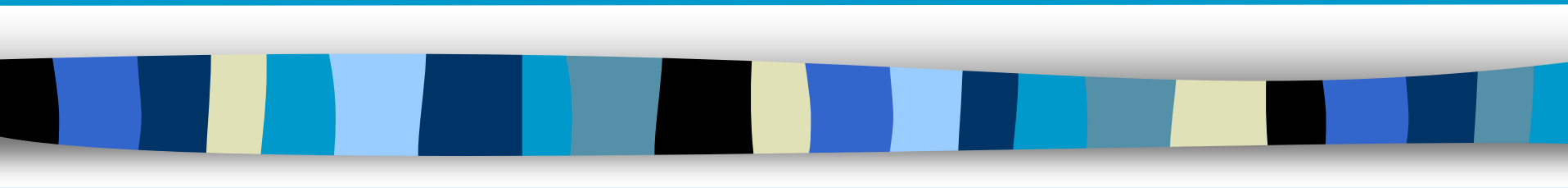
LIPID BIOSYNTHESIS



Outline

1. Fatty Acid Biosynthesis & Degradation
2. Biosynthesis of Complex Lipids
3. Eicosanoid Biosynthesis and Function
4. Cholesterol Biosynthesis
5. Transport via Lipoprotein Complexes
6. Biosynthesis of Bile Acids
7. Synthesis and Metabolism of Steroids

Fatty Acid Pathways

The Biosynthesis and Degradation Pathways are Different

- As in cases of glycolysis/ gluconeogenesis and glycogen synthesis/breakdown, fatty acid synthesis and degradation go by different routes
- There are four major differences between fatty acid breakdown and biosynthesis

  - **Intermediates in synthesis are linked to -SH groups of acyl carrier proteins (as compared to -SH groups of CoA)**
  - **Synthesis in cytosol; breakdown in mitochondria**
  - **Enzymes of synthesis are one polypeptide**
  - **Biosynthesis uses NADPH/$NADP^+$; breakdown uses NADH/NAD^+**

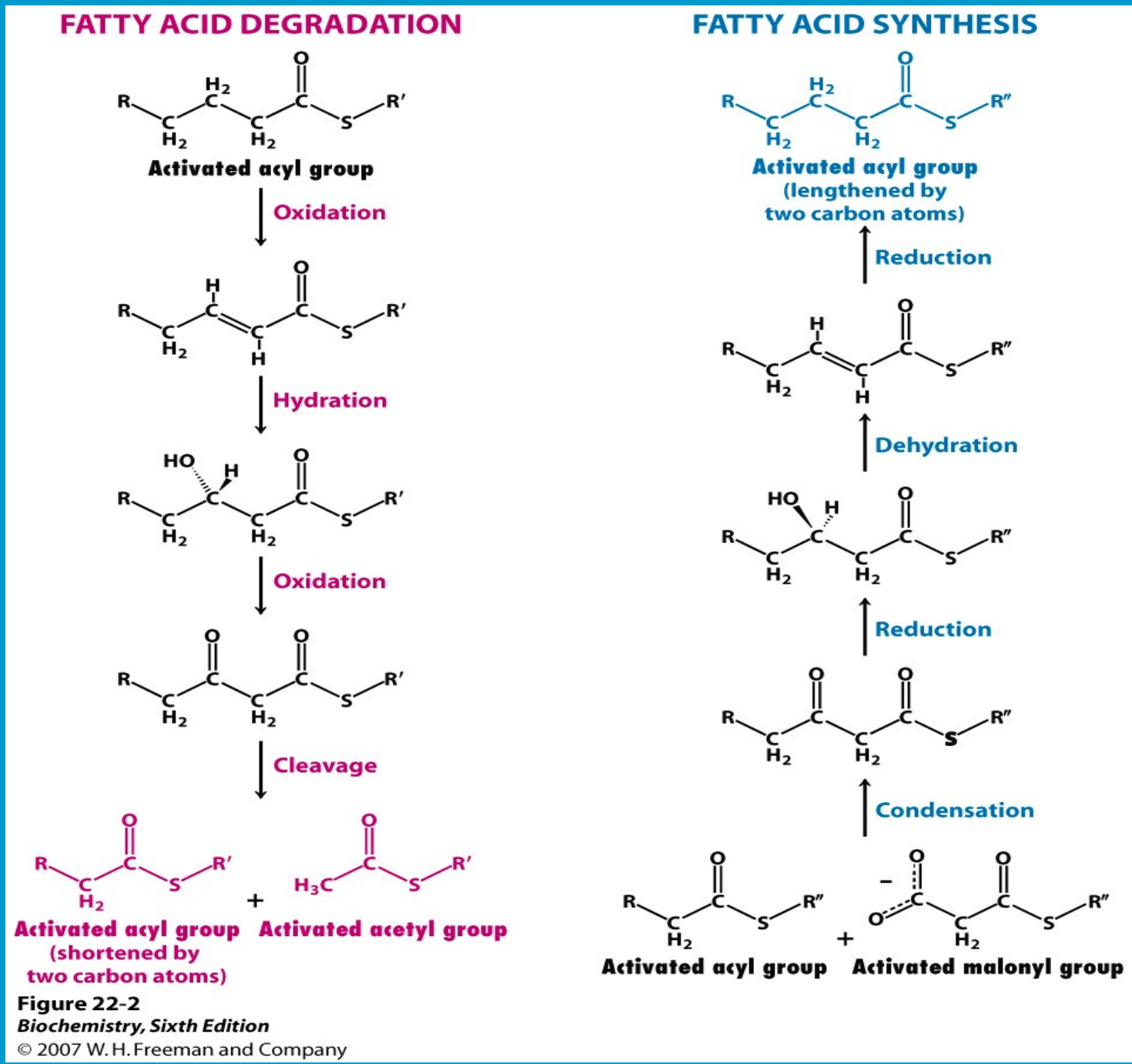


Figure 22-2
Biochemistry, Sixth Edition

Activation by Malonyl-CoA

Acetate Units are Activated for Transfer in Fatty Acid Synthesis by Malonyl-CoA

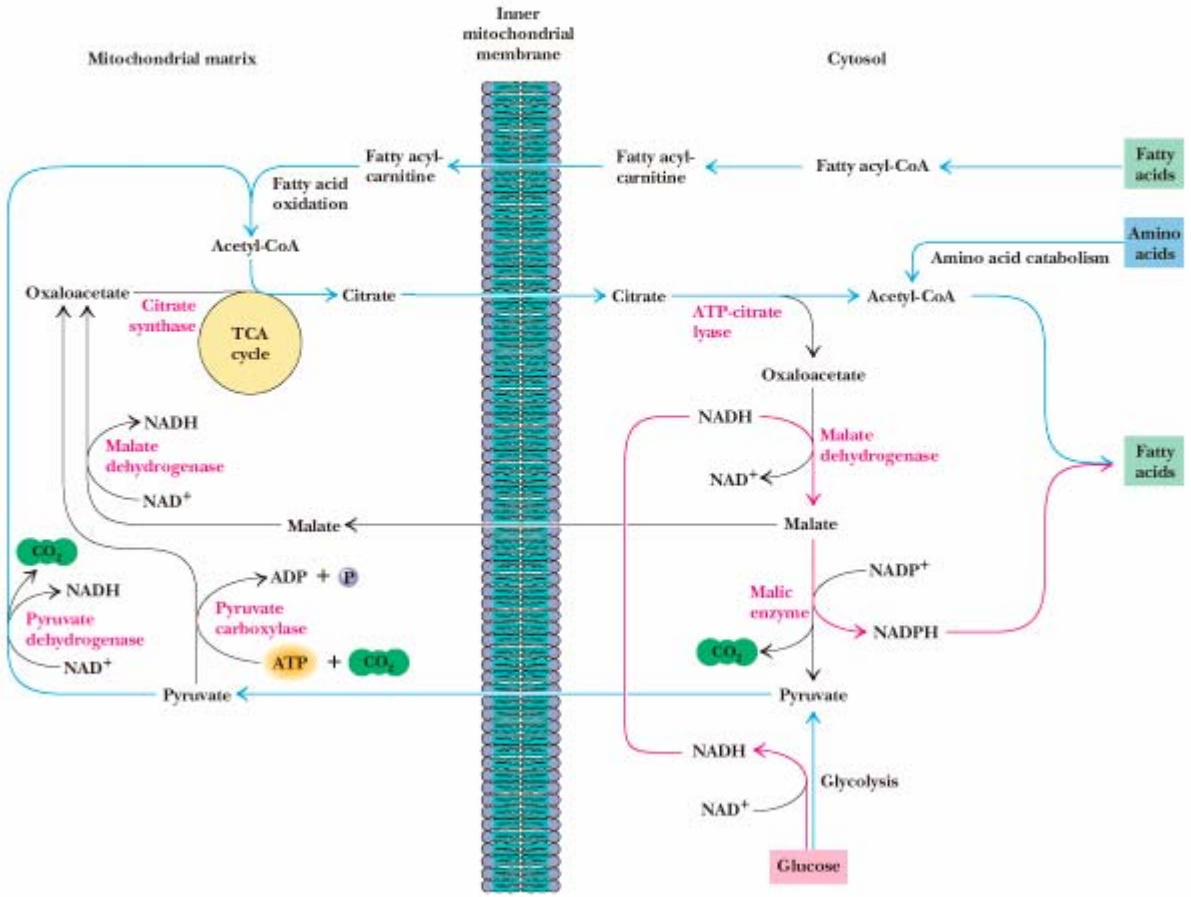
- Fatty acids are built from 2-C units - acetyl-CoA
- Acetate units are activated for transfer by conversion to malonyl-CoA
- Decarboxylation of malonyl-CoA and reducing power of NADPH drive chain growth
- Chain grows to 16-carbons
- Other enzymes add double bonds and more Cs

Challenge: Ac-CoA in Cytosol

What are the sources?

- Amino acid degradation produces cytosolic acetyl-CoA
- FA oxidation produces mitochondrial acetyl-CoA
- Glycolysis yields cytosolic pyruvate which is converted to acetyl-CoA in mitochondria
- Citrate-malate-pyruvate shuttle provides cytosolic acetate units and reducing equivalents for fatty acid synthesis

Garrett & Grisham: Biochemistry, 2/e
Figure 25.1



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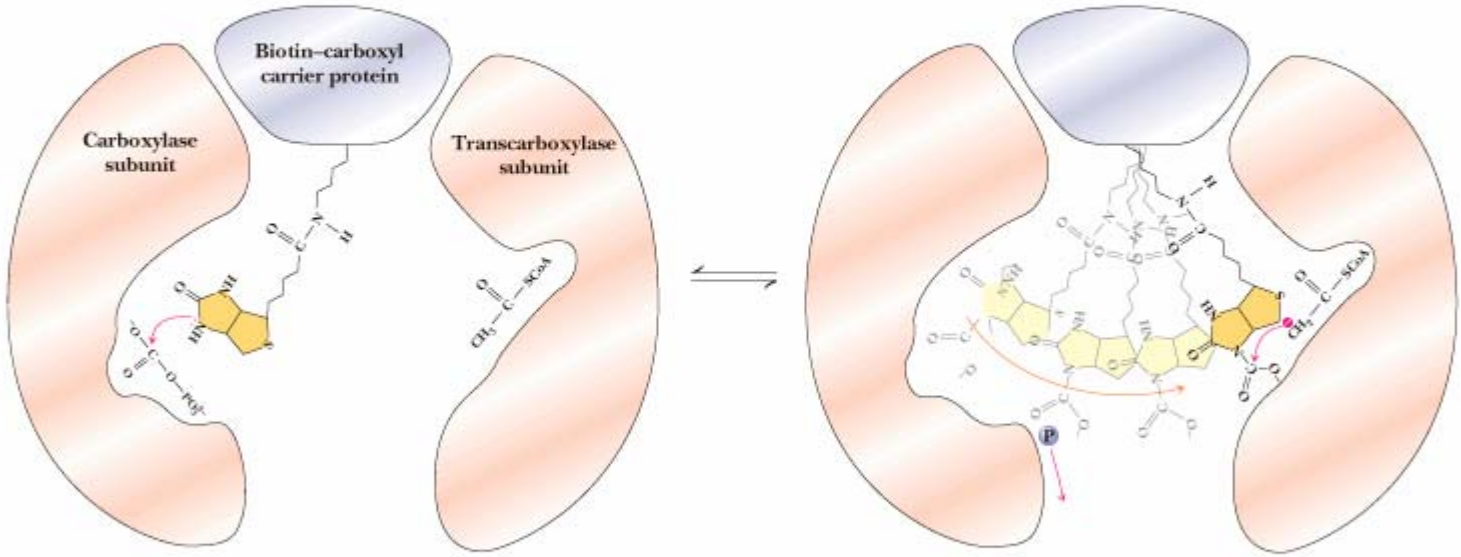
Acetyl-CoA Carboxylase

