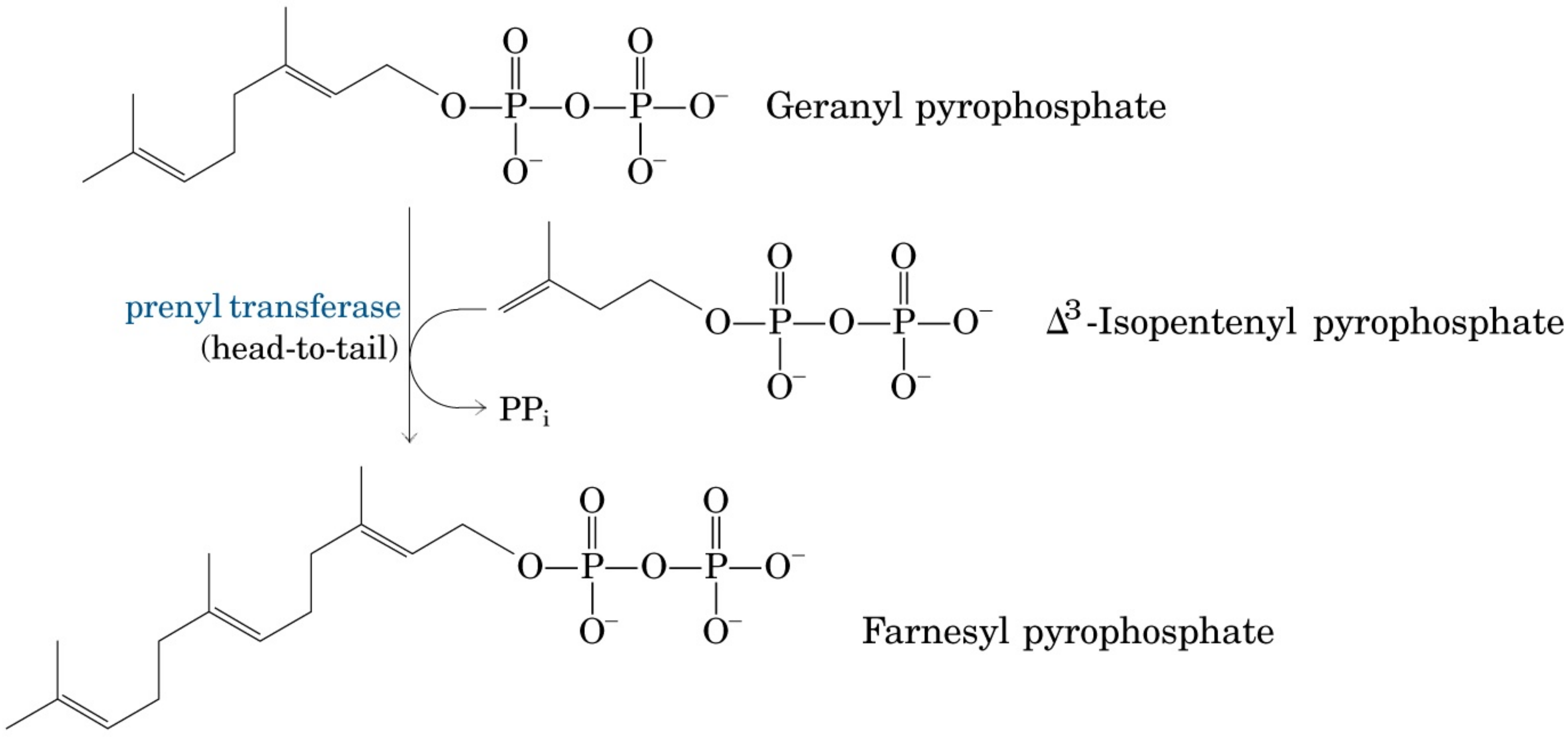
3. Condensación de 6 unidades activadas de isopreno para formar escualeno



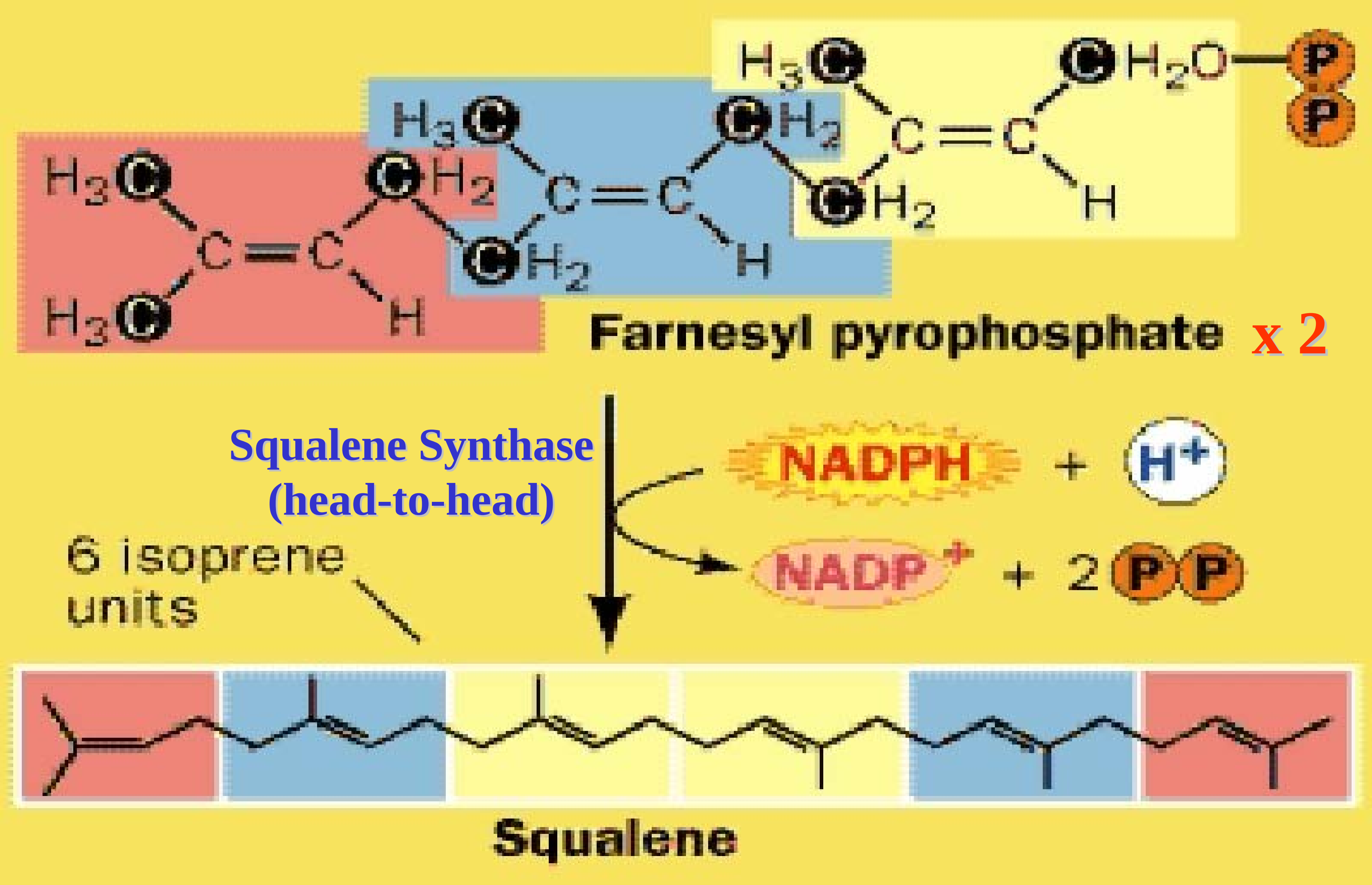

Dimethylallyl pyrophosphate
Δ3-Isopentenyl pyrophosphate
prenyl transferase
(head-to-tail
condensation)
PPi
Geranyl pyrophosphate



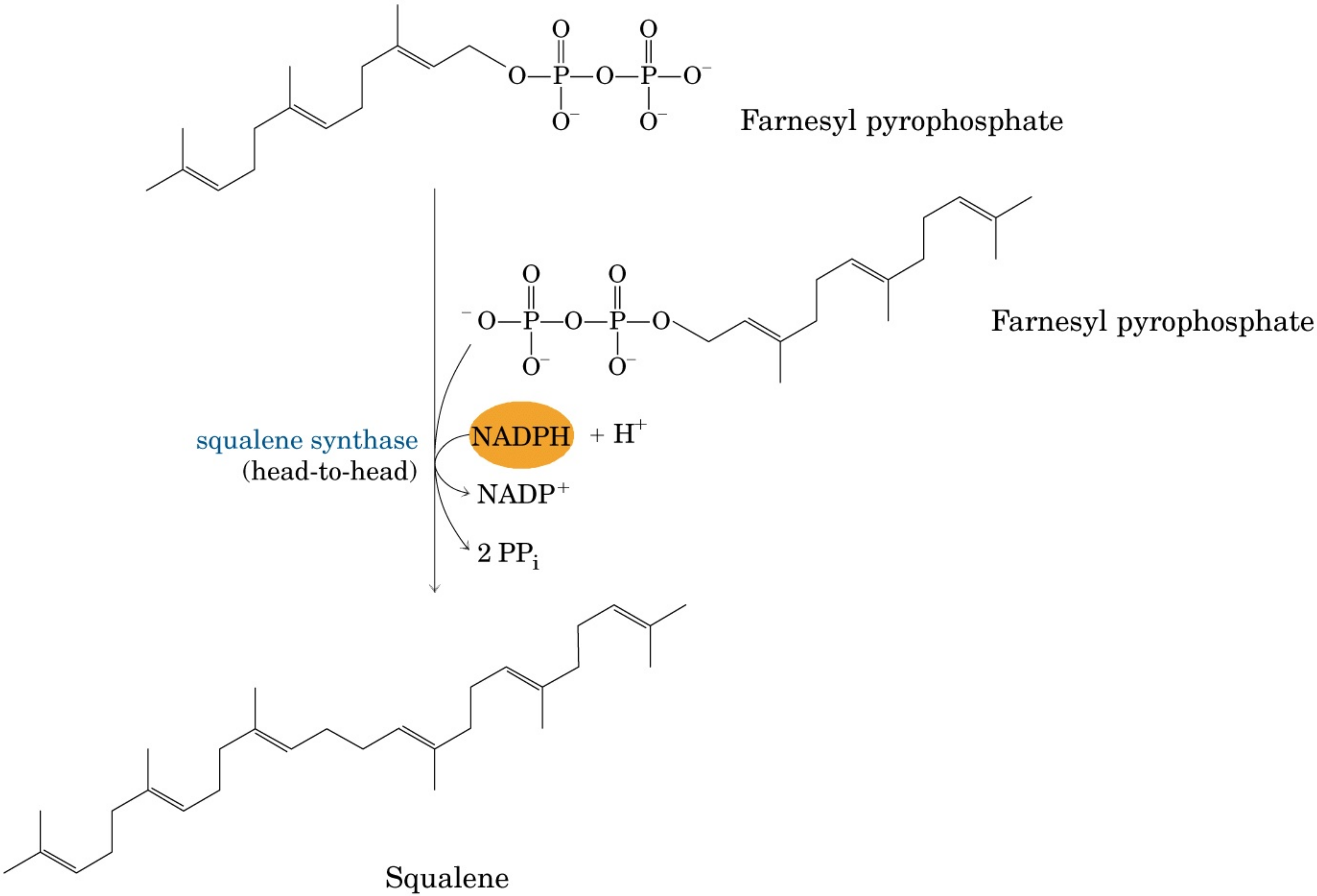
Geranyl pyrophosphate
Prenyl Transferase
(head-to-tail)
Isopentenyl pyrophosphate
3 isoprene units
Farnesyl pyrophosphate



Geranyl pyrophosphate
prenyl transferase
(head-to-tail)
PP_i
Δ^3-Isopentenyl pyrophosphate
Farnesyl pyrophosphate