The "ACC enzyme" commits acetate to fatty acid synthesis

- Carboxylation of acetyl-CoA to form malonyl-CoA is the irreversible, committed step in fatty acid biosynthesis
- ACC uses bicarbonate and ATP (AND biotin!)
- *E.coli* enzyme has three subunits
- Animal enzyme is one polypeptide with all three functions - biotin carboxyl carrier, biotin carboxylase and transcarboxylase

Garrett & Grisham: Biochemistry, 2/e
Figure 25.3



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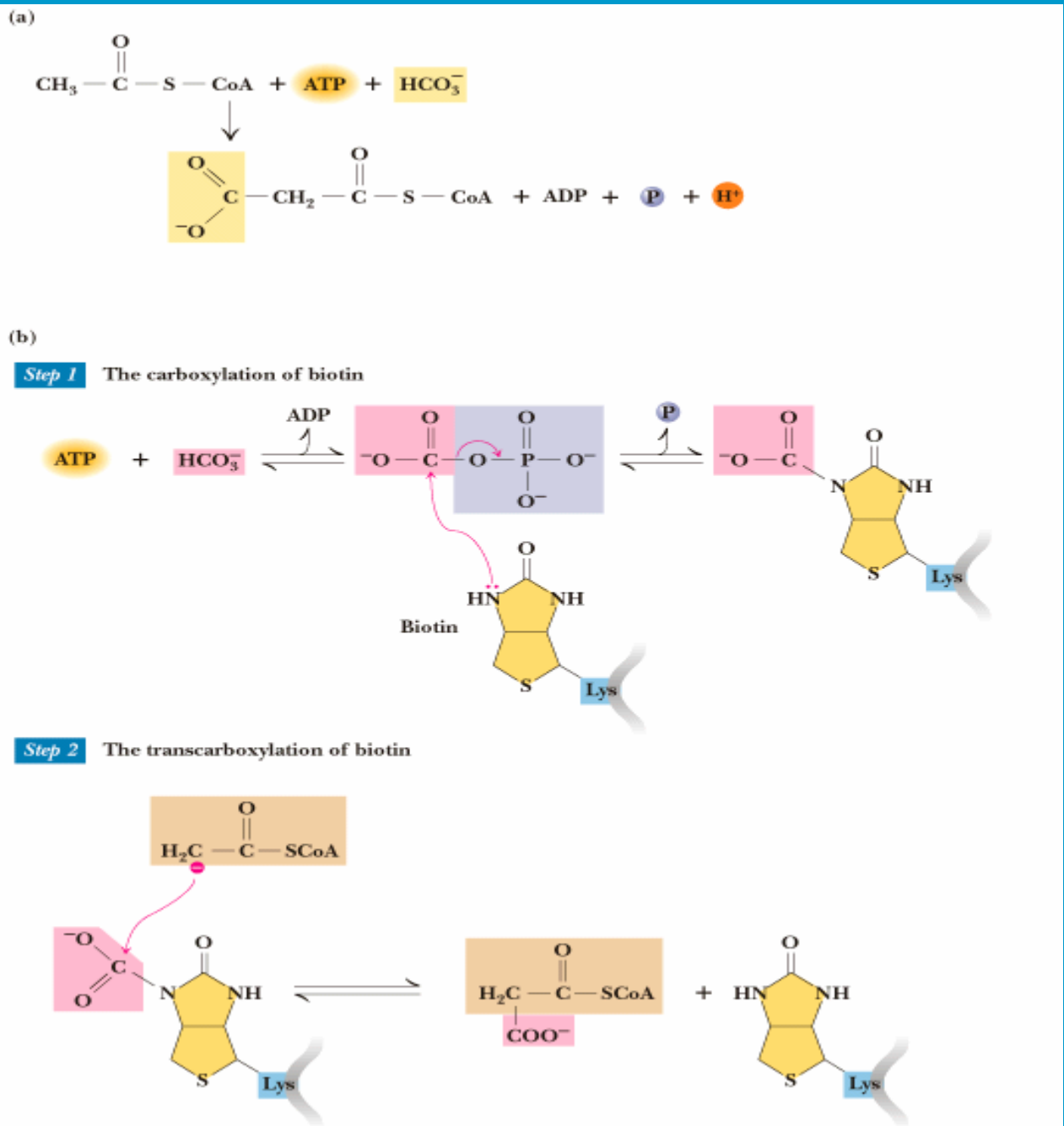

(a)
CH3 — C — S — CoA + ATP + HCO3−
ADP + P + H+
(b)
Step 1 The carboxylation of biotin
ATP + HCO3−
ADP
P
Biotin
Lys
Step 2 The transcarboxylation of biotin
H2C — C — SCoA
COO−
Lys

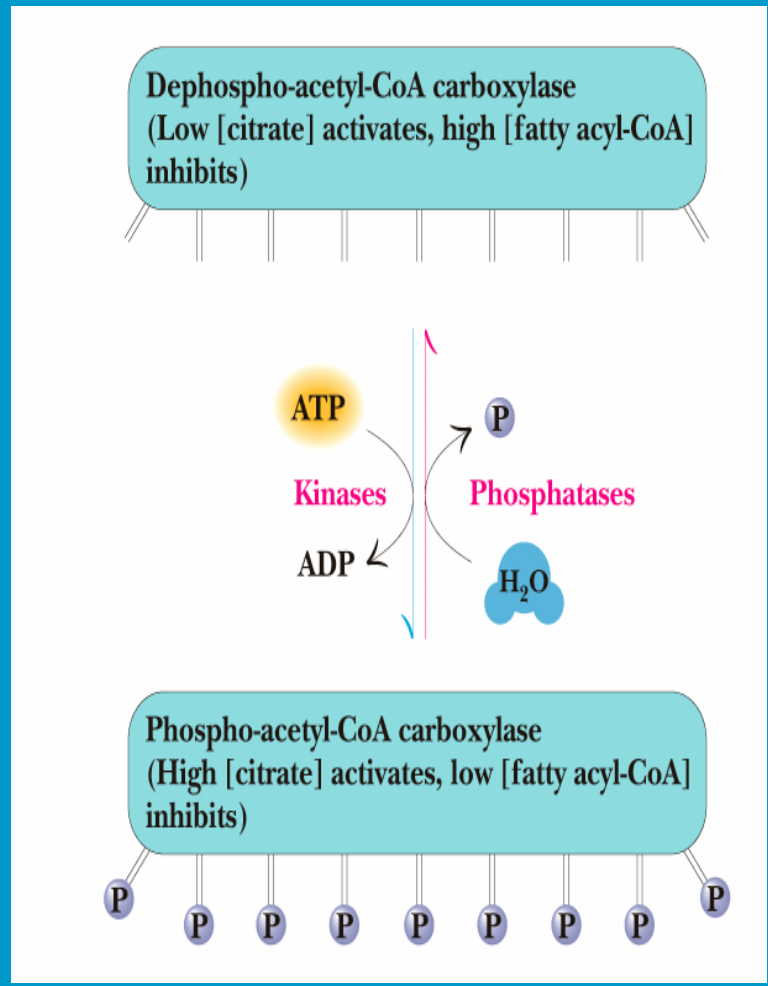
Acetyl-CoA Carboxylase II

ACC forms long, active filamentous polymers from inactive protomers

- As a committed step, ACC is carefully regulated
- Palmitoyl-CoA (product) favors monomers
- Citrate favors the active polymeric form
- Phosphorylation modulates citrate activation and palmitoyl-CoA inhibition

The Effect of Phosphorylation

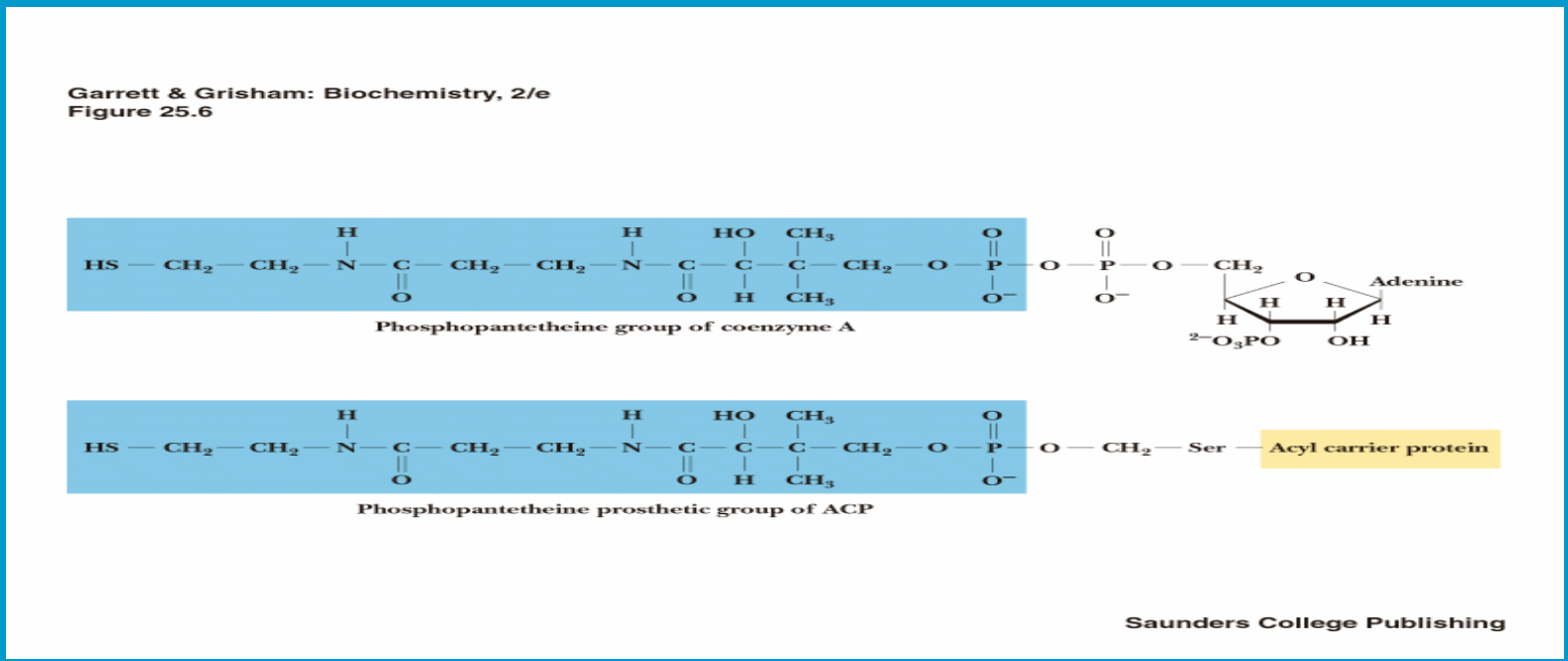
- Unphosphorylated E has low K_m for citrate and is active at low citrate
- Unphosphorylated E has high K_i for palm-CoA and needs high palm-CoA to inhibit
- Phosphorylated E has high K_m for citrate and needs high citrate to activate
- Phosphorylated E has low K_i for palm-CoA and is inhibited at low palm-CoA



The Acyl Carrier Protein

Carrier of intermediates in fatty acid synthesis

- Discovered by P. Roy Vagelos - a 77 residue protein in E.coli - with a phosphopantetheine
- In terms of function, it's a large CoA
- See Figure below to compare ACP and CoA



Fatty Acid Synthesis

BACTERIA AND PLANTS

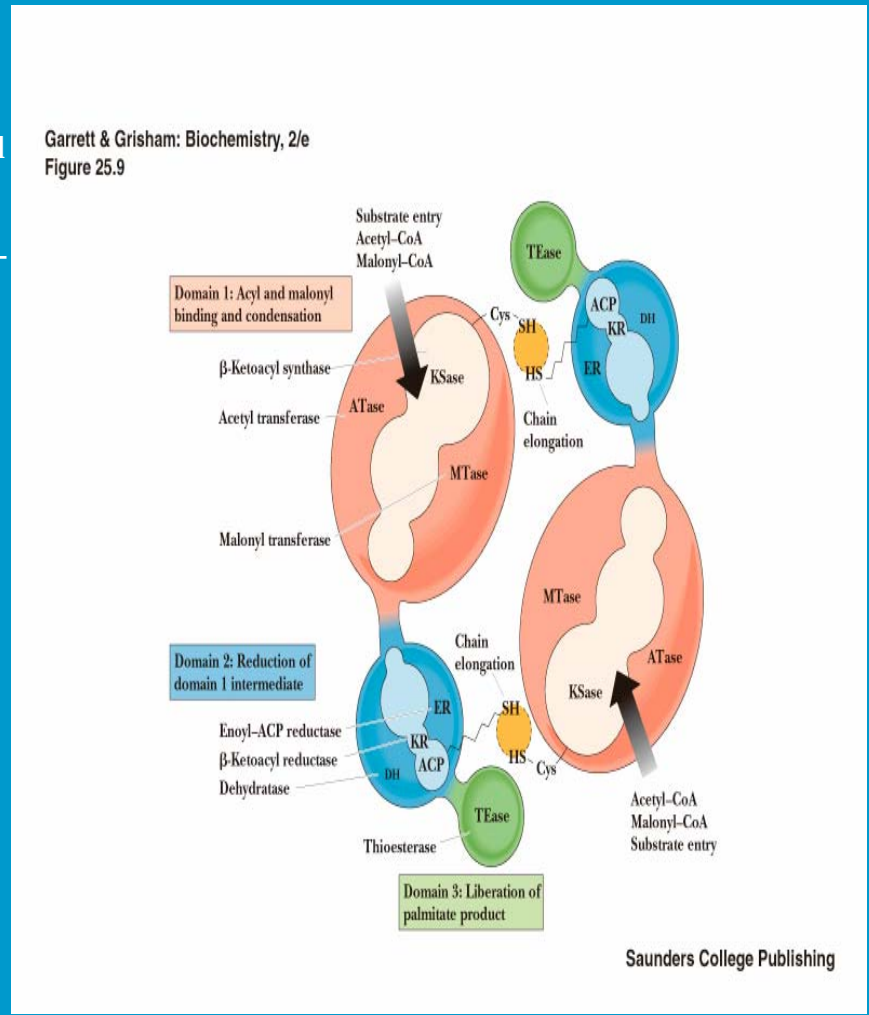
Separate enzymes in a complex

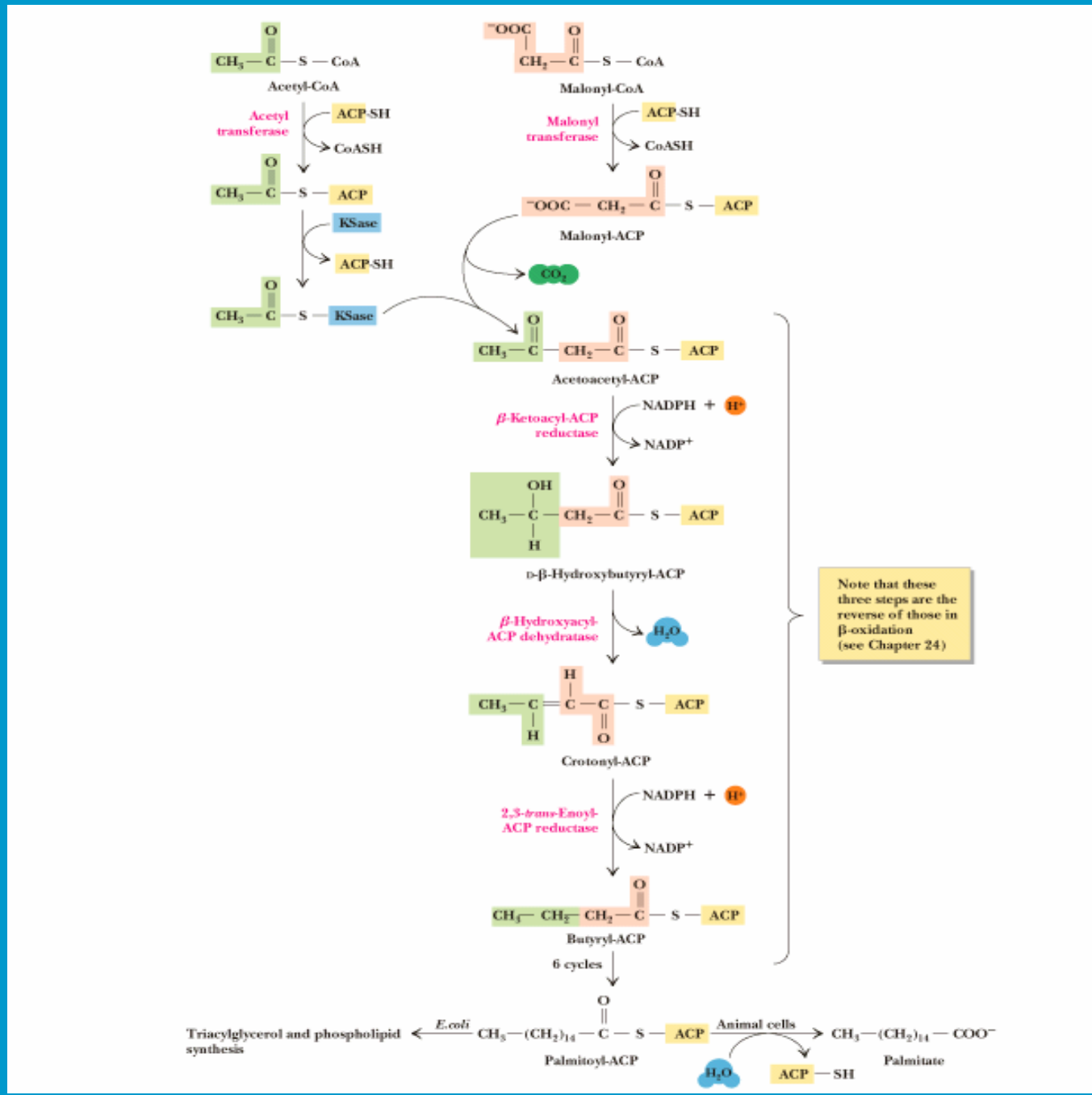
- Pathway initiated by formation of acetyl-ACP and malonyl-ACP by transacylases
- Decarboxylation drives the condensation of acetyl-CoA and malonyl-CoA
- Other three steps are VERY familiar!
- Only differences: D configuration and NADPH

ANIMALS

Fatty Acid Synthase - a multienzyme complex

- Head to tail dimer of 250 kD multifunctional polypeptides
- Steps 3-6 repeat to elongate the chain



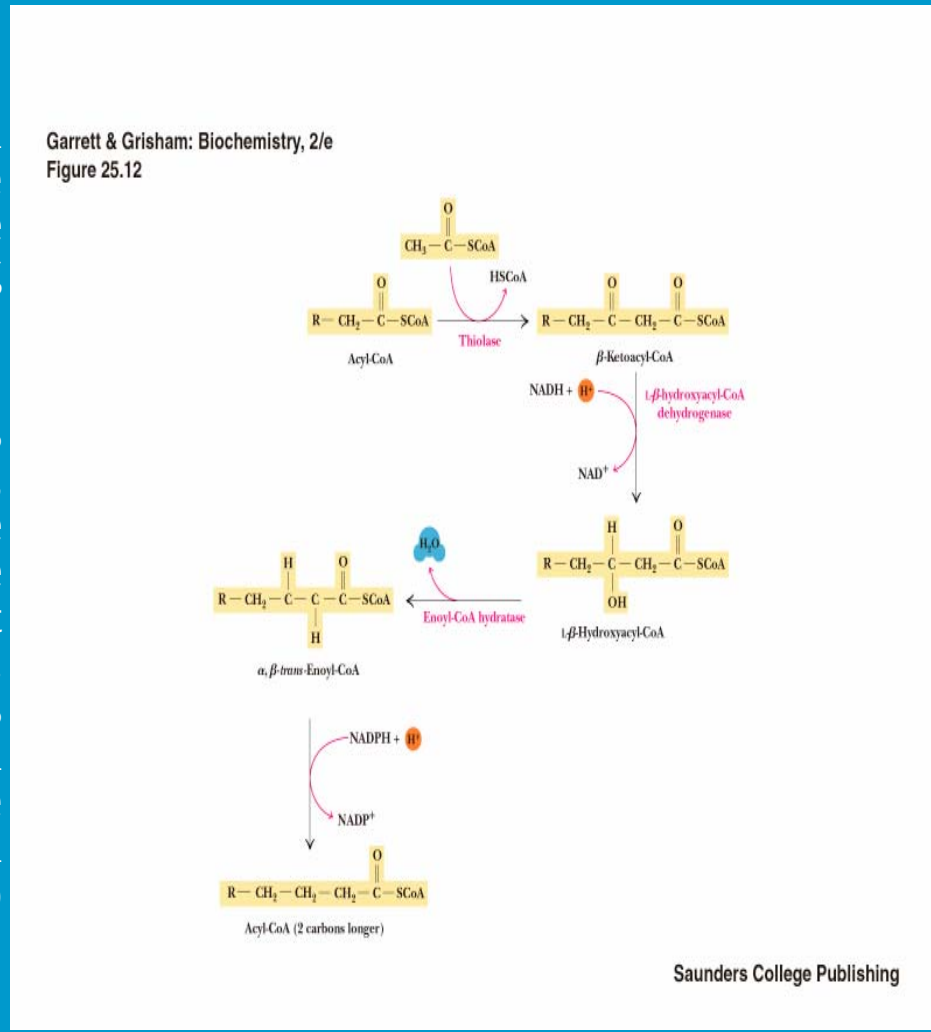

Acetyl-CoA
Malonyl-CoA
Acetyl transferase
ACP-SH
CoASH
Malonyl transferase
ACP-SH
CoASH
Malonyl-ACP
KSase
ACP-SH
CO2
Acetoacetyl-ACP
β-Ketoacyl-ACP reductase
NADPH + H+
NADP+
D-β-Hydroxybutyryl-ACP
β-Hydroxyacyl-ACP dehydratase
H2O
Crotonyl-ACP
2,3-trans-Enoyl-ACP reductase
NADPH + H+
NADP+
Butyryl-ACP
6 cycles
Note that these three steps are the reverse of those in β-oxidation (see Chapter 24)
Palmitoyl-ACP
E.coli
Triacylglycerol and phospholipid synthesis
Animal cells
Palmitate
H2O
ACP — SH

Further Processing of FAs

1. Elongation of FAs

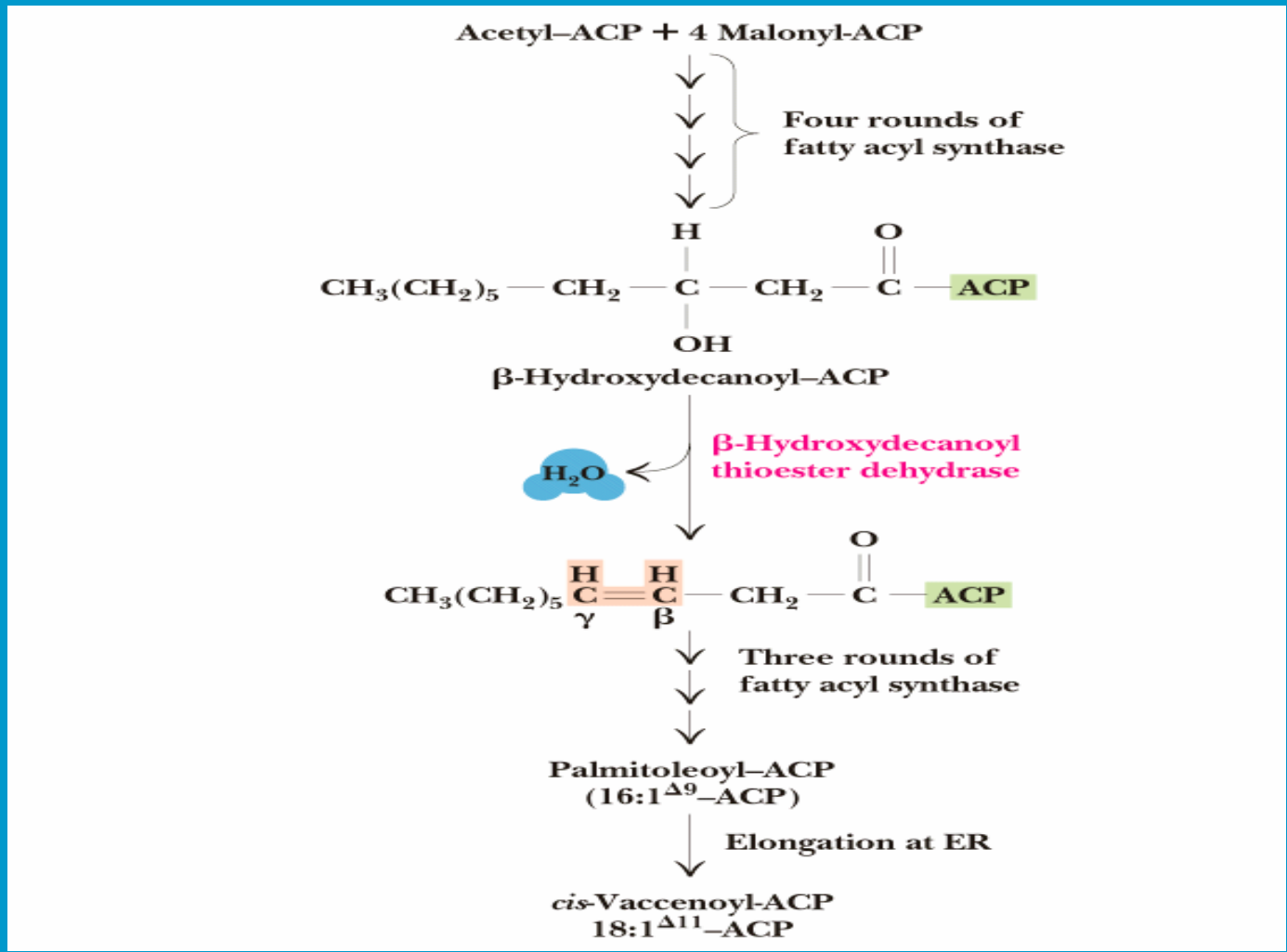
- **In ER: addition of two-carbon units at the carboxyl end of the chain by means of oxidative decarboxylations involving malonyl CoA.**