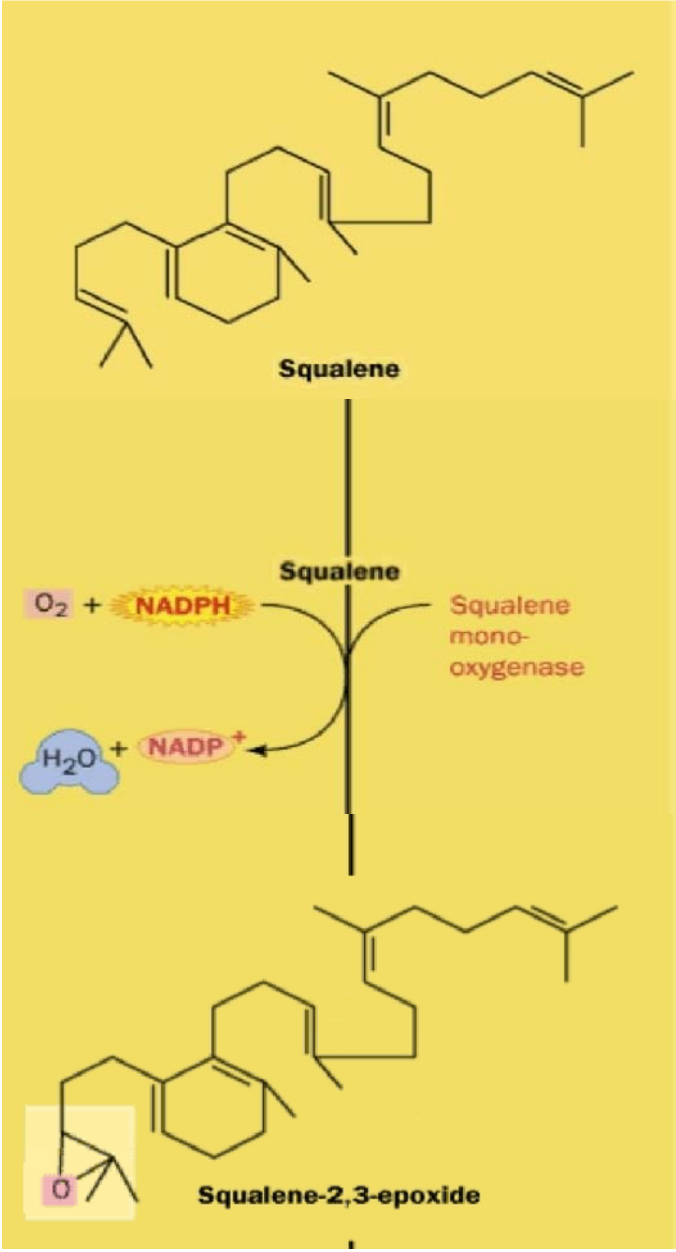


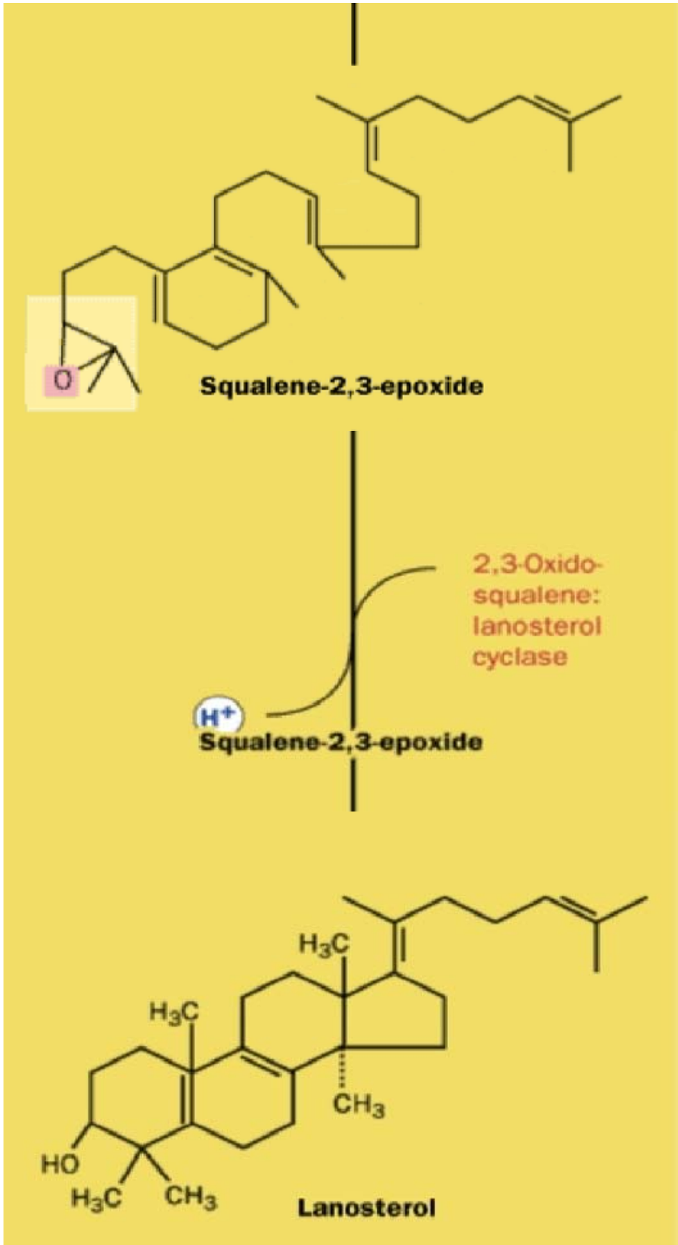
H_3C
CH_2
$C=C$
H
CH_2O
P
Farnesyl pyrophosphate
x 2
Squalene Synthase
(head-to-head)
NADPH
+
H^+
$NADP^+$
+ 2 P P
6 isoprene units
Squalene

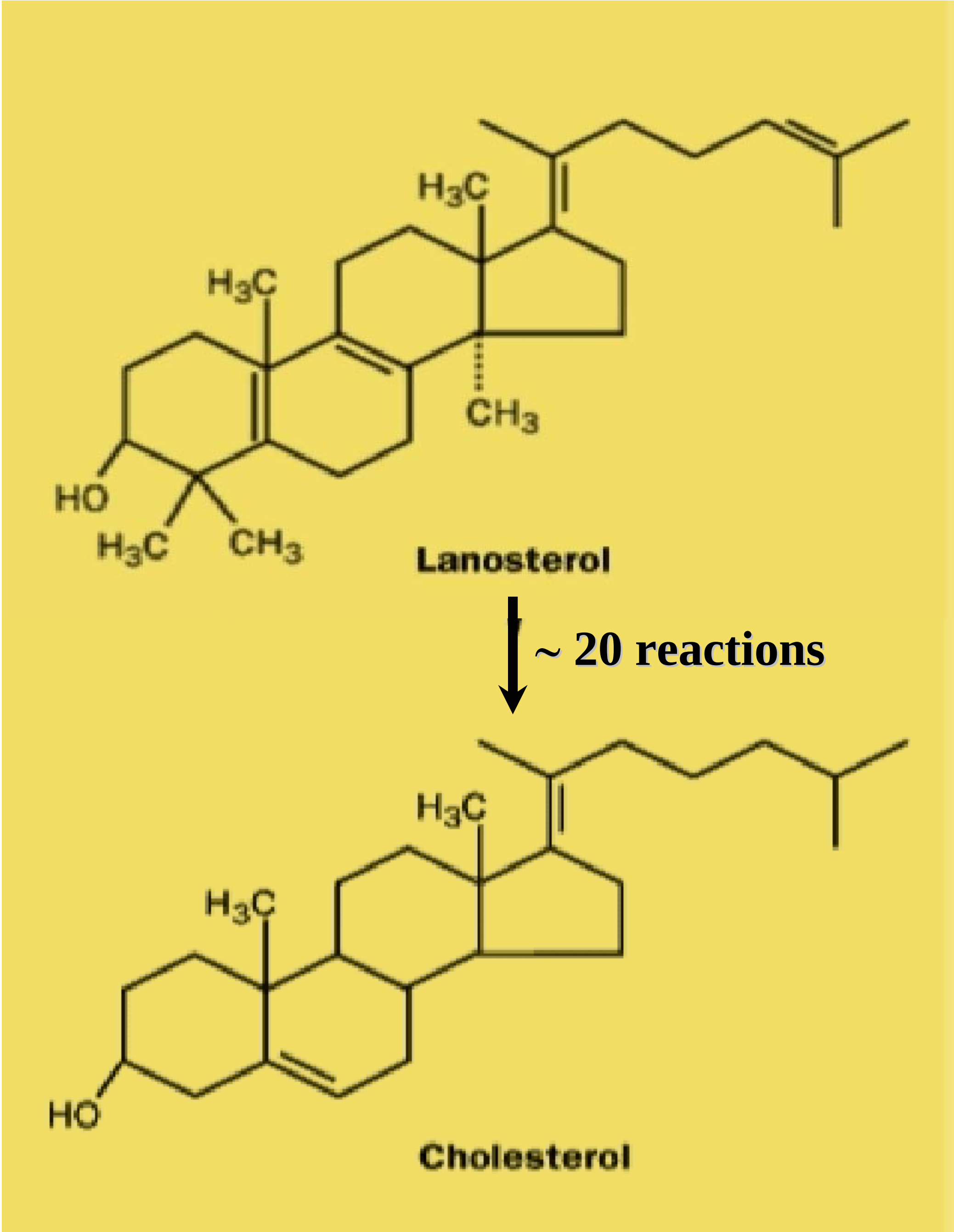


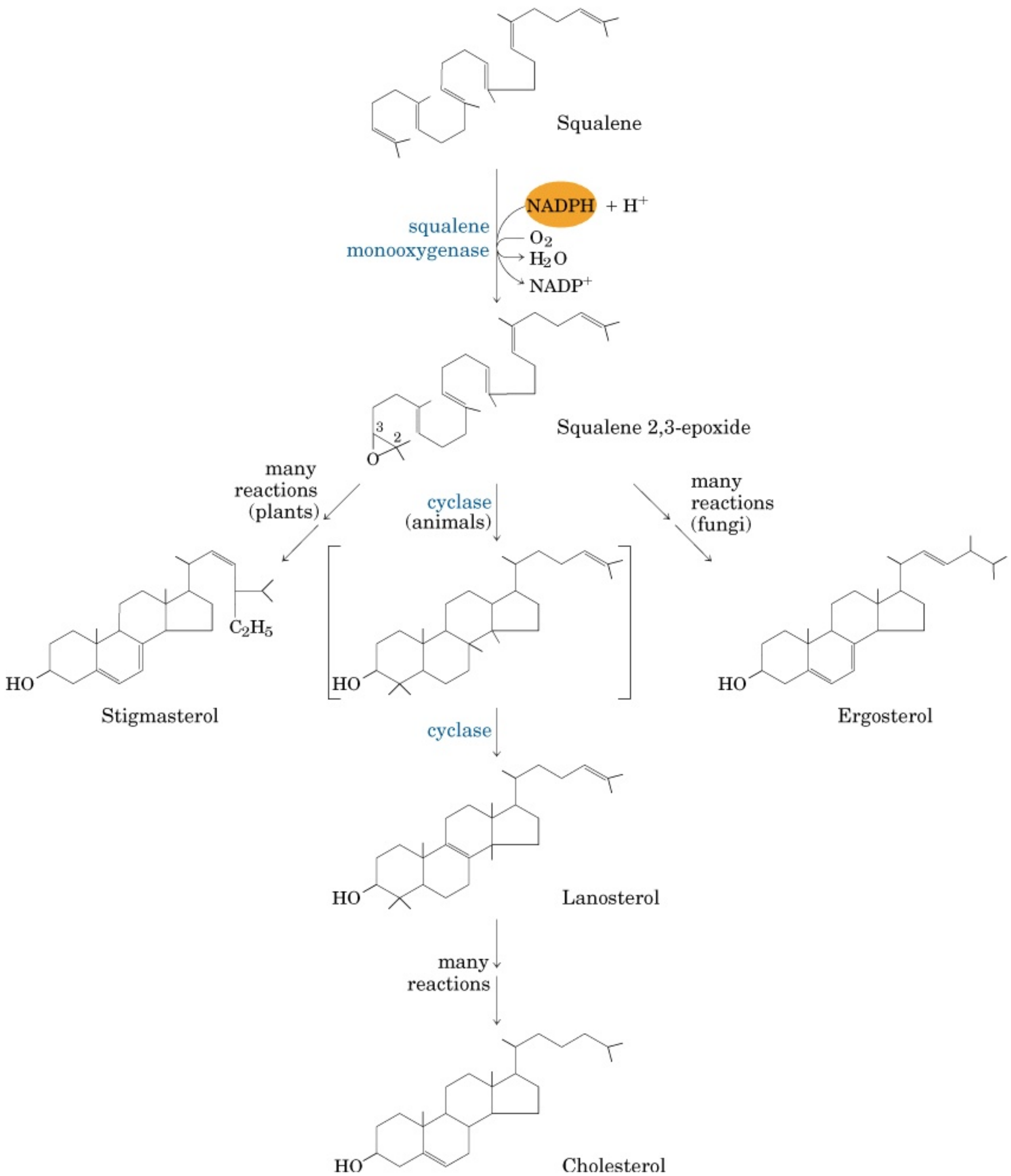
O
O
O
P
O
P
O⁻
O⁻
O⁻
Farnesyl pyrophosphate
Farnesyl pyrophosphate
squalene synthase
(head-to-head)
NADPH
+ H+
NADP+
2 PPi
Squalene

4. Conversión del escualeno en el núcleo esteroideo de 4 anillos

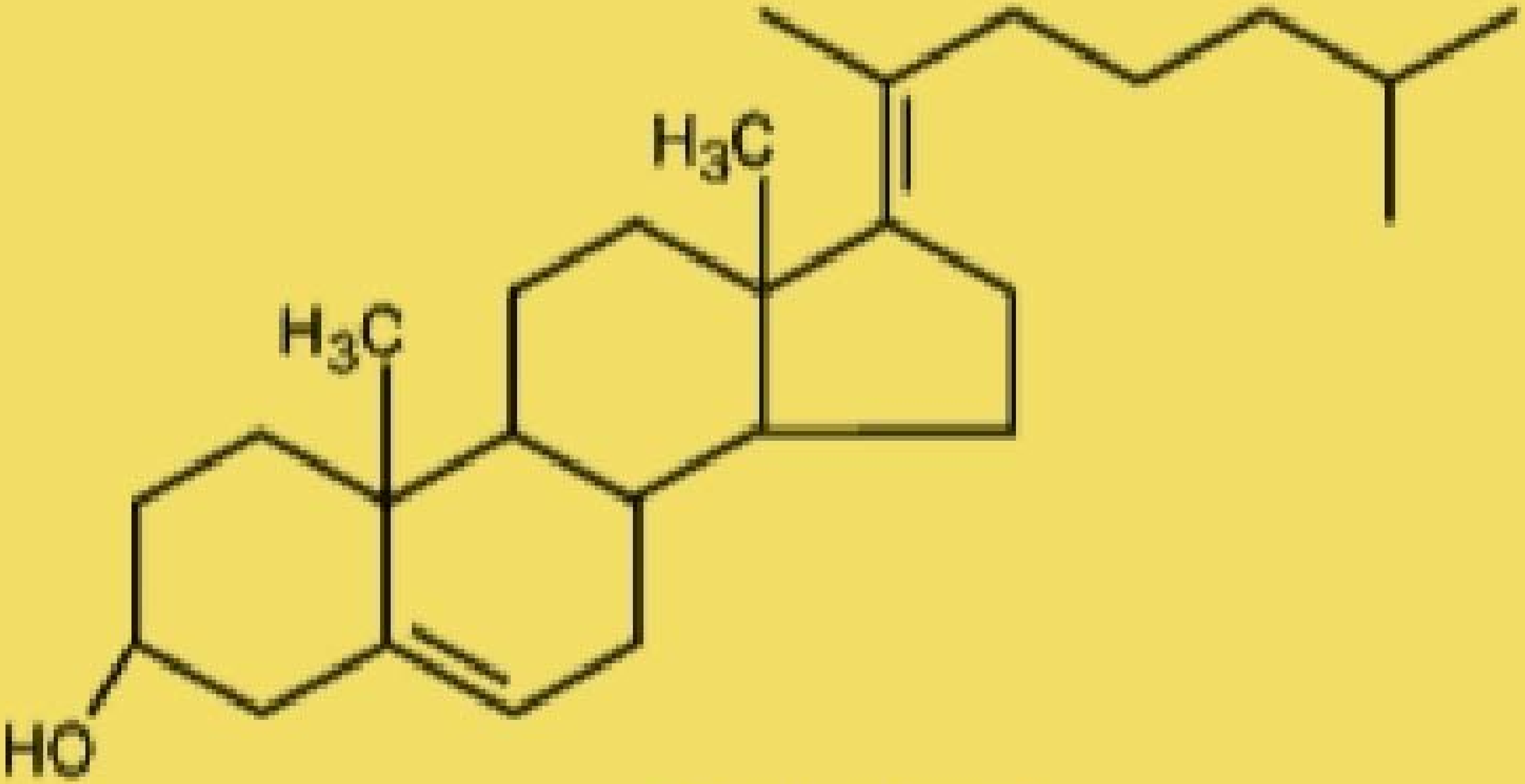





H3C
H3C
CH3
HO
H3C
CH3
Lanosterol
~ 20 reactions
H3C
H3C
HO
Cholesterol



Squalene
squalene
monooxygenase
NADPH
+ H+
O2
H2O
NADP+
Squalene 2,3-epoxide
3
2
O
many
reactions
(plants)
cyclase
(animals)
many
reactions
(fungi)
C2H5
HO
Stigmasterol
HO
HO
Ergosterol
cyclase
HO
Lanosterol
many
reactions
HO
Cholesterol



Cholesterol

Regulation of the mevalonate pathway

Joseph L. Goldstein & Michael S. Brown

The mevalonate pathway produces isoprenoids that are vital for diverse cellular functions, ranging from cholesterol synthesis to growth control. Several mechanisms for feedback regulation of low-density-lipoprotein receptors and of two enzymes involved in mevalonate biosynthesis ensure the production of sufficient mevalonate for several end-products. Manipulation of this regulatory system could be useful in treating certain forms of cancer as well as heart disease.