- **In mitochondria: elongation is initiated by the thiolase reaction, the beta-ketoacyl intermediate thus formed undergoes the same 3 reactions (in reverse order) that are the basis of b-oxidation. Reduction of b-keto group is followed by dehydration to form a double bond. Reduction of the double bond yields a fatty acyl CoA that is elongated by two carbons**



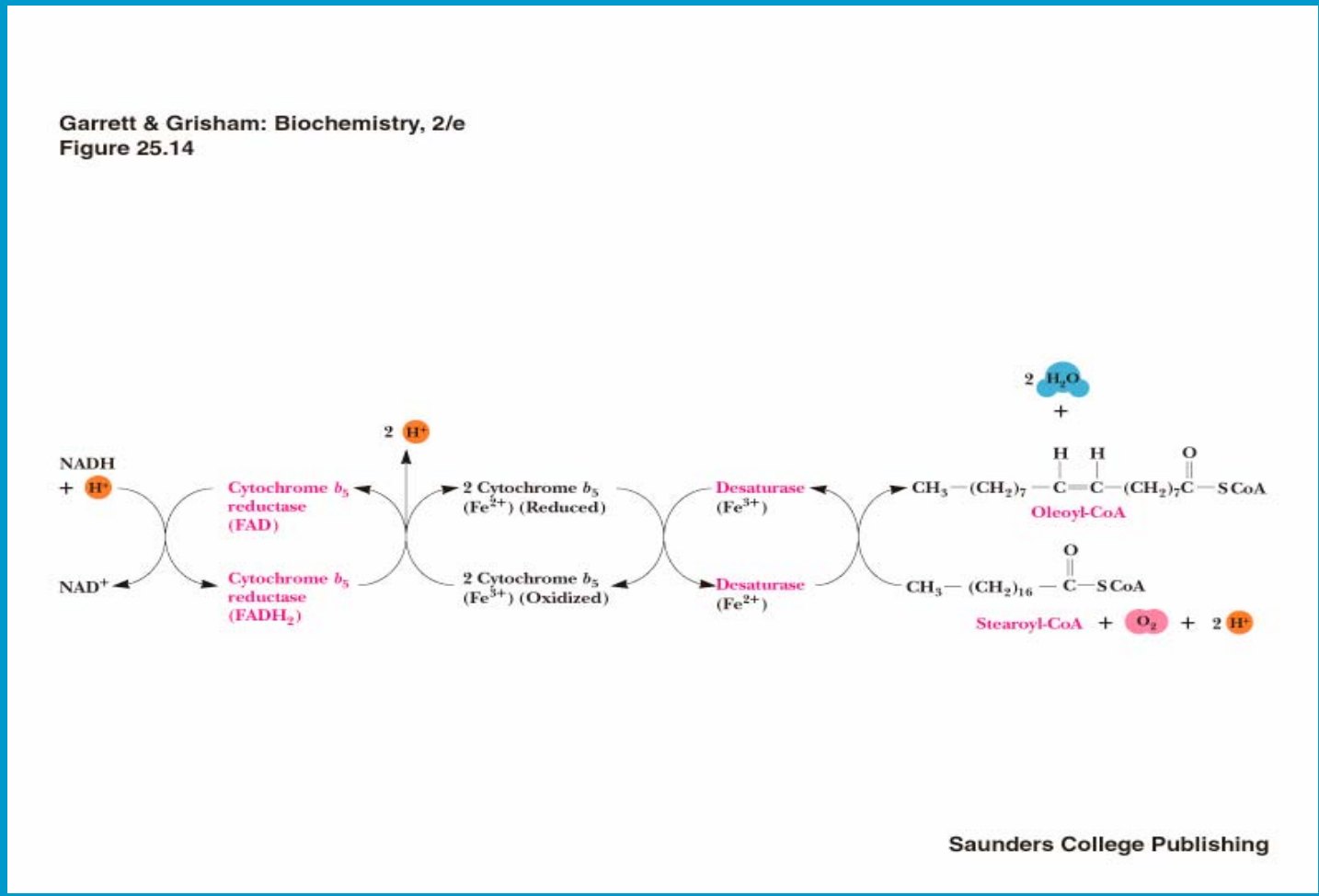
2. Addition of double bonds

EColi: ***E.coli*** **add double bonds independent of O2 and therefore to active the bond, subsequent dehydrogenation reaction occurs while the site of attack is still near something functional**

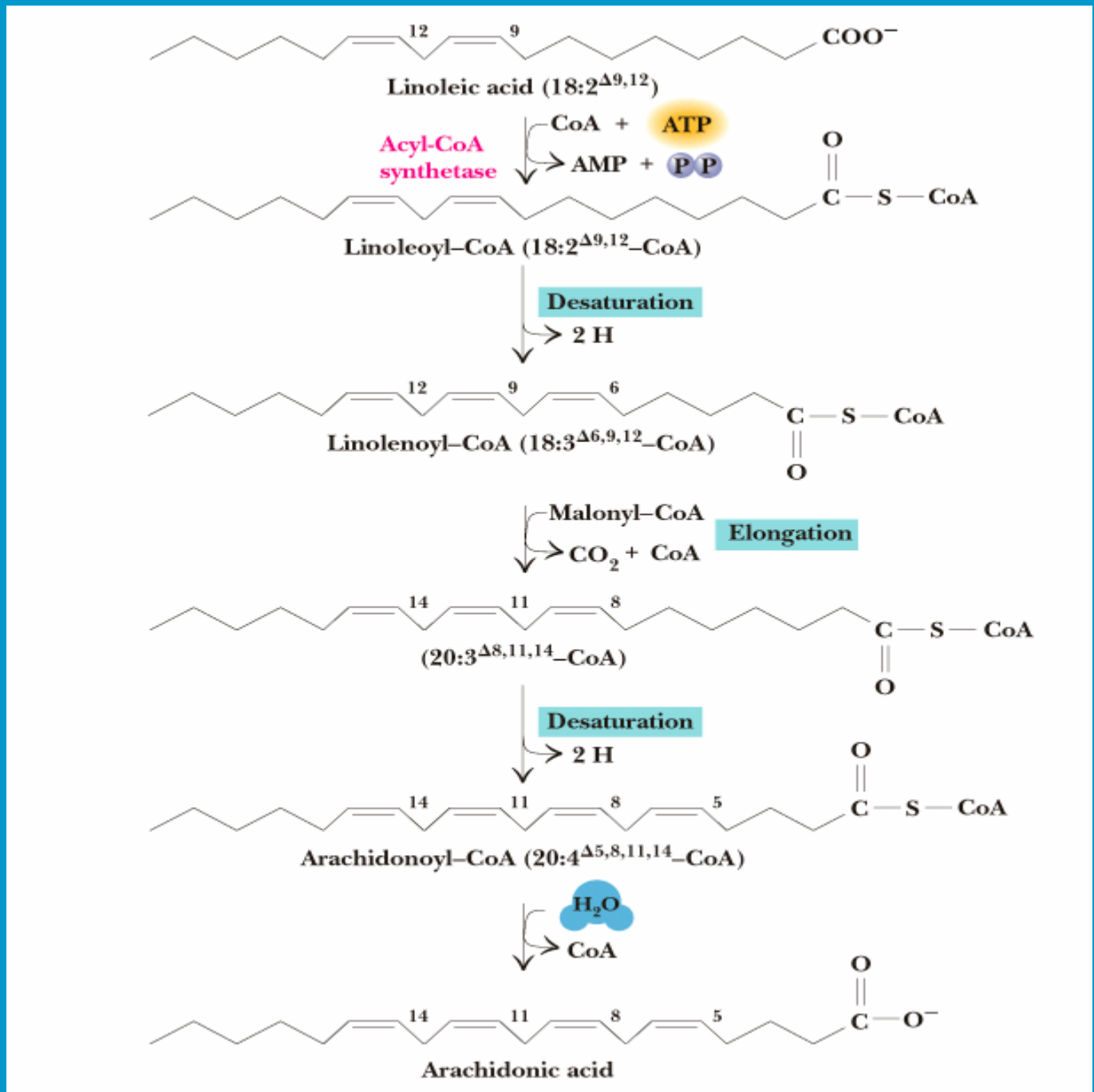


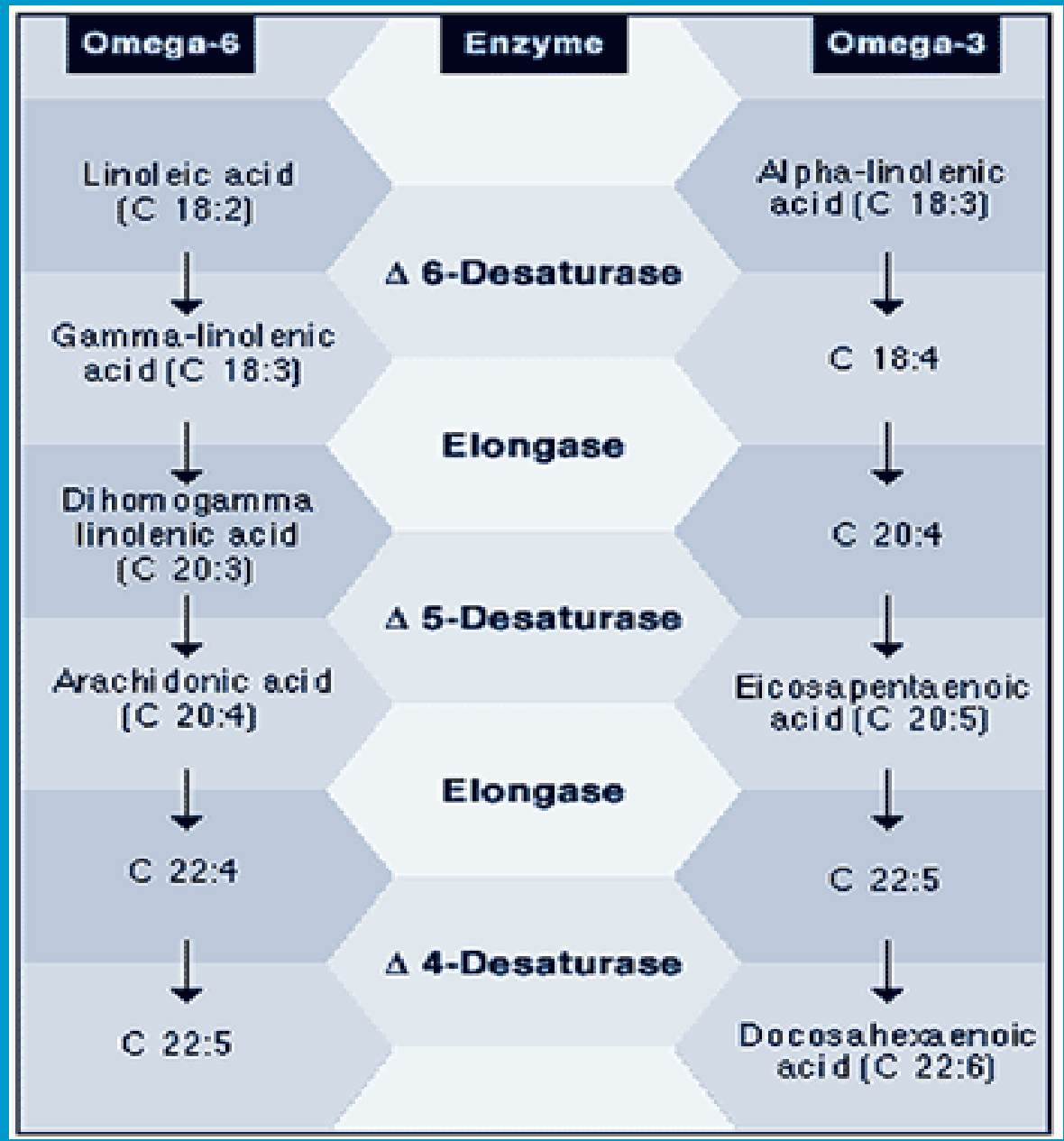
Eukaryotes: Eukaryotes add double bond to middle of the chain - and need power of O2 to do it

Conversion of stearoyl-CoA to oleoyl-CoA in eukaryotes is catalyzed by stearoyl-CoA desaturase in a reaction sequence that involves cytochrome b5 and cytochrome b5 reductase. Two electrons are passed from NADH through the chain of reactions shown below, and two electrons are also derived from fatty acid substrate