A FINELY tuned mechanism regulates the biosynthesis of mevalonate, the precursor of isoprenoid groups that are incorporated into more than a dozen classes of end-products (Fig. 1). These include: sterols, especially cholesterol, involved in membrane structure; haem A and ubiquinone, which partake in electron transport; dolichol, required for glycoprotein synthesis; isopentyladenine, present in some transfer RNAs; and intercellular messengers, such as cytokines in plants, farnesylated mating factors in fungi, juvenile hormones in insects, and steroid hormones in animals. Interest in the regulatory importance of mevalonate was heightened recently by the discovery that growth-regulating $p21^{ras}$ proteins[1-3], encoded by *ras* proto-oncogenes and oncogenes, and nuclear envelope proteins[4-6], are covalently attached to farnesyl residues, which anchor them to cell membranes. Inhibition of mevalonate synthesis prevents farnesylation of these proteins[1-6] and blocks cell growth[7].

To ensure a constant production of the multiple isoprenoid compounds at all stages of growth, cells must precisely regulate mevalonate synthesis while avoiding overaccumulation of potentially toxic products such as cholesterol[7]. In the past few years the crucial genes that control mevalonate synthesis have been cloned, and the molecular mechanisms for mevalonate regulation are beginning to be unravelled. We review recent progress in this area and discuss the relation between mevalonate synthesis, cholesterol homeostasis and cell proliferation.

Balancing external and internal cholesterol

Cells from higher animals face a complex problem in regulating mevalonate synthesis because cholesterol, the bulk end-product of mevalonate metabolism, is derived from plasma low-density lipoprotein (LDL), which enters the cell by receptor-mediated endocytosis, as well as from synthesis within the cell (Fig. 1). Each cell must balance these external and internal sources so as to sustain mevalonate synthesis while avoiding sterol overaccumulation. This balance is achieved through feedback regulation of at least two sequential enzymes in mevalonate synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase and HMG-CoA reductase, and also of LDL receptors (Fig. 1). In the absence of LDL, animal cells maintain high activities of the two enzymes, thereby synthesizing mevalonate for production of cholesterol as well as the nonsterol products. When LDL is present, HMG-CoA synthase and reductase activities decline by more than 90%, and the cells produce only the small amounts of mevalonate needed for the nonsterol end-products[7]. If excess mevalonate is supplied externally together with LDL, the residual activity of HMG-CoA reductase is abolished and mevalonate production is terminated. When cellular sterols rise or when cell growth ceases and cholesterol demand declines, the LDL receptor gene is repressed, further averting cholesterol overaccumulation[8].

In addition to regulating mevalonate synthesis, cells regulate mevalonate disposition. The enzymes of the non-sterol pathways generally have higher affinities than those of the sterol pathway for mevalonate-derived substrates[7]. So, when mevalonate is limiting, it is preferentially shunted into the high-affinity non-sterol pathways. After prolonged incubation of cells with sterols, squalene synthetase, the first committed enzyme of sterol synthesis, is suppressed, further limiting the incorporation of mevalonate into sterols[7]. Other enzymes of mevalonate metabolism, including acetoacetyl-CoA thiolase[9] and prenyltransferase[10], are also regulated by sterols, but the quantitative role of these regulatory steps has not yet been established.

Sterol-mediated regulation of transcription

The cloning from animals cells in recent years of the genes for the LDL receptor[11], HMG-CoA synthase[12] and HMG-CoA reductase[13] has made it possible to investigate the mechanisms by which these gene products are regulated by sterols. One mechanism is transcriptional regulation. Figure 2 illustrates the coordinate induction and repression of the messenger RNAs for the LDL receptor and HMG-CoA synthase and reductase in animal cells that were cultured in the absence and presence of sterols.

To study in detail how transcriptional regulation is achieved, we focused our attention on the 5' flanking regions of the genes,

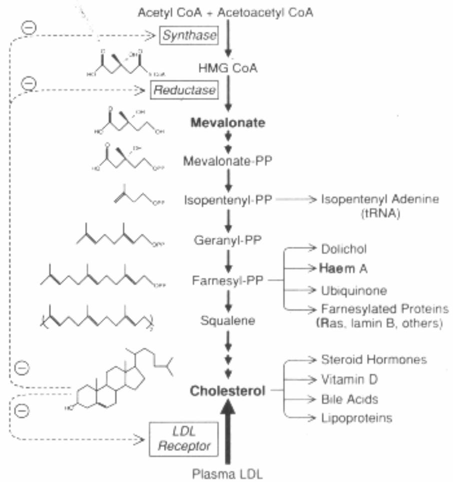


FIG. 1 The mevalonate pathway in animal cells. The bulk product of mevalonate metabolism, cholesterol, is obtained from two sources: (1) endogenously, by synthesis from acetyl-CoA through mevalonate; and (2) exogenously, from receptor-mediated uptake of plasma LDL. Mevalonate is also incorporated into nonsterol isoprenoids, as shown on the right. Mevalonate homeostasis is achieved through: (1) sterol-mediated feedback repression of the genes for HMG-CoA synthase, HMG-CoA reductase and the LDL receptor, as shown on the left; and (2) post-transcriptional regulation of HMG-CoA reductase by one of the nonsterol isoprenoids shown on the right.

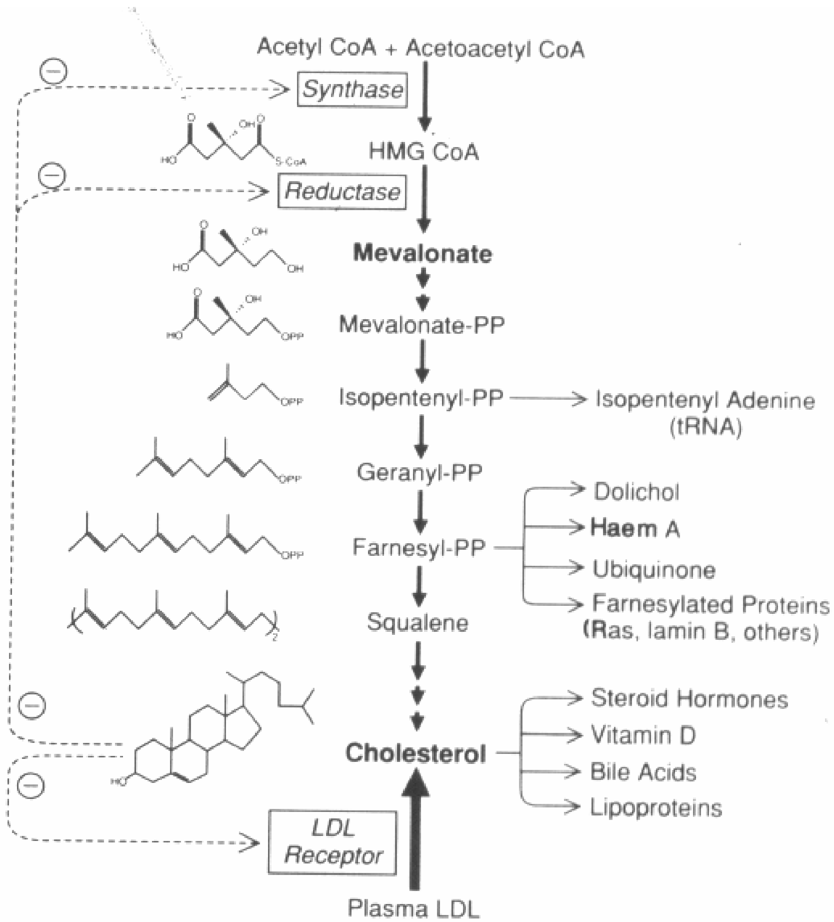


FIG. 1 The mevalonate pathway in animal cells. The bulk product of mevalonate metabolism, cholesterol, is obtained from two sources: (1) endogenously, by synthesis from acetyl-CoA through mevalonate; and (2) exogenously, from receptor-mediated uptake of plasma LDL. Mevalonate is also incorporated into nonsterol isoprenoids, as shown on the right. Mevalonate homeostasis is achieved through: (1) sterol-mediated feedback repression of the genes for HMG-CoA synthase, HMG-CoA reductase and the LDL receptor, as shown on the left; and (2) post-transcriptional regulation of HMG-CoA reductase by one of the nonsterol isoprenoids shown on the right.

Regulación de la HMG-CoA reductasa

- **A. Control a "largo plazo": *regulación de la [E]***
  - 1a. Regulación de la Síntesis:
    - Regulación de la transcripción: inhibición de la transcripción mediada por esteroles y a través de los SER/SRE-BP
    - Regulación post-transcripcional: control de la traducción del RNAm mediada por intermediarios de la ruta no esteroles (mevalonato?, isopenteniladenina?)
  - 1b. Regulación de la Degradación: por productos esteroideos y no esteroideos
- **B. Control a "corto plazo": *reg. actividad enzimática***
  - 2. Inhibición por fosforilzación:
    - 2a. Fosforilación por PK hormono dependientes: (+) insulina; (-) glucagón
    - 2b. Fosforilación regulada por carga energética: PK AMP dependientes

1a. Regulación de la Síntesis de la HMG-CoA reductasa: regulación transcripcional

Cell, Vol. 89, 331–340, May 2, 1997, Copyright ©1997 by Cell Press

The SREBP Pathway: Regulation of Cholesterol Metabolism by Proteolysis of a Membrane-Bound Transcription Factor

Review

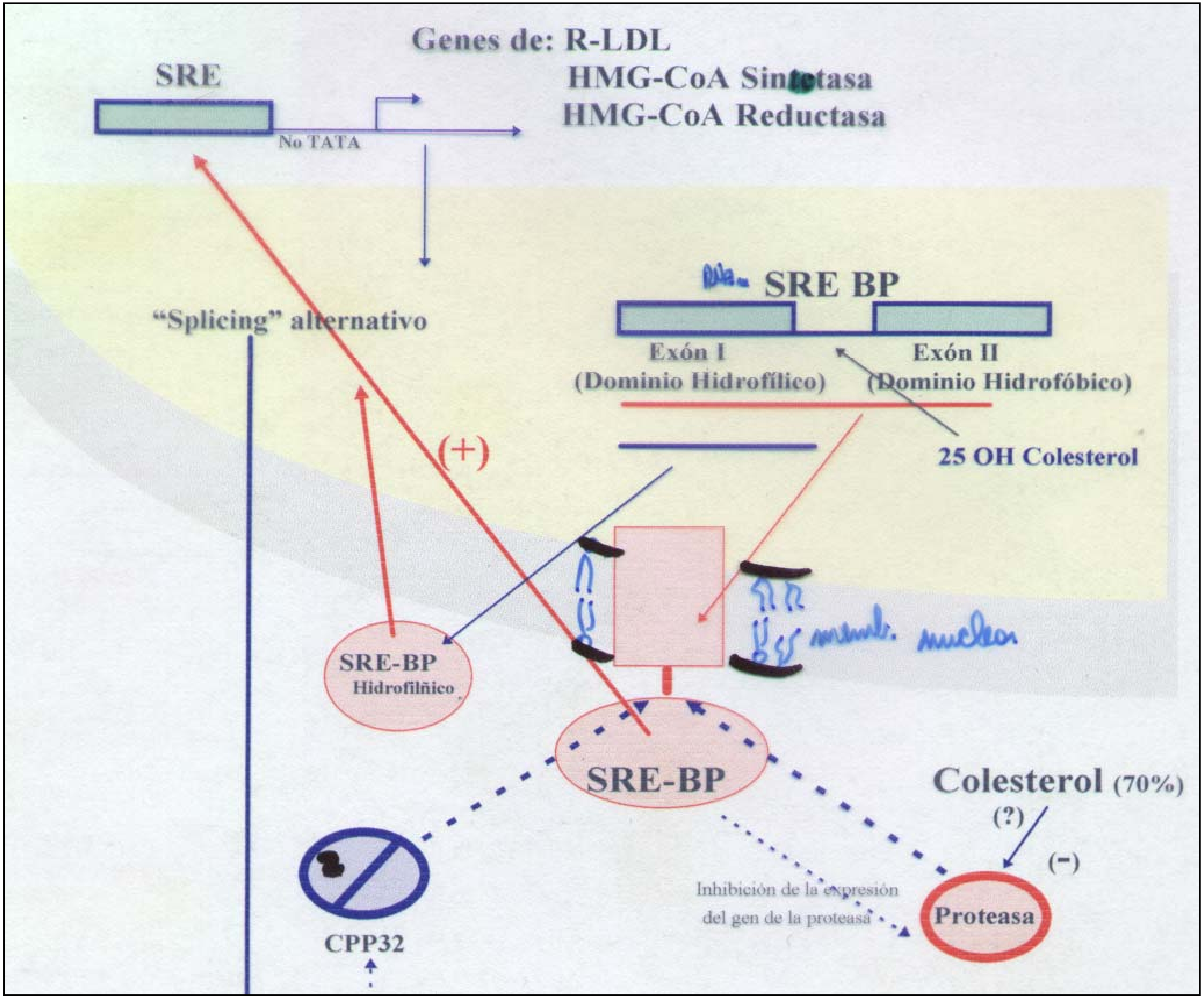
Michael S. Brown and Joseph L. Goldstein
Department of Molecular Genetics
University of Texas
Southwestern Medical Center
Dallas, Texas 75235

Animal cells must regulate their biosynthetic pathways so as to produce the required amounts of end-products without risking overproduction. Such control is particularly important in cholesterol homeostasis because cholesterol must be supplied for many cellular functions, including two recently recognized ones: formation of caveolae (Smart et al., 1994; Murata et al., 1995) and covalent modification of embryonic signaling proteins (Porter et al., 1996). Excess cholesterol must be avoided

CoA reductase), which converts HMG CoA to the 6-carbon intermediate, mevalonate (Bucher et al. 1960; Siperstein and Fagan, 1966). The latter is converted to isopentenyl pyrophosphate, which is polymerized and modified to form the 27 carbons of cholesterol. Cholesterol accumulation lowers the activity of HMG CoA reductase and several other enzymes in the cholesterol biosynthetic pathway, thereby limiting the production of cholesterol (reviewed in Goldstein and Brown, 1990).