Unsaturation reaction may be followed by chain elongation

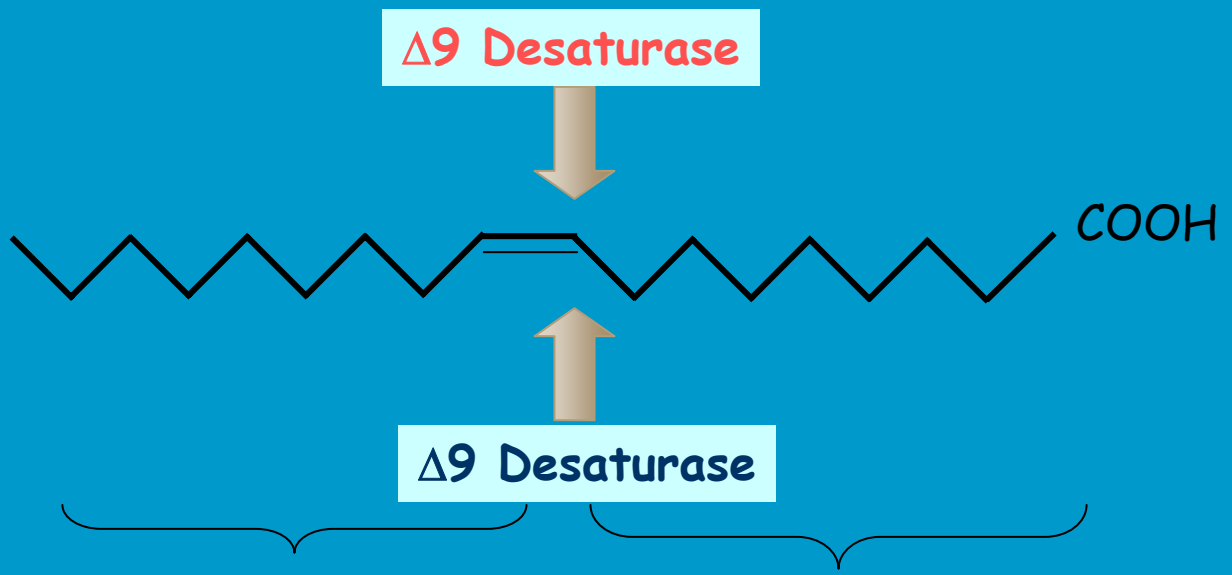

12
9
COO⁻
Linoleic acid (18:2^Δ9,12)
Acyl-CoA synthetase
CoA + ATP
AMP + P P
O
C — S — CoA
Linoleoyl–CoA (18:2^Δ9,12–CoA)
Desaturation
2 H
12
9
6
C — S — CoA
O
Linolenoyl–CoA (18:3^Δ6,9,12–CoA)
Malonyl–CoA
CO2 + CoA
Elongation
14
11
8
C — S — CoA
O
(20:3^Δ8,11,14–CoA)
Desaturation
2 H
O
14
11
8
5
C — S — CoA
Arachidonoyl–CoA (20:4^Δ5,8,11,14–CoA)
H2O
CoA
O
14
11
8
5
C — O⁻
Arachidonic acid


Omega-6
Enzyme
Omega-3
Linoleic acid (C 18:2)
Δ 6-Desaturase
Alpha-linolenic acid (C 18:3)
Gamma-linolenic acid (C 18:3)
C 18:4
Elongase
Dihomogamma linolenic acid (C 20:3)
C 20:4
Δ 5-Desaturase
Arachidonic acid (C 20:4)
Eicosapentaenoic acid (C 20:5)
Elongase
C 22:4
C 22:5
Δ 4-Desaturase
C 22:5
Docosahexaenoic acid (C 22:6)

Polyunsaturated Fatty acid synthesis: Differences between animals and plants

- E.coli, does not have any PUFA
- Eukaryotes do synthesize a variety of PUFAs.
- Plants manufacture double bonds between Δ9 and the methyl end of the chain, BUT mammals can introduce double bonds between the double bond at the 8 or 9 position and the carboxyl group.
- Plants readily desaturate oleic acid at the 12 position (linoleic acid) or at both the 12 and 15 positions (linolenic acid), but mammals cannot (because they lack the desaturase required for the synthesis for Δ12 (n-6) and n-3 fatty acids.

First double bonds always inserted at 9th C



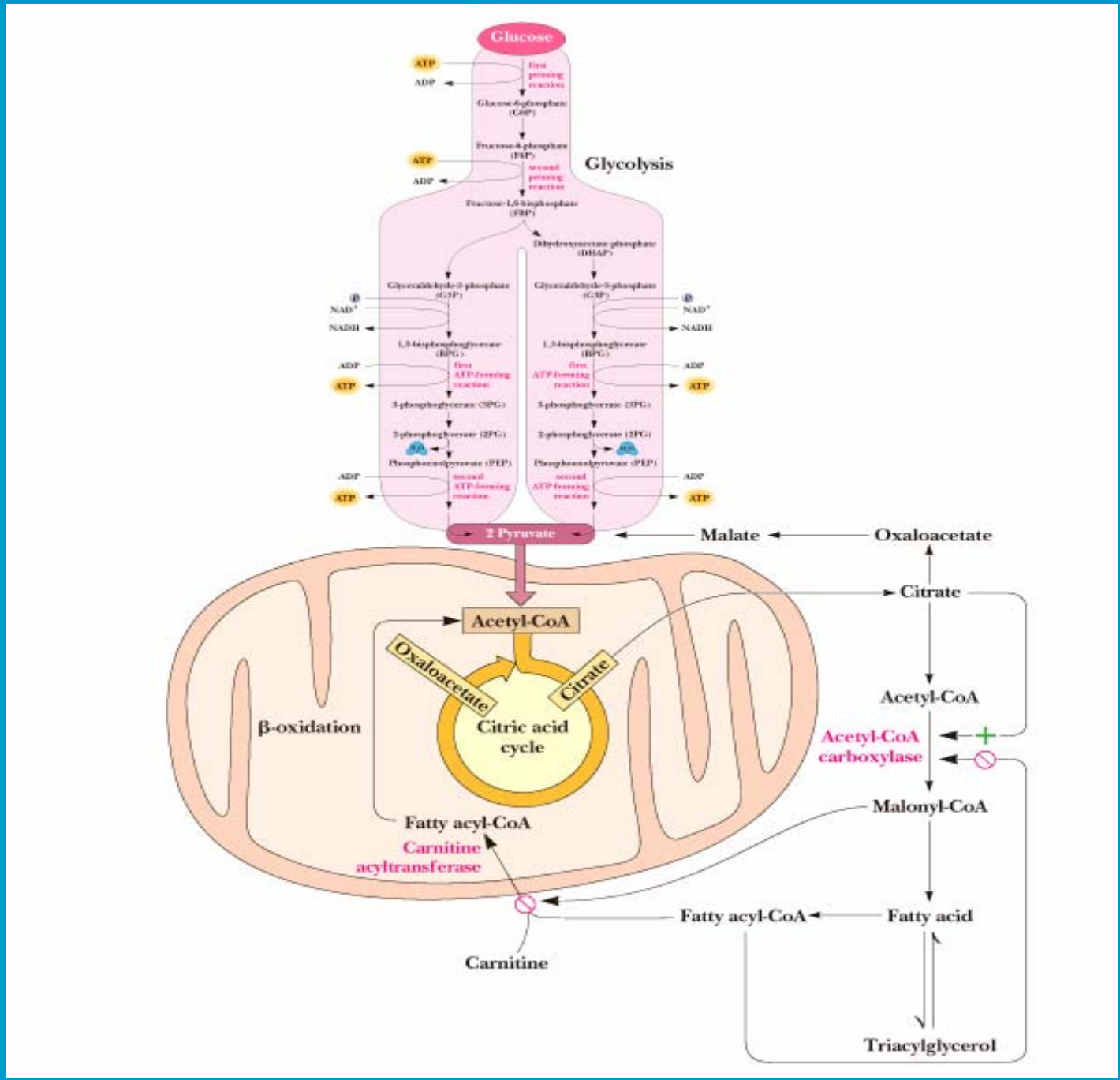
Regulation of FA Synthesis

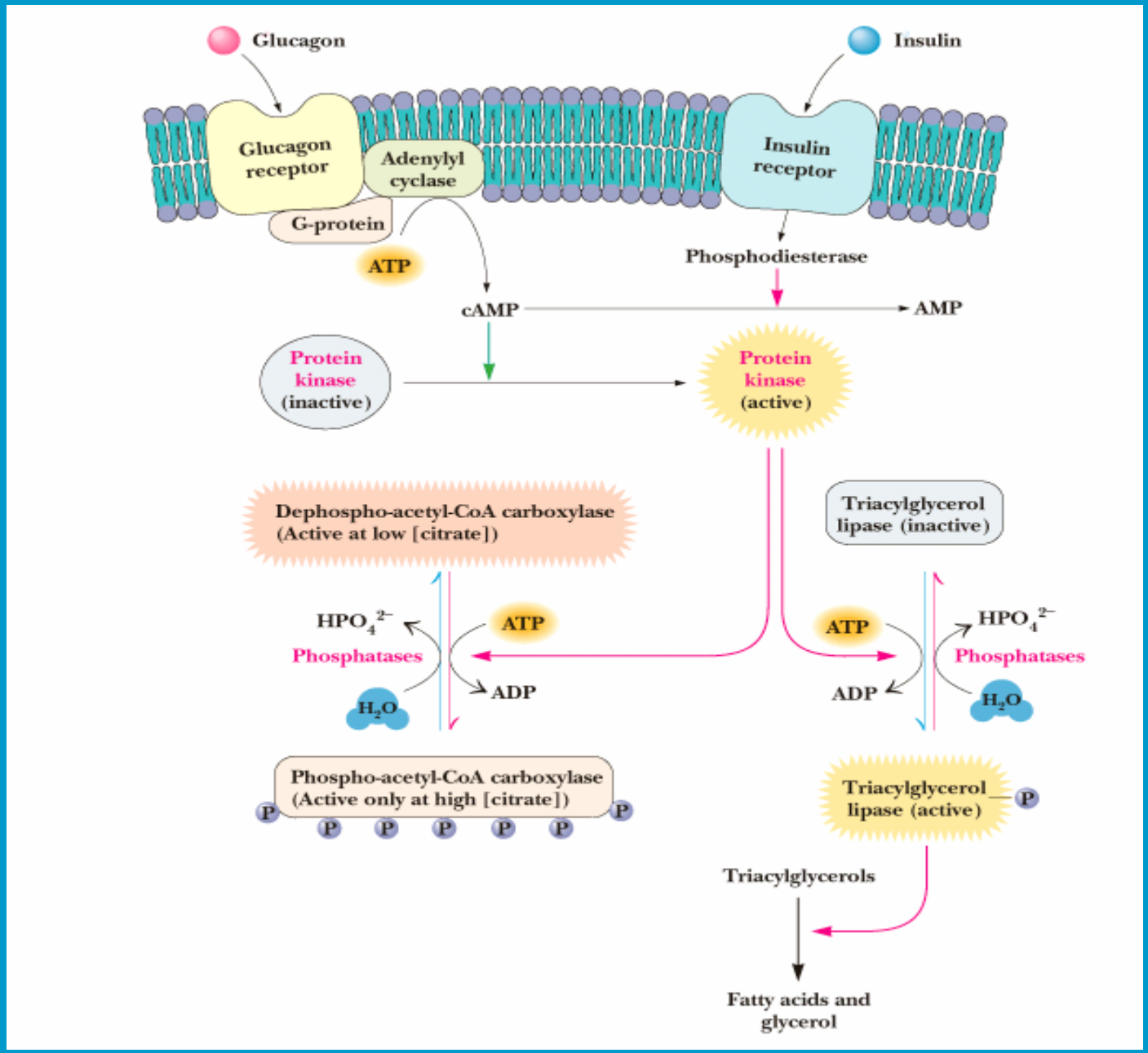
Allosteric modifiers, phosphorylation and hormones

- Malonyl-CoA blocks the carnitine acyltransferase and thus inhibits beta-oxidation
- Citrate activates acetyl-CoA carboxylase
- Fatty acyl-CoAs inhibit acetyl-CoA carboxylase
- Hormones regulate ACC:

  Glucagon activates lipases/inhibits ACC

  Insulin inhibits lipases/activates ACC


Glucose
ATP
ADP
Glycolysis
NAD+
NADH
2 Pyruvate
Malate
Oxaloacetate
Citrate
Acetyl-CoA
Oxaloacetate
Citrate
Citric acid cycle
β-oxidation
Acetyl-CoA
Acetyl-CoA carboxylase
Malonyl-CoA
Fatty acyl-CoA
Carnitine acyltransferase
Carnitine
Fatty acyl-CoA
Fatty acid
Triacylglycerol


Glucagon
Insulin
Glucagon receptor
Adenylyl cyclase
G-protein
Insulin receptor
ATP
Phosphodiesterase
cAMP
AMP
Protein kinase (inactive)
Protein kinase (active)
Dephospho-acetyl-CoA carboxylase (Active at low [citrate])
Triacylglycerol lipase (inactive)
HPO_4^{2-}
Phosphatases
H_2O
ATP
ADP
ATP
ADP
HPO_4^{2-}
Phosphatases
H_2O
Phospho-acetyl-CoA carboxylase (Active only at high [citrate])
P
Triacylglycerol lipase (active)
P
Triacylglycerols
Fatty acids and glycerol