The importance of the cholesterol feedback system to human health was established by the finding that diets rich in cholesterol and saturated fatty acids raise blood cholesterol levels and cause heart attacks (Keys, 1975). In addition to suppressing synthesis of cholesterol, high cholesterol diets act through the feedback system to reduce the liver's uptake of cholesterol by

Cell 89: 331-340; 1997



Genes de: R-LDL
SRE
HMG-CoA Sintetasa
HMG-CoA Reductasa
No TATA
SRE BP
"Splicing" alternativo
Exón I
(Dominio Hidrofílico)
Exón II
(Dominio Hidrofóbico)
(+)
25 OH Colesterol
memb. nuclear
SRE-BP
Hidrofilñico
SRE-BP
Colesterol (70%)
(?)
(-)
Inhibición de la expresión
del gen de la proteasa
Proteasa
CPP32

Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 11603–11607, December 1993
Biochemistry

SREBP-2, a second basic–helix–loop–helix–leucine zipper protein that stimulates transcription by binding to a sterol regulatory element

(cDNA cloning/cholesterol/low density lipoprotein receptor/3-hydroxy-3-methylglutaryl-coenzyme A synthase)

Xianxin Hua, Chieko Yokoyama, Jian Wu, Michael R. Briggs*, Michael S. Brown, Joseph L. Goldstein, and Xiaodong Wang

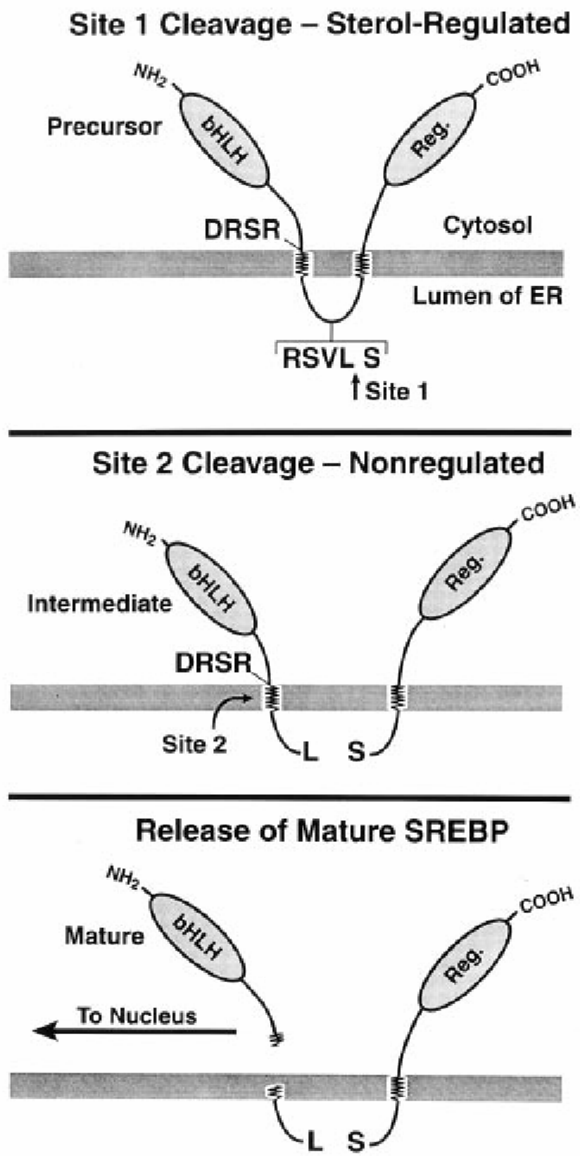
Department of Molecular Genetics, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235

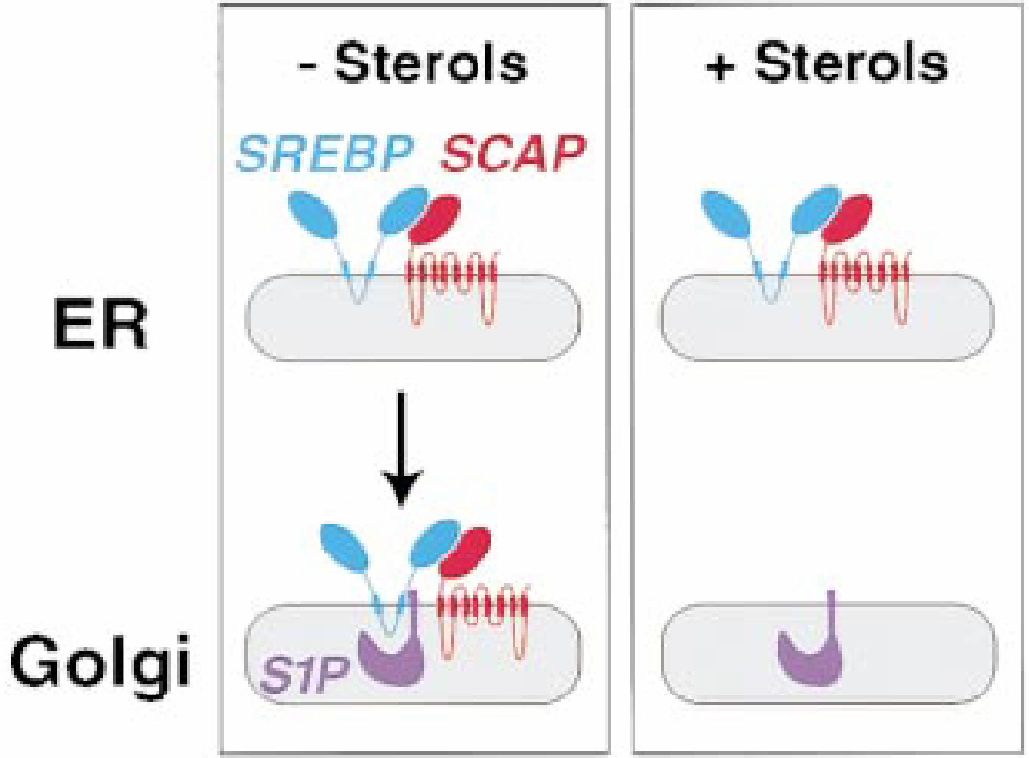
Contributed by Michael S. Brown, September 9, 1993

ABSTRACT **We report the cDNA cloning of SREBP-2, the second member of a family of basic–helix–loop–helix–leucine zipper (bHLH-Zip) transcription factors that recognize sterol regulatory element 1 (SRE-1). SRE-1, a conditional enhancer in the promoters for the low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl-coenzyme A synthase genes, increases transcription in the absence of sterols and is inactivated when sterols accumulate. Human SREBP-2 contains 1141 amino acids and is 47% identical to human SREBP-1a, the first recognized member of this family. The resemblance includes an acidic NH_2 terminus, a highly conserved bHLH-Zip motif (71% identical), and an unusually long extension of 740 amino acids**

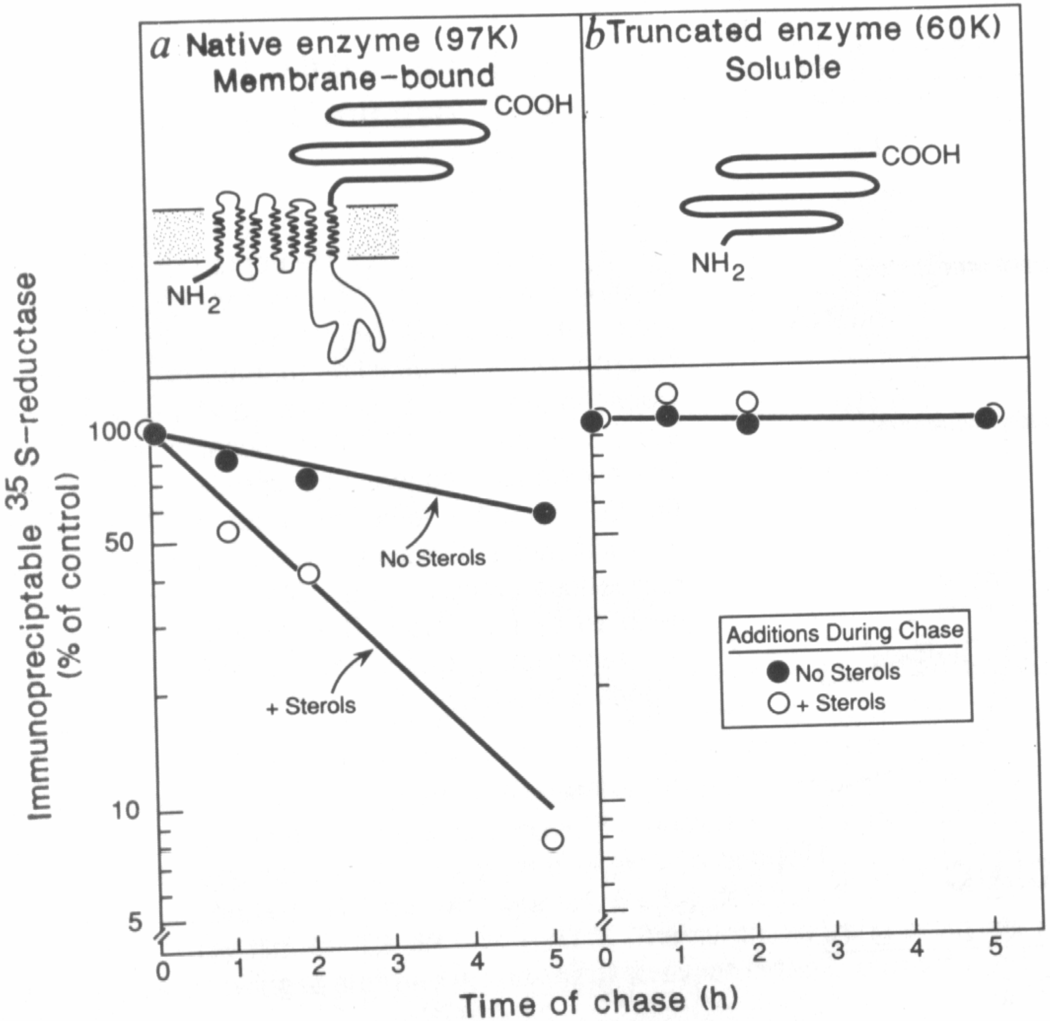
Sequences of six peptides were obtained from a mixed preparation of SREBPs, and a cDNA that encoded a protein containing five of the peptides was isolated (5). The protein, designated SREBP-1, is a member of the basic–helix–loop–helix–leucine zipper (bHLH-Zip) family of transcription factors. SREBP-1 and its bHLH-Zip domain were produced by recombinant methods and shown to bind SRE-1 with perfect specificity as defined by the 16 point mutants (5). A cDNA encoding SREBP-1a activated transcription of reporter genes containing the SRE-1 when introduced into simian CV-1 cells or human embryonic kidney 293 cells by cotransfection. Surprisingly, SREBP-1a activated transcription in sterol-

PNAS 90: 11603 – 07; 1993


Site 1 Cleavage – Sterol-Regulated
NH_2
COOH
Precursor
bHLH
Reg.
DRSR
Cytosol
Lumen of ER
RSVL S
Site 1
Site 2 Cleavage – Nonregulated
NH_2
COOH
Intermediate
bHLH
Reg.
DRSR
Site 2
L S
Release of Mature SREBP
NH_2
COOH
Mature
bHLH
Reg.
To Nucleus
L S


- Sterols
+ Sterols
SREBP
SCAP
ER
Golgi
S1P

1b. Regulación de la Degradación de la HMG-CoA Red.



Nature 343: 425 – 430; 1990

2b. Regulación de la HMG-CoA reductasa por carga energética

Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 9261–9265, October 1993
Biochemistry

Replacement of serine-871 of hamster 3-hydroxy-3-methylglutaryl-CoA reductase prevents phosphorylation by AMP-activated kinase and blocks inhibition of sterol synthesis induced by ATP depletion

(cholesterol/end-product feedback regulation/protein degradation/energy metabolism)

RYUICHIRO SATO, JOSEPH L. GOLDSTEIN, AND MICHAEL S. BROWN

Department of Molecular Genetics, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-9046

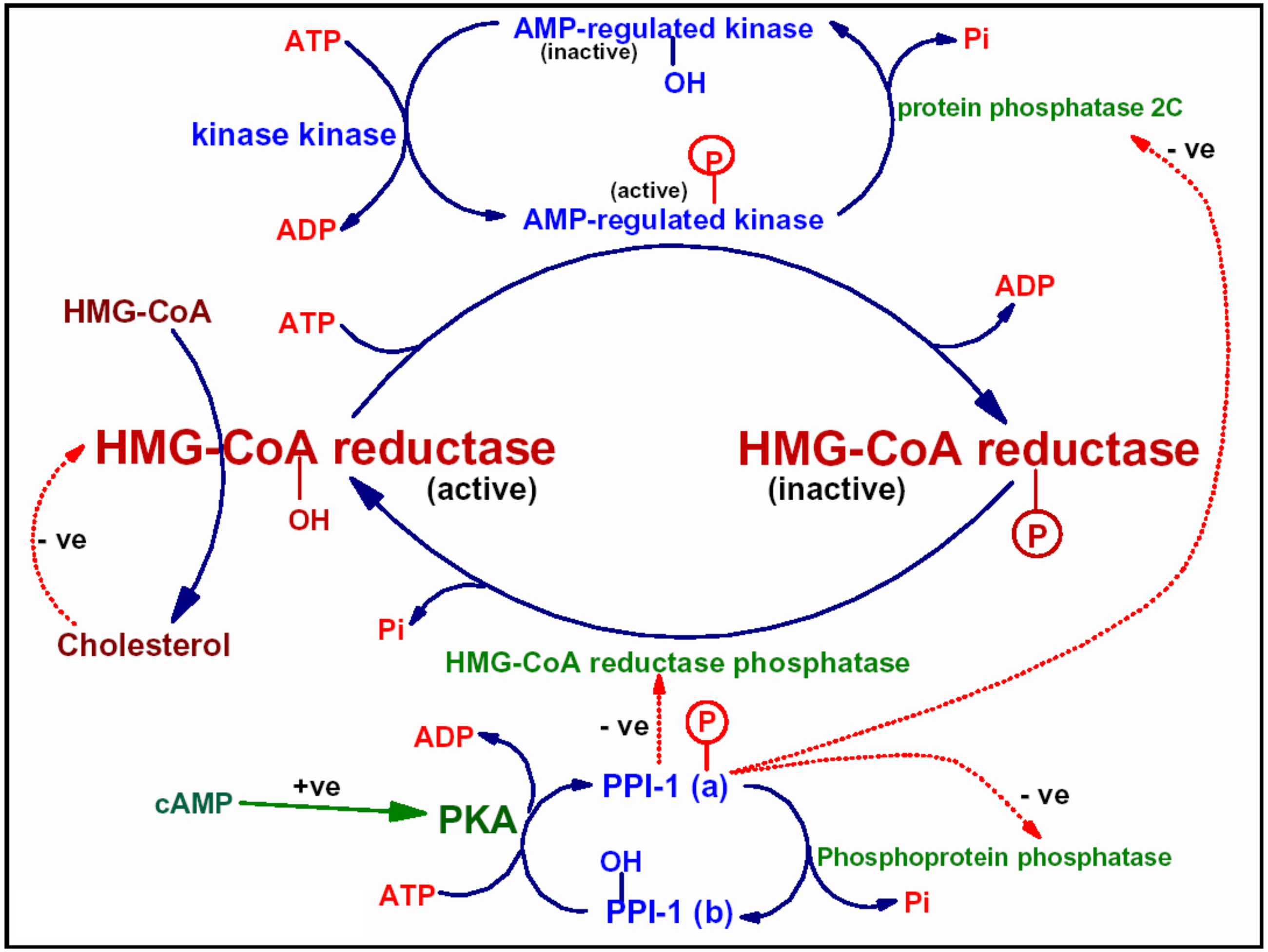
Contributed by Joseph L. Goldstein, June 30, 1993

ABSTRACT An AMP-activated protein kinase has been reported to phosphorylate rodent 3-hydroxy-3-methylglutaryl-coenzyme A reductase [HMG-CoA reductase; (*S*)-mevalonate:-NAD$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.88] at Ser-871, thereby lowering its catalytic activity [Clarke, P. R. & Hardie, D. G. (1990) *EMBO J.* 9, 2439–2446]. To explore the physiologic role of this reaction, we prepared a cDNA encoding a mutant form of hamster HMG-CoA reductase with alanine substituted for serine at residue 871. When overexpressed in transfected cells, the wild-type enzyme, but not the Ser-871 to Ala mutant, was labeled with [^{32}P]phosphate, confirming Ser-871 as the site of phosphorylation. The wild-type enzyme, but

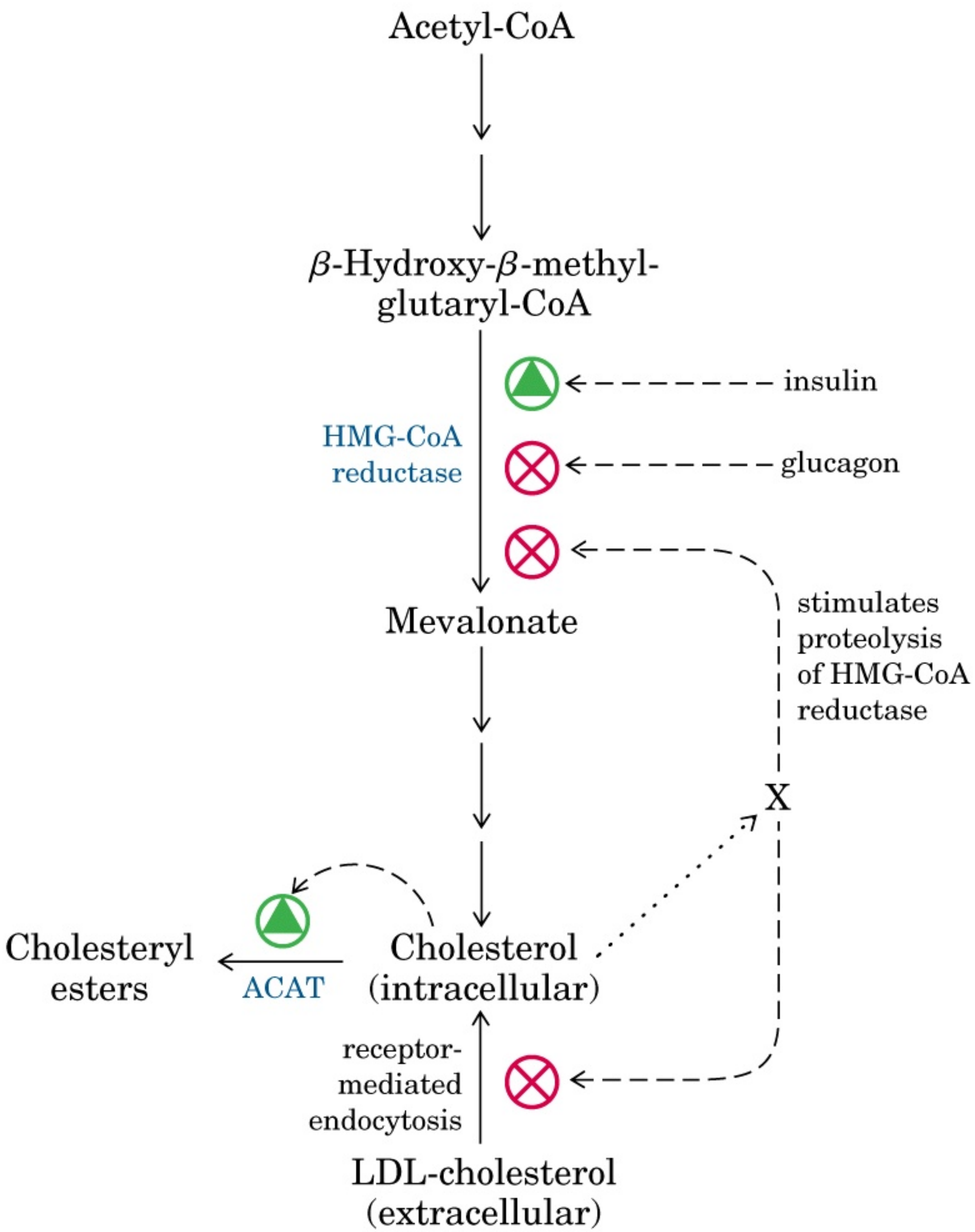
is near the C terminus of the protein (3). The reaction is catalyzed by a unique kinase that uses ATP as a phosphate donor and requires AMP as an activator (4, 5). Phosphorylation decreases the catalytic activity of the enzyme by ≈80%. Although some groups have speculated that phosphorylation accelerates the degradation of HMG-CoA reductase protein (6, 7), other groups have found no evidence for such an effect (8).

The AMP-activated kinase also phosphorylates and inactivates acetyl-CoA carboxylase, thereby potentially inhibiting the synthesis of fatty acids as well as cholesterol (4, 5). Hardie (4, 5) has suggested that the AMP-activated kinase

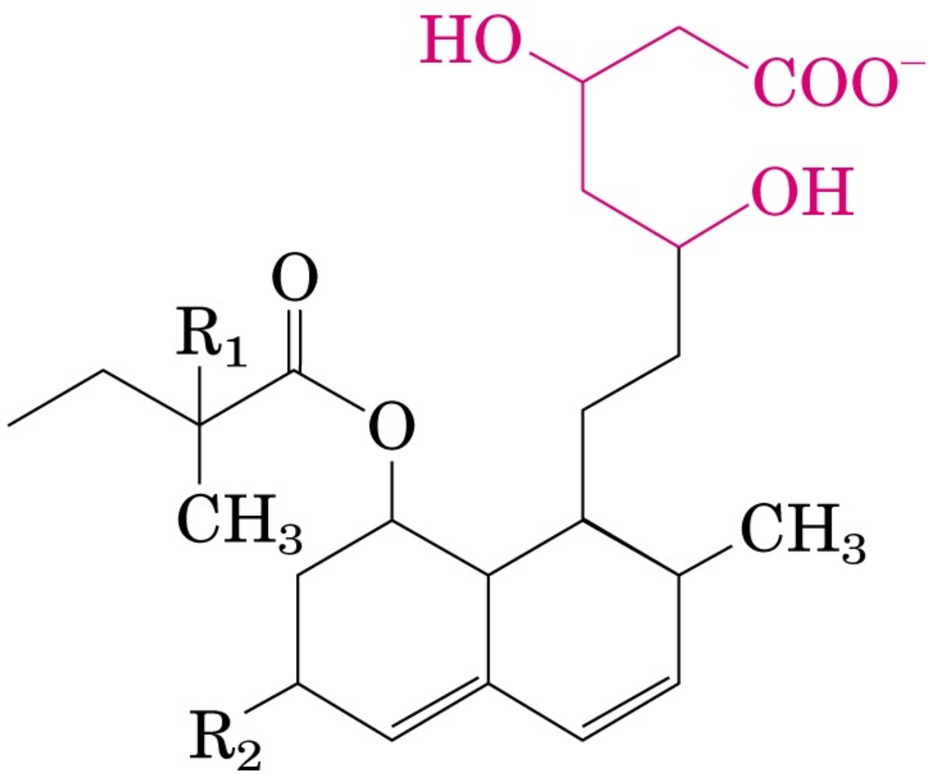
2b. Regulación de la HMG-CoA reductasa por carga energética