Biosynthesis of Complex Lipids

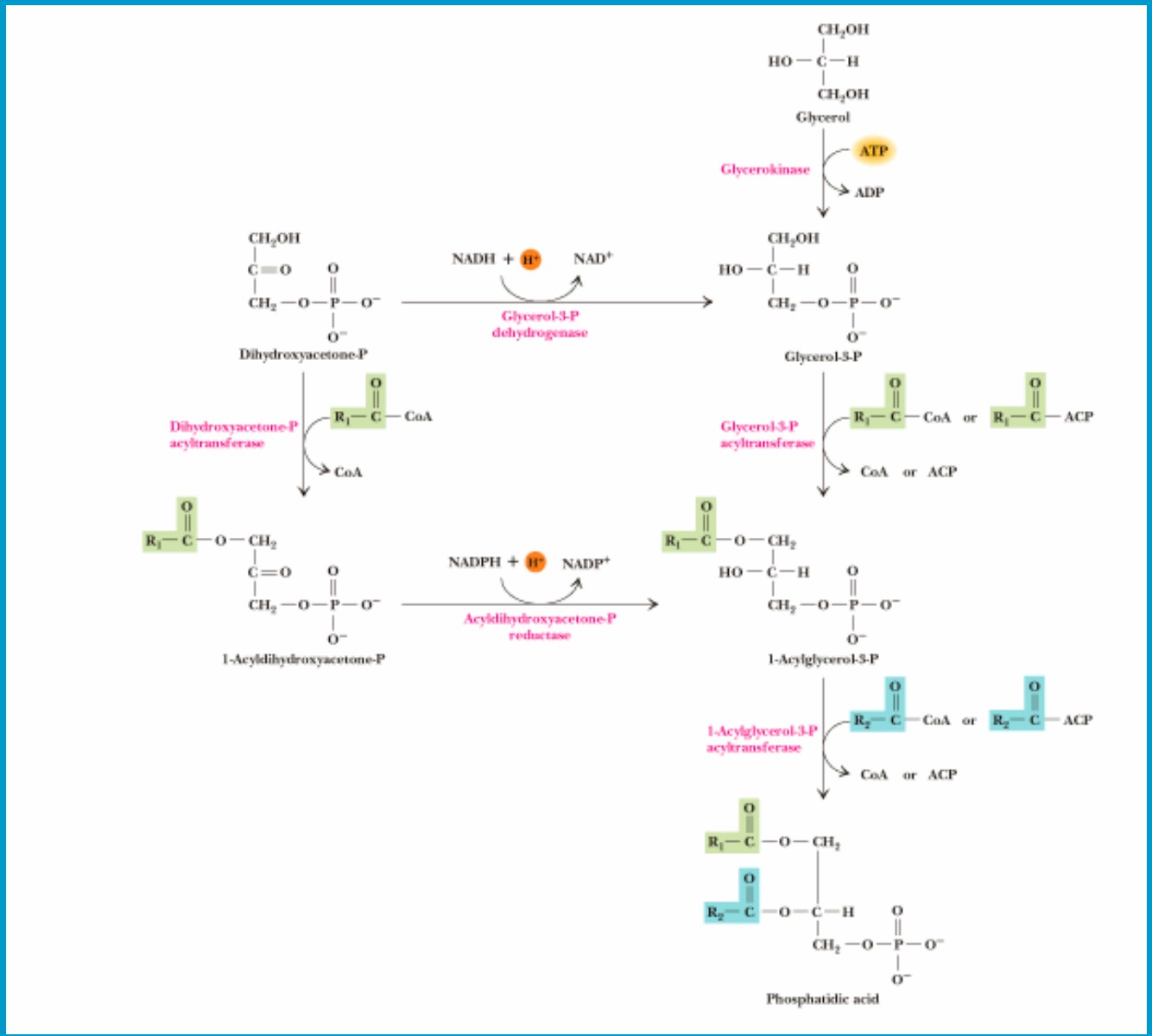
Synthetic pathways depend on organism

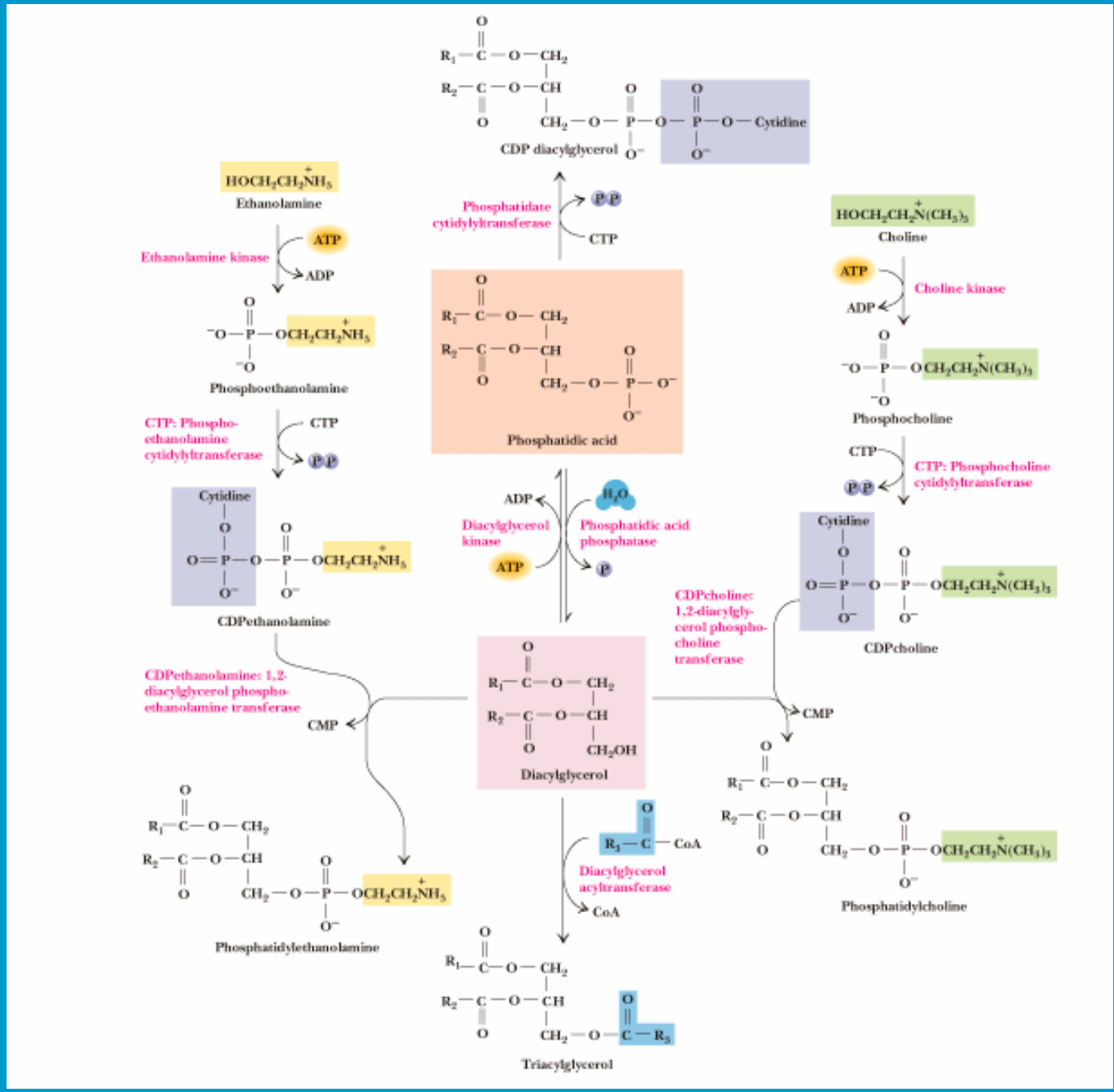
- Sphingolipids and triacylglycerols are only made in eukaryotes
- PE accounts for about 75% of PLs in *E.coli*
- No PC, PI, sphingolipids, cholesterol in *E.coli*
- But some bacteria do produce PC

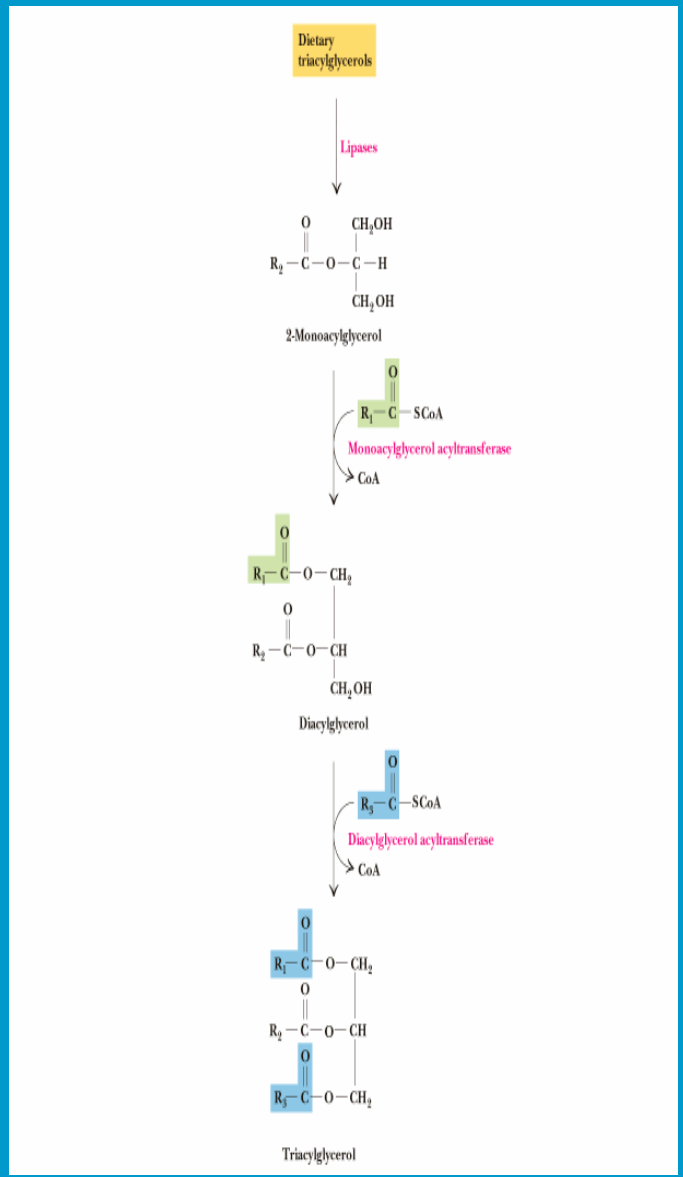
Glycerolipid Biosynthesis

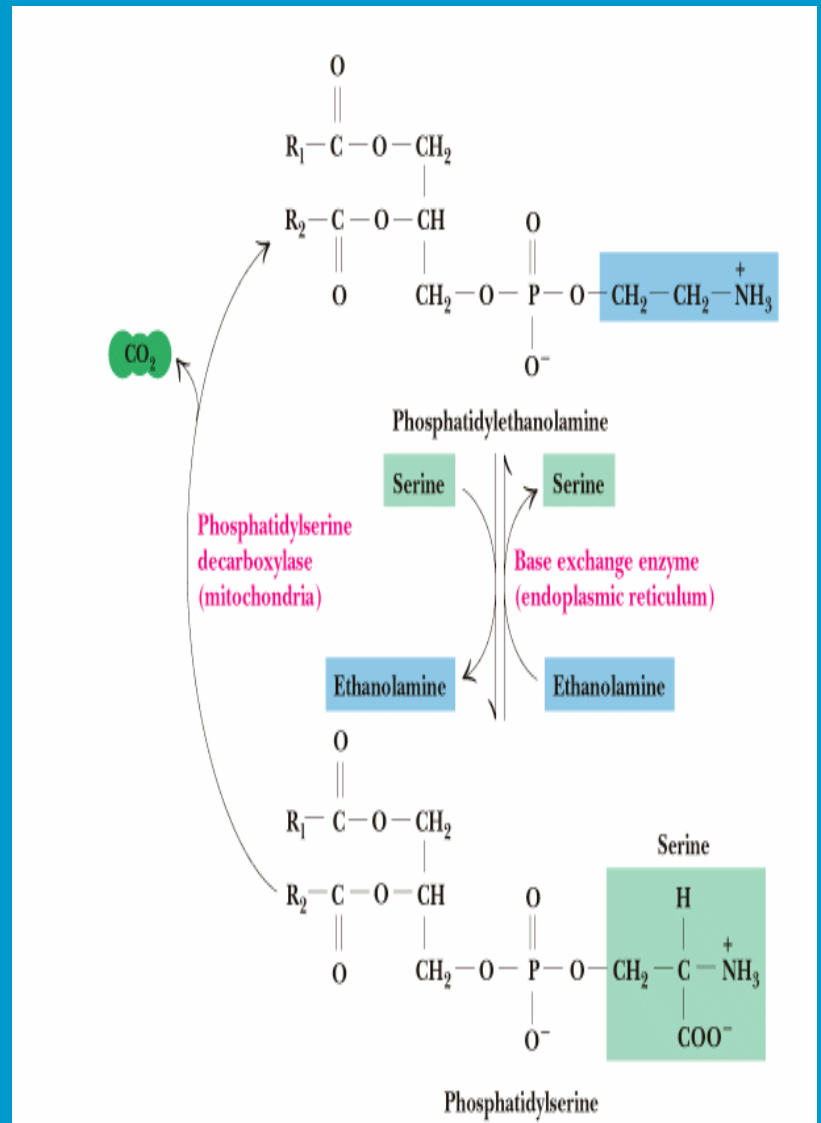
CTP drives formation of CDP complexes

- Phosphatidic acid is the precursor for all other glycerolipids in eukaryotes
- PA is made either into DAG or CDP-DAG
- Note the roles of CDP-choline and CDP-ethanolamine in synthesis of PC and PE
- Note exchange of ethanolamine for serine