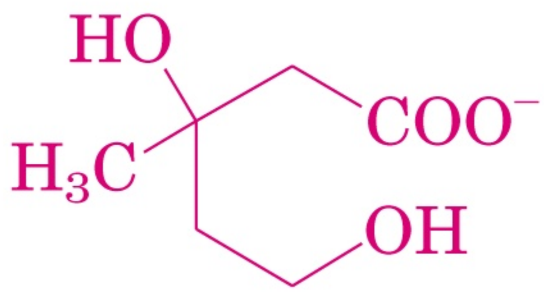


Panorámica regulación del metabolismo del colesterol



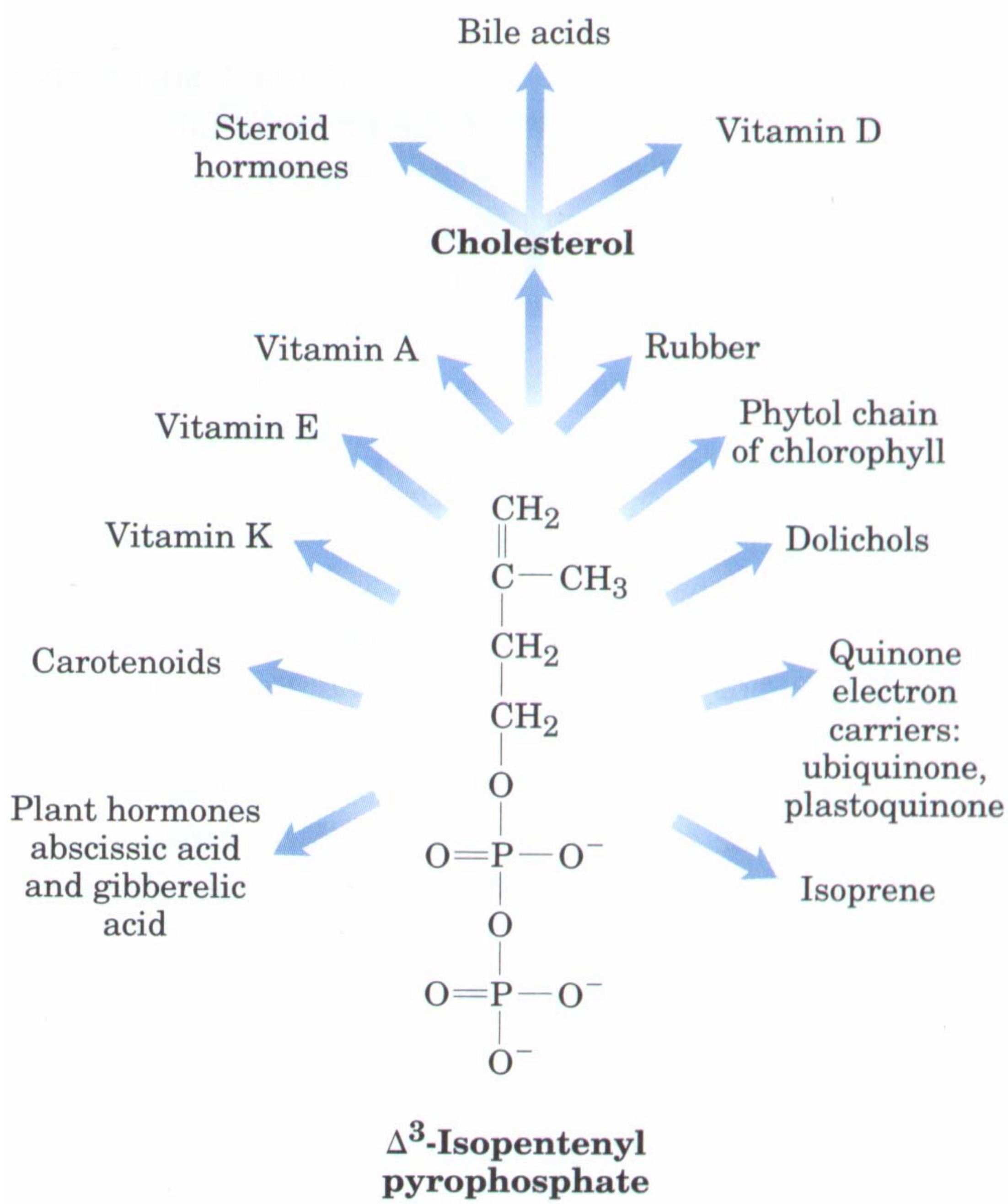
Farmacología - Terapéutica



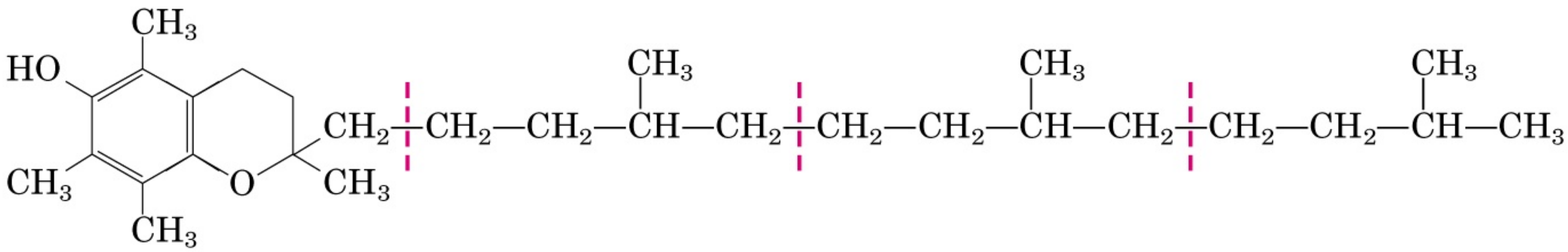


Mevalonate

$R_1 = H$	$R_2 = H$	Compactin
$R_1 = CH_3$	$R_2 = CH_3$	Simvastatin (Zocov)
$R_1 = H$	$R_2 = OH$	Pravastatin (Pravacol)
$R_1 = H$	$R_2 = CH_3$	Lovastatin (Mevacor)

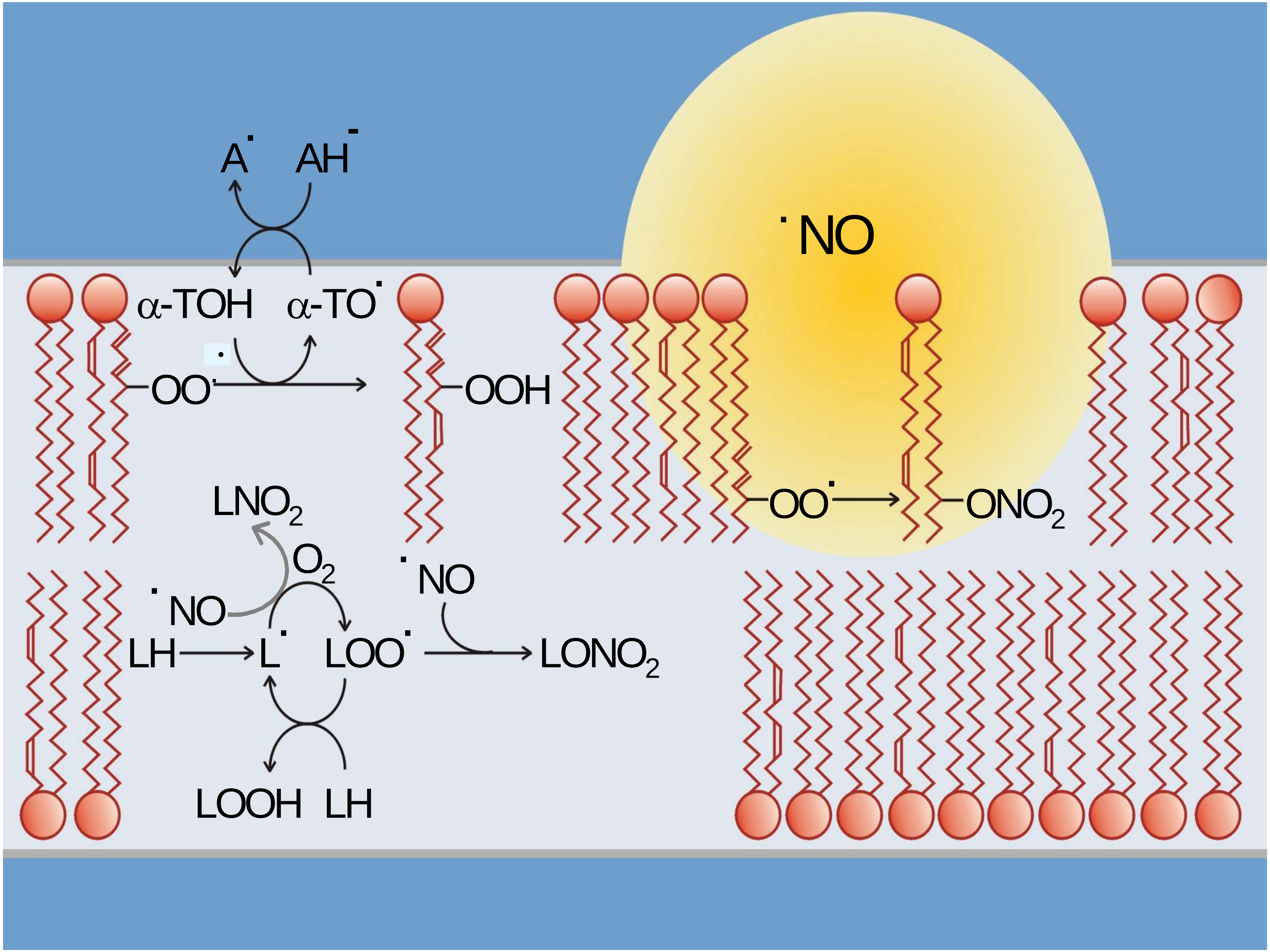


Bile acids
Steroid hormones
Vitamin D
Cholesterol
Vitamin A
Rubber
Vitamin E
Phytol chain of chlorophyll
Vitamin K
Dolichols
Carotenoids
Quinone electron carriers: ubiquinone, plastoquinone
Plant hormones abscissic acid and gibberelic acid
Isoprene
CH_2
C
CH_3
CH_2
CH_2
O
$O{=}P{-}O^-$
O
$O{=}P{-}O^-$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

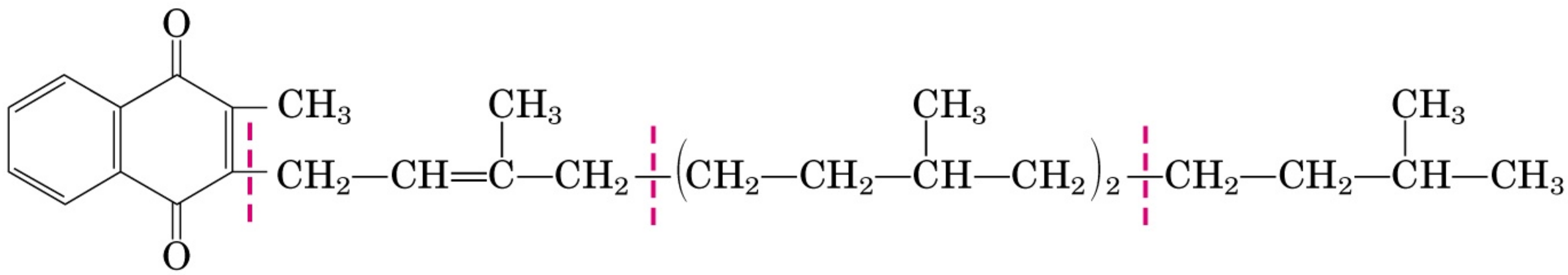


(a)

Vitamin E: an antioxidant



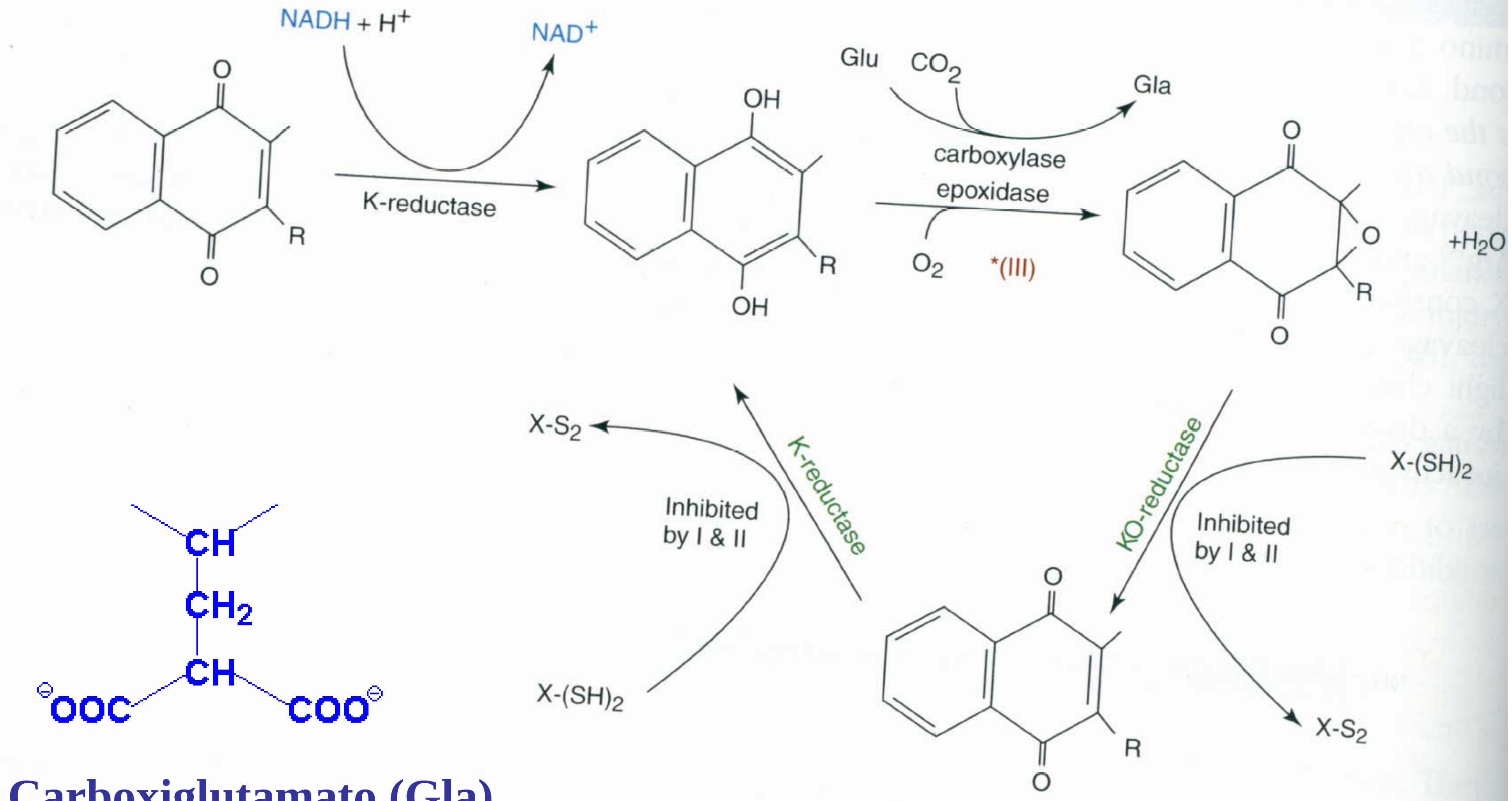
A˙ AH⁻
α-TOH α-TO˙
OO˙
OOH
˙NO
OO˙
ONO2
LNO2
O2
˙NO
˙NO
LH
L˙
LOO˙
LONO2
LOOH LH



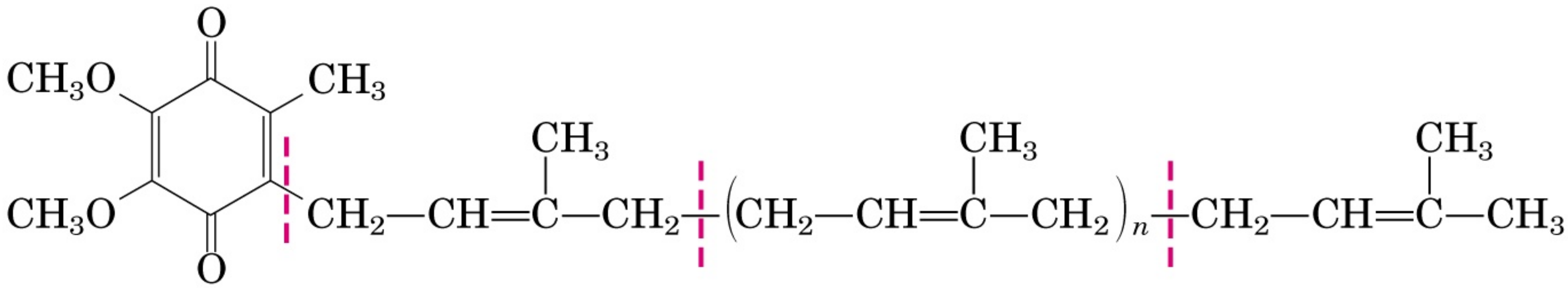
(b)

Vitamin K_1: a blood-clotting cofactor (phylloquinone)

Mecanismo de acción de la vitamina K



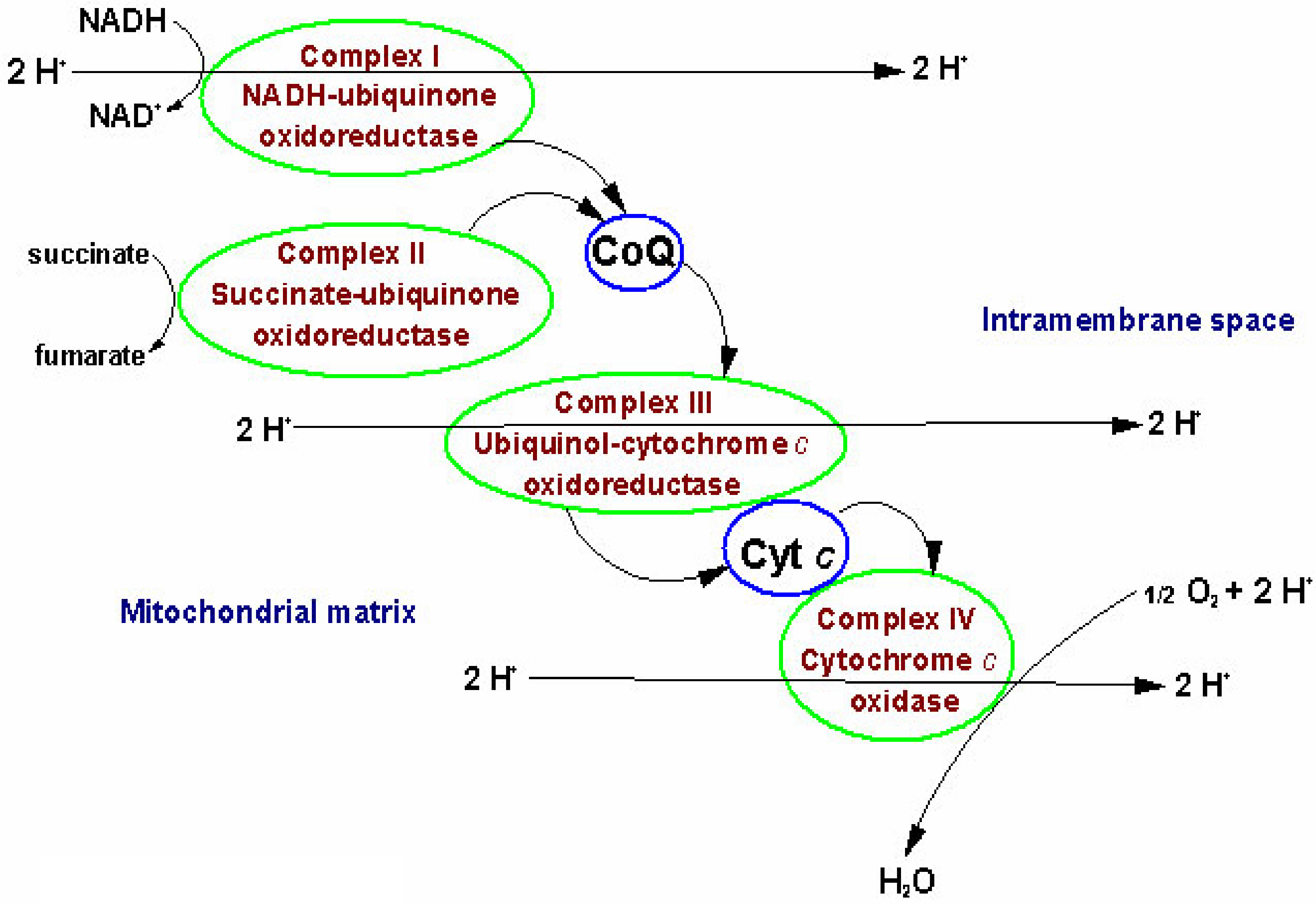
γ- Carboxiglutamato (Gla)

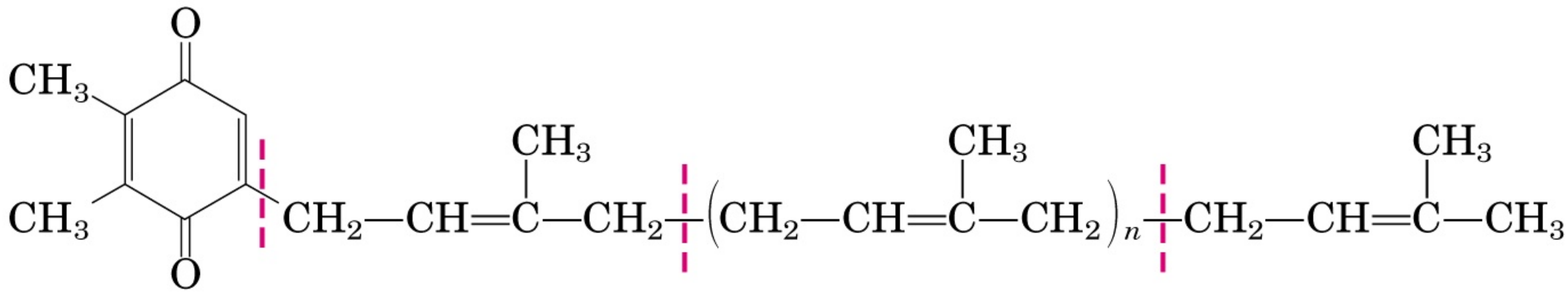


(d)

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

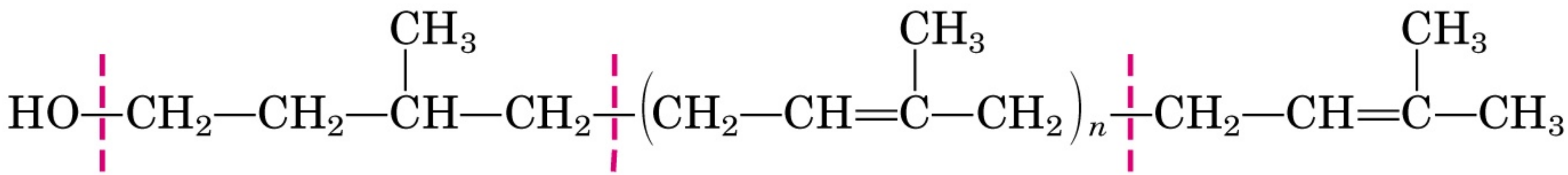
Flow of Electrons During Oxidative Phosphorylation





(e)

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

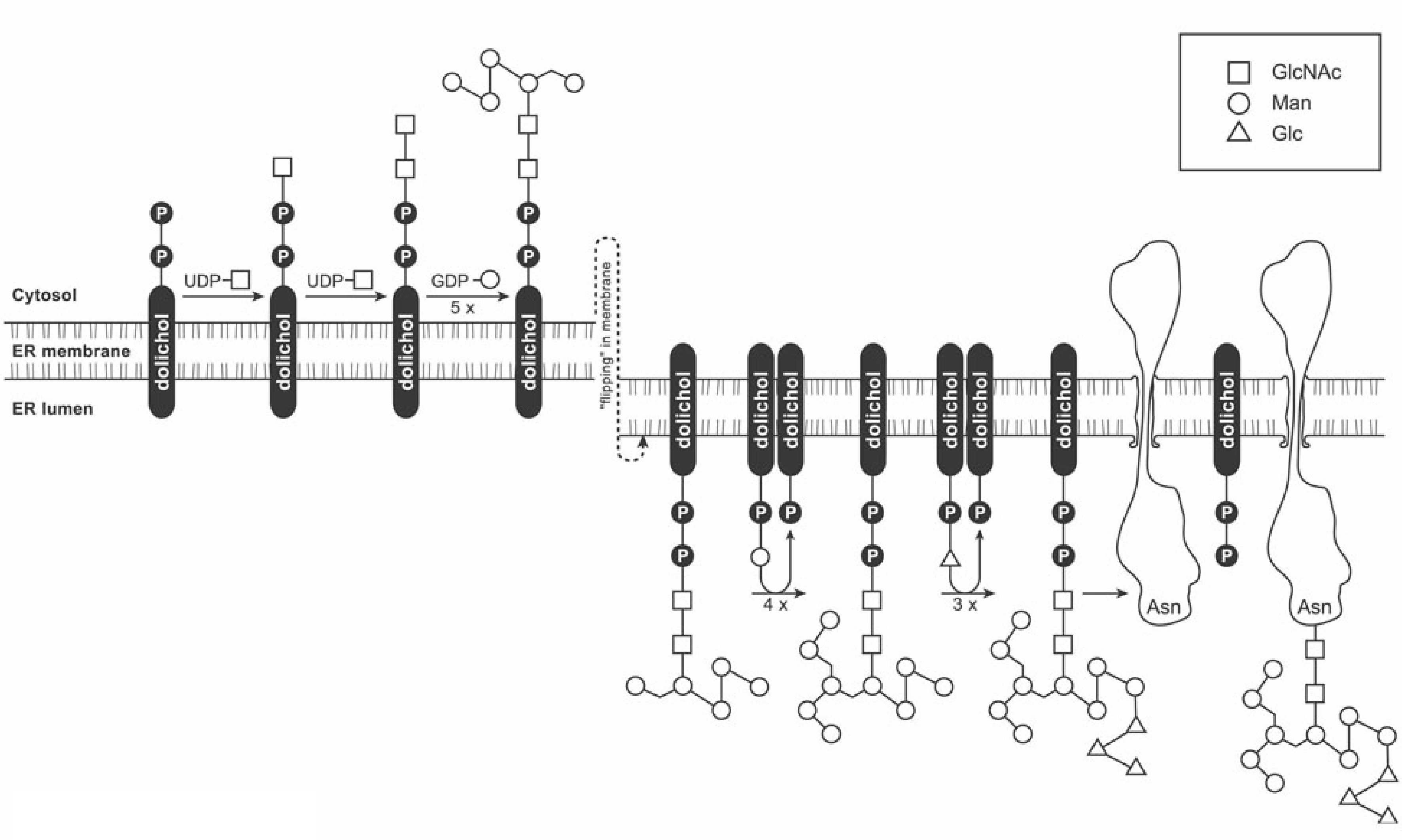


(f)

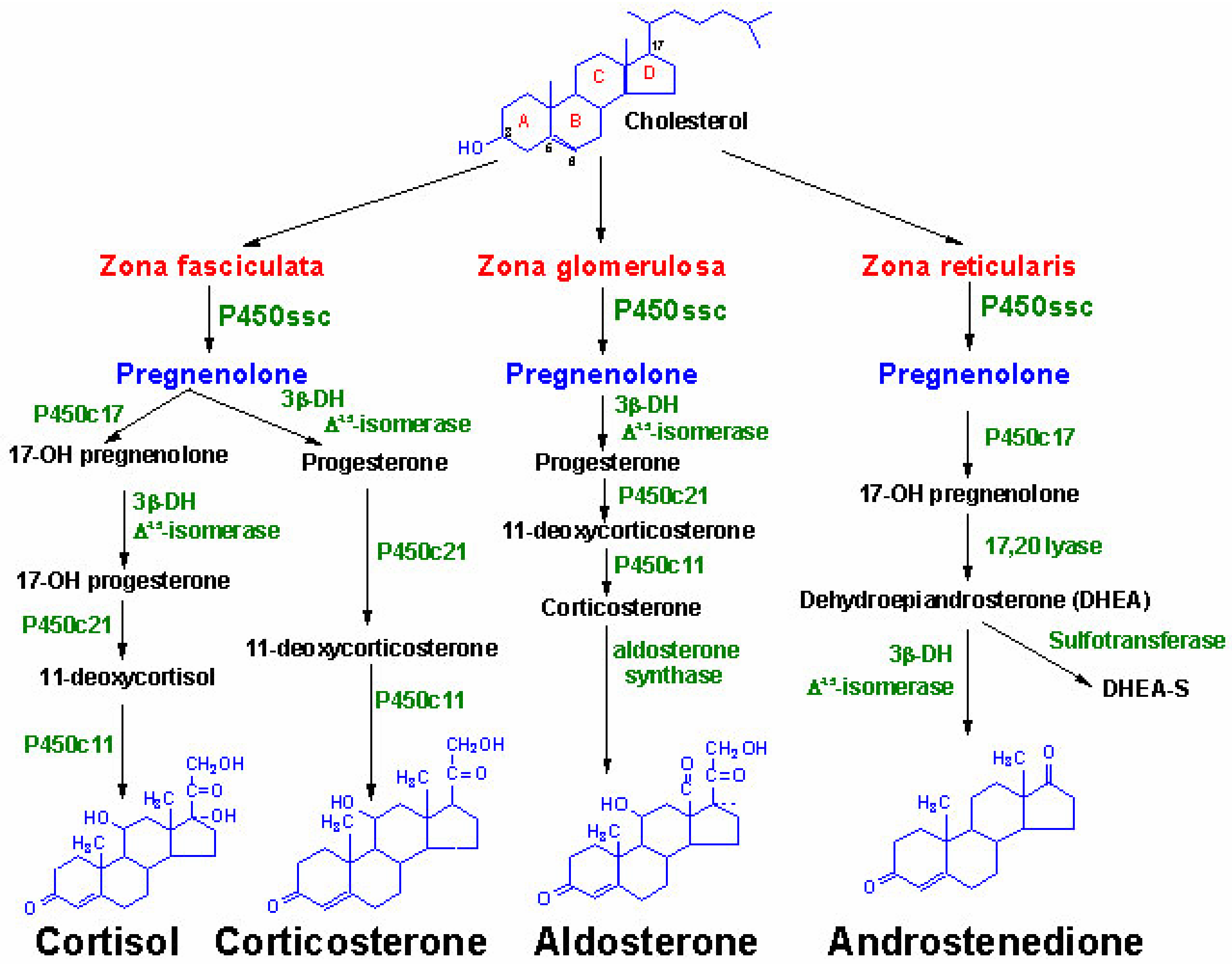
Dolichol: a sugar carrier

(n = 9–22)