Glycerol
Glycerokinase
ATP
ADP
Dihydroxyacetone-P
NADH + H+
NAD+
Glycerol-3-P dehydrogenase
Glycerol-3-P
Dihydroxyacetone-P acyltransferase
CoA
Glycerol-3-P acyltransferase
CoA or ACP
1-Acyldihydroxyacetone-P
NADPH + H+
NADP+
Acyldihydroxyacetone-P reductase
1-Acylglycerol-3-P
1-Acylglycerol-3-P acyltransferase
Phosphatidic acid


CDP diacylglycerol
Cytidine
Phosphatidate cytidylyltransferase
CTP
Ethanolamine
ATP
Ethanolamine kinase
ADP
Phosphoethanolamine
CTP: Phospho-ethanolamine cytidylyltransferase
CTP
CDPethanolamine
Phosphatidic acid
ADP
Diacylglycerol kinase
ATP
H_2O
Phosphatidic acid phosphatase
Choline
ATP
Choline kinase
ADP
Phosphocholine
CTP
CTP: Phosphocholine cytidylyltransferase
CDPcholine
CDPcholine: 1,2-diacylgly-cerol phospho-choline transferase
CDPethanolamine: 1,2-diacylglycerol phospho-ethanolamine transferase
CMP
Diacylglycerol
CMP
Phosphatidylethanolamine
Diacylglycerol acyltransferase
CoA
CoA
Phosphatidylcholine
Triacylglycerol

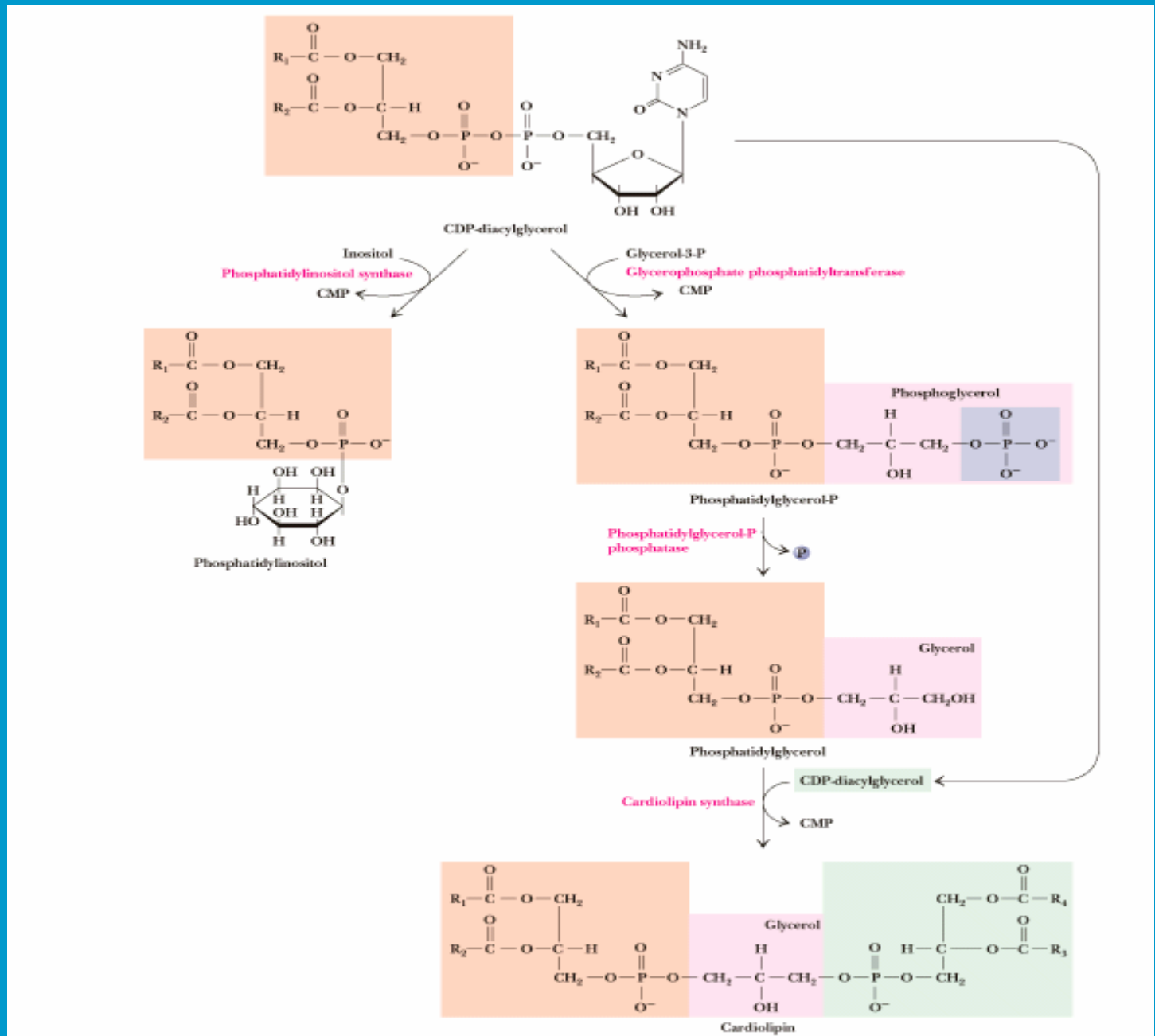

Dietary triacylglycerols
Lipases
2-Monoacylglycerol
Monoacylglycerol acyltransferase
CoA
Diacylglycerol
Diacylglycerol acyltransferase
CoA
Triacylglycerol


Phosphatidylethanolamine
Serine
Serine
Phosphatidylserine decarboxylase (mitochondria)
Base exchange enzyme (endoplasmic reticulum)
Ethanolamine
Ethanolamine
Serine
Phosphatidylserine

Other PLs from CDP-DAG

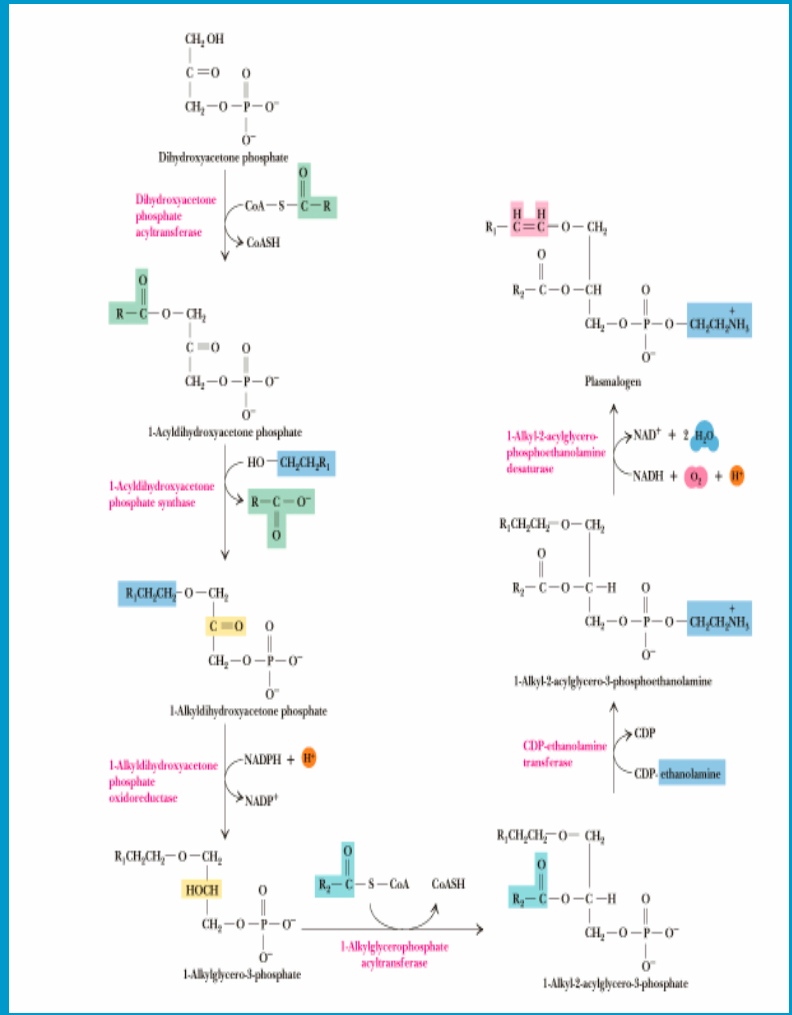
CDP-diacylglycerol is used in eukaryotes to produce:

- PI in one step
- PG in two steps
- Cardiolipin in three steps


CDP-diacylglycerol
Inositol
Phosphatidylinositol synthase
CMP
Glycerol-3-P
Glycerophosphate phosphatidyltransferase
CMP
Phosphatidylinositol
Phosphoglycerol
Phosphatidylglycerol-P
Phosphatidylglycerol-P phosphatase
Glycerol
Phosphatidylglycerol
CDP-diacylglycerol
Cardiolipin synthase
CMP
Glycerol
Cardiolipin

Plasmalogen Biosynthesis

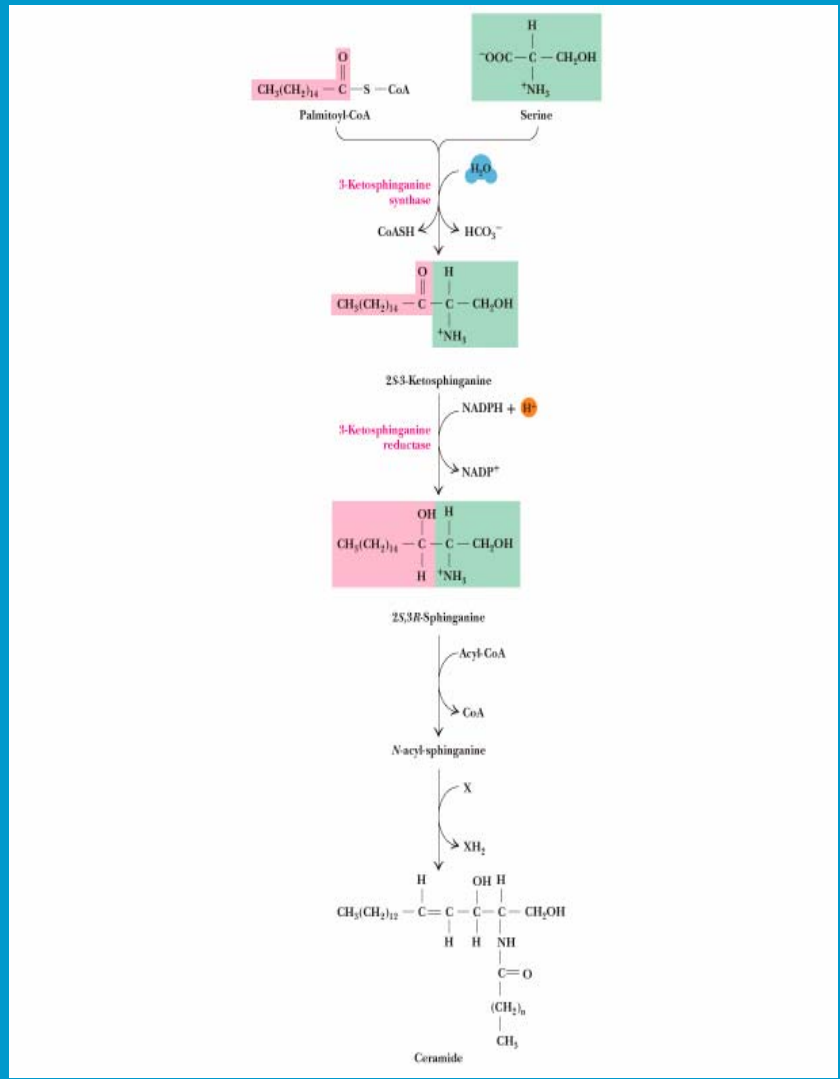
- Dihydroxyacetone phosphate is the precursor
- Acylation at C-1 activates and an exchange of the acyl group for a long-chain alcohol produces the ether linkage
- Reduction of the Ketone group at C-2 transferase reactions which add an acyl group at C-2 and a polar head group moiety
- CDP-ethanolamine delivers the headgroup
- A desaturase produces the double bond in the alkyl chain