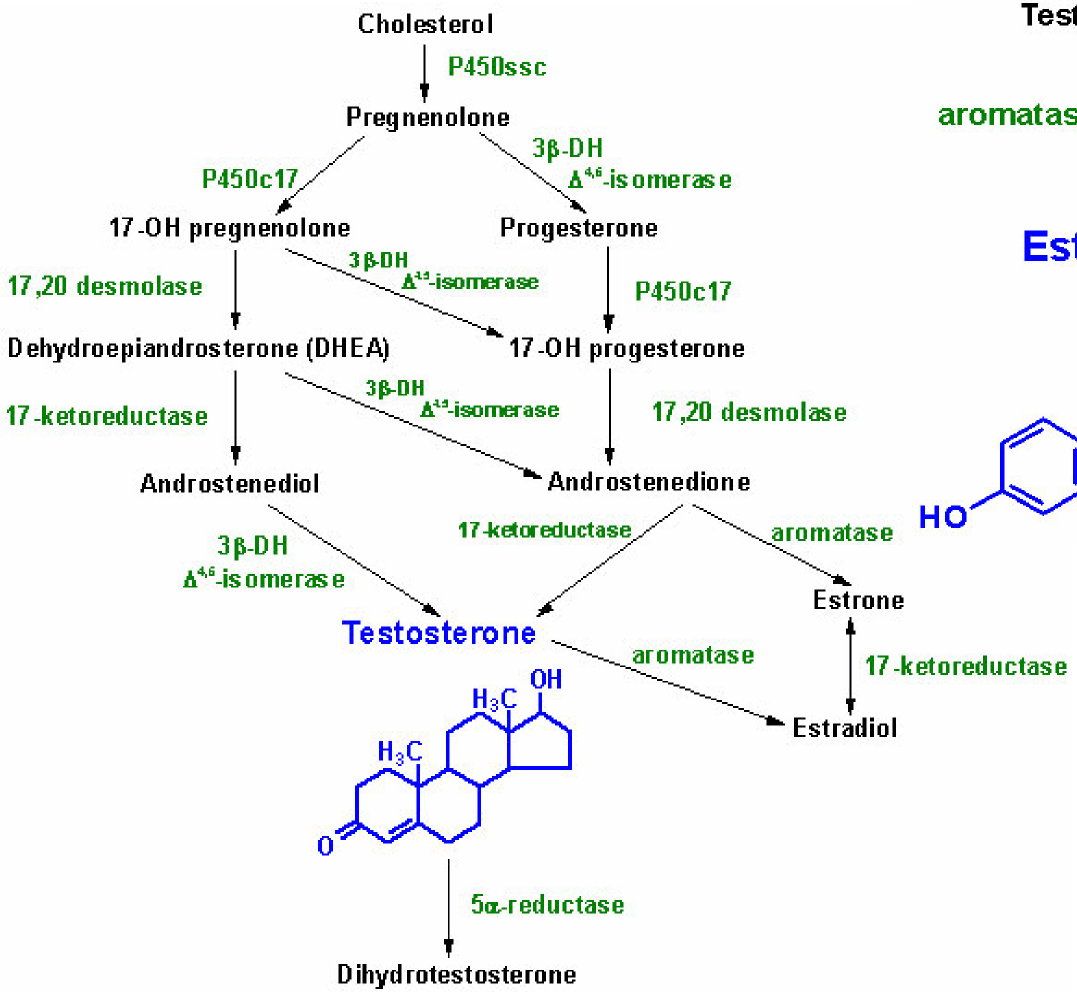
Mecanismo de acción del dolicol

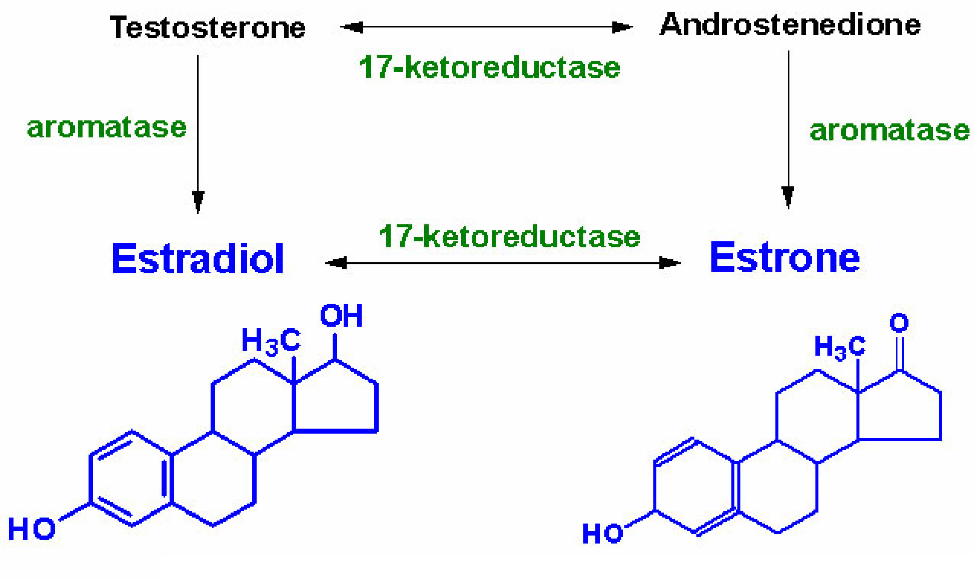


Formación de hormonas esteroideas a partir del colesterol

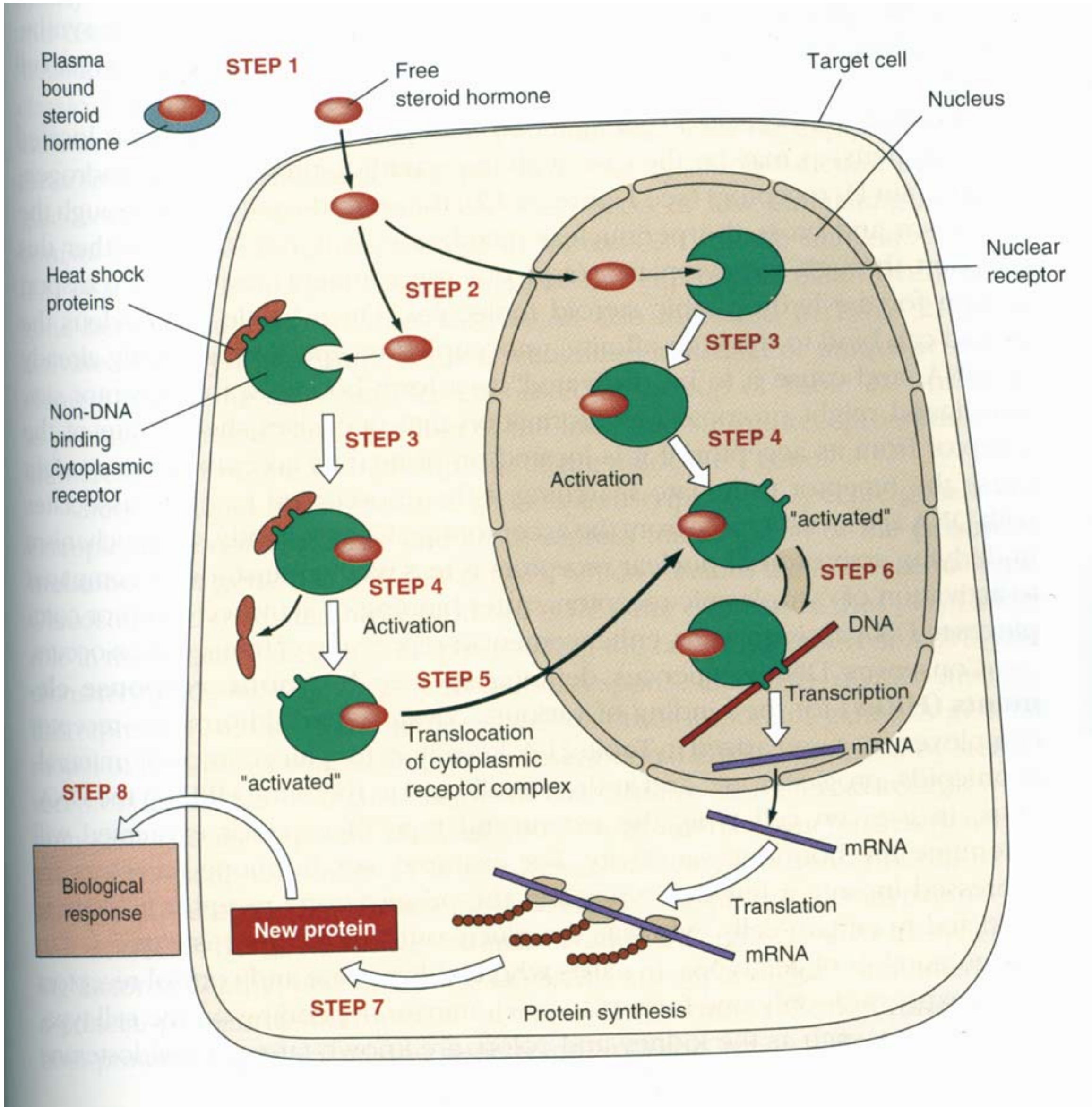


Formación de hormonas esteroideas a partir del colesterol

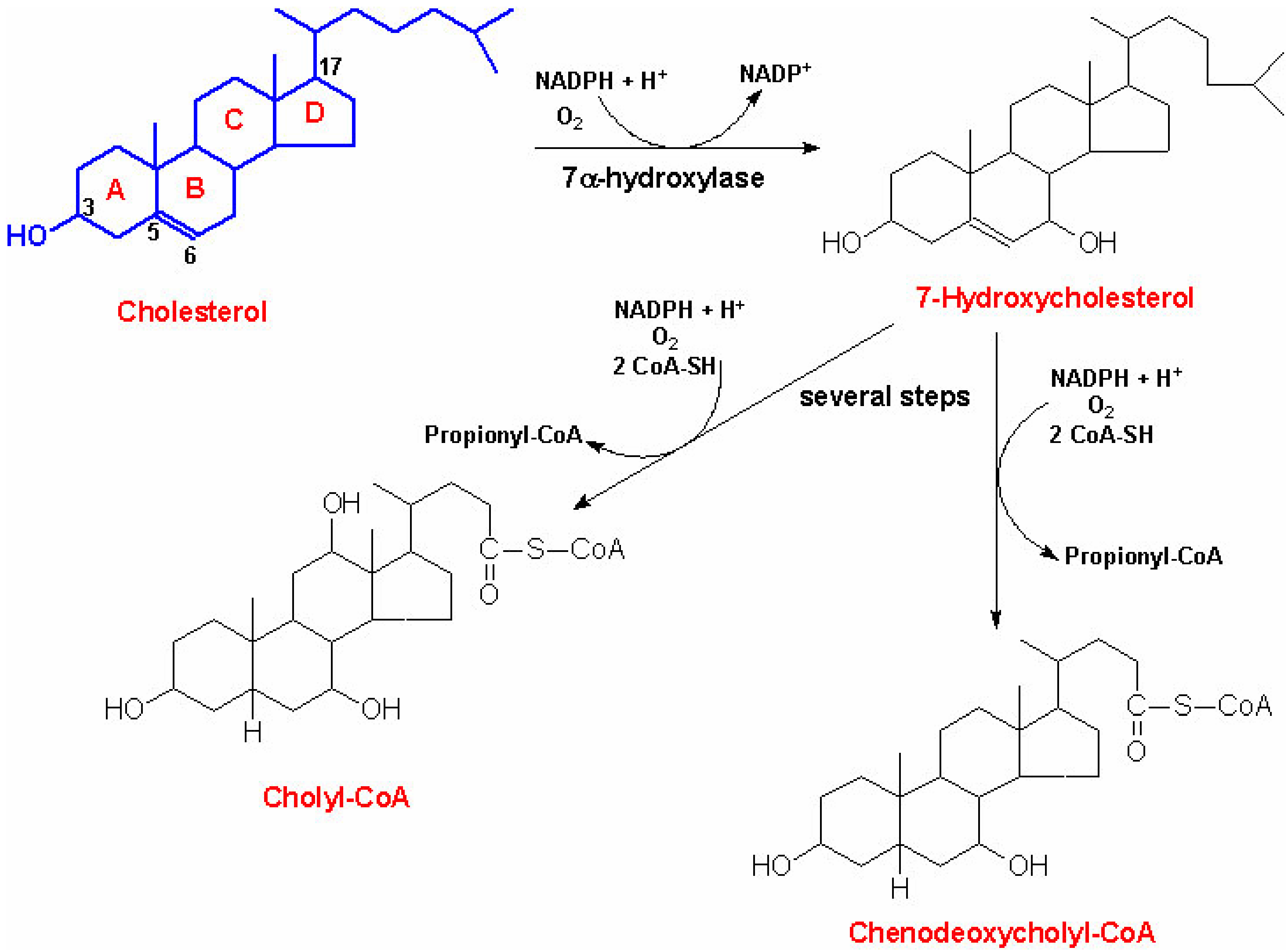




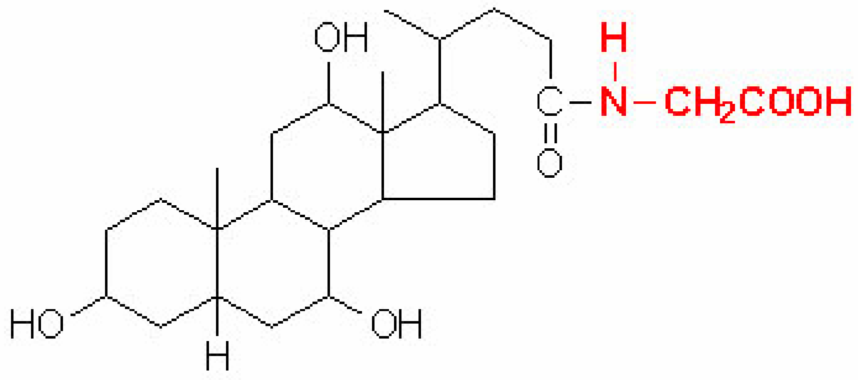
Mecanismo de acción de las hormonas esteroideas



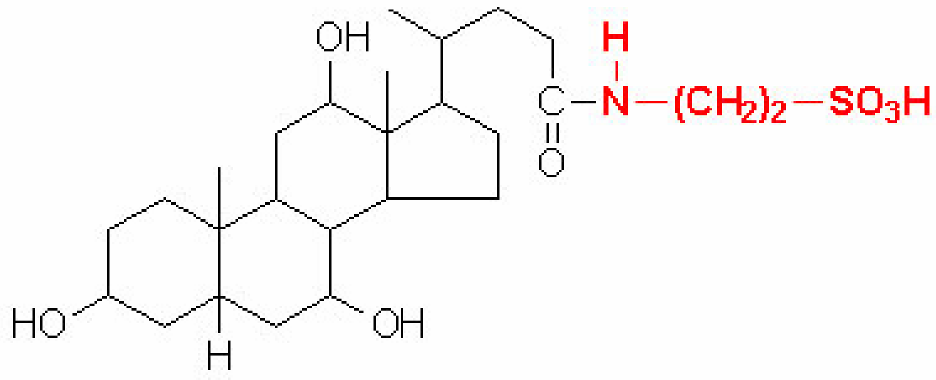
Formación de Sales Biliares



Sales Biliares Primarias



Glycocholic acid



Taurocholic acid

Sales Biliares Secundarias