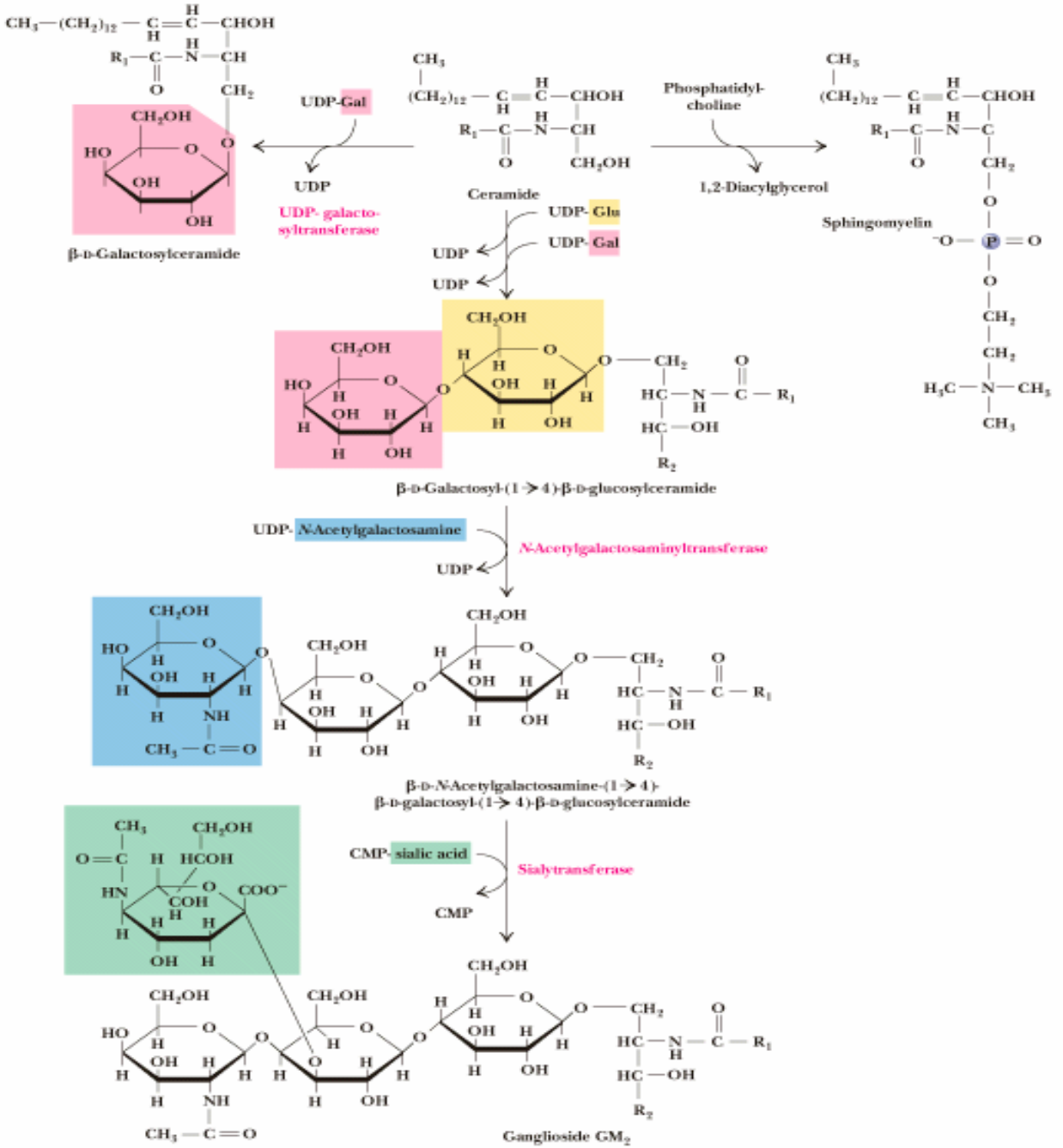


Sphingolipid Biosynthesis

High levels made in neural tissue

- Initial reaction is a condensation of serine and palmitoyl-CoA
- 3-ketosphinganine synthase is PLP-dependent
- Ketone is reduced with help of NADPH
- Acylation is followed by double bond formation
- Resulting ceramide is precursor for other sphingolipids

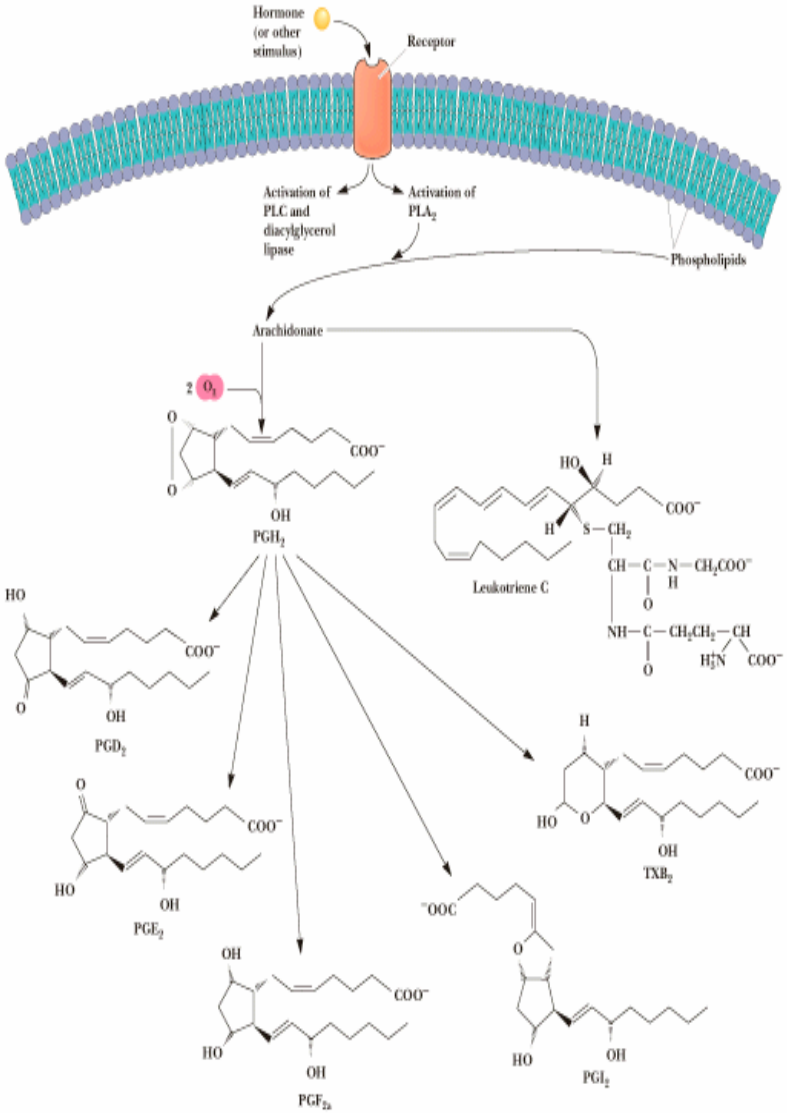


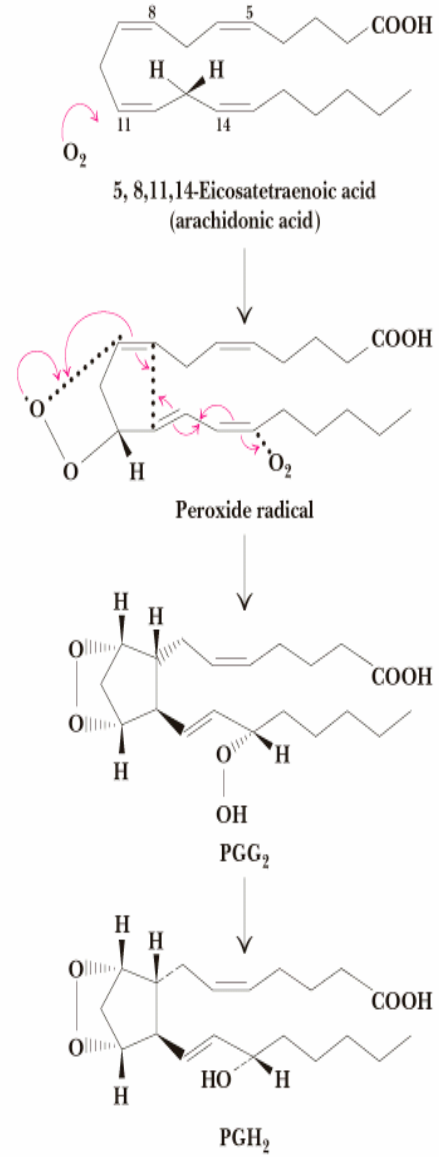

UDP-Gal
UDP
UDP- galacto-syltransferase
β-D-Galactosylceramide
Phosphatidyl-choline
1,2-Diacylglycerol
Sphingomyelin
Ceramide
UDP- Glu
UDP- Gal
UDP
UDP
β-D-Galactosyl-(1→4)-β-D-glucosylceramide
UDP- N-Acetylgalactosamine
N-Acetylgalactosaminyltransferase
UDP
β-D-N-Acetylgalactosamine-(1→4)-β-D-galactosyl-(1→4)-β-D-glucosylceramide
CMP- sialic acid
Sialytransferase
CMP
Ganglioside GM2

Eicosanoid Biosynthesis

PLA_2 releases arachidonic acid - a precursor of eicosanoids

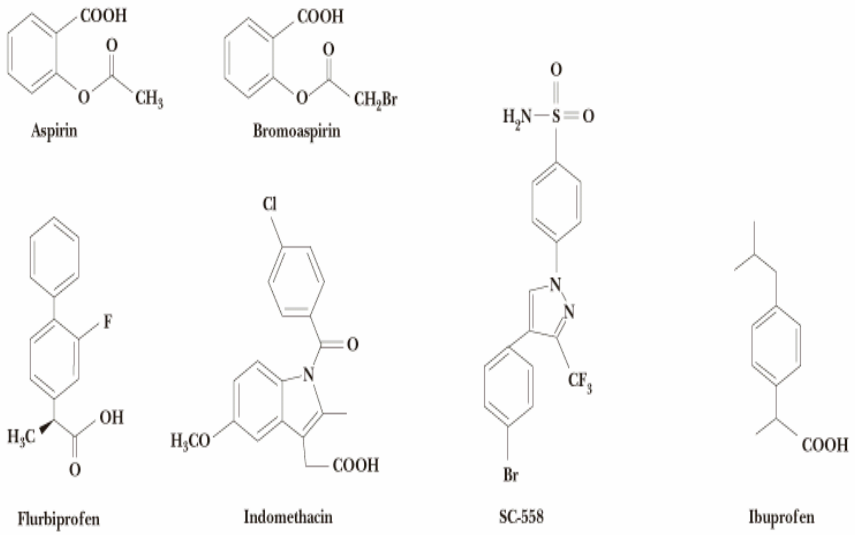
- **Eicosanoids are local hormones**
- **The PG endoperoxide synthase [*What is the other name for this enzyme*] oxidizes and cyclizes**
- **Tissue injury and inflammation triggers arachidonate release and eicosanoid synthesis**


Hormone (or other stimulus)
Receptor
Activation of PLC and diacylglycerol lipase
Activation of PLA_2
Phospholipids
Arachidonate
$2\ O_2$
PGH_2
Leukotriene C
PGD_2
PGE_2
TXB_2
$PGF_{2\alpha}$
PGI_2


5, 8,11,14-Eicosatetraenoic acid
(arachidonic acid)
Peroxide radical
PGG_2
PGH_2

Aspirin (covalently) and other nonsteroid anti-inflammatory agents inhibit the cyclooxygenase

Garrett & Grisham: Biochemistry, 2/e
Unnumbered Figure p.835



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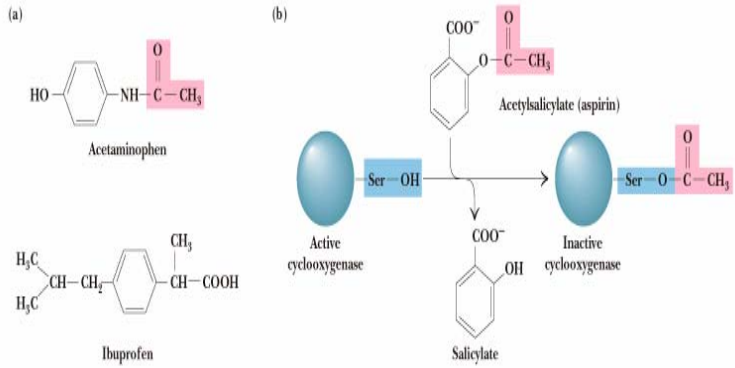
Garrett & Grisham: Biochemistry, 2/e
Figure 25.29



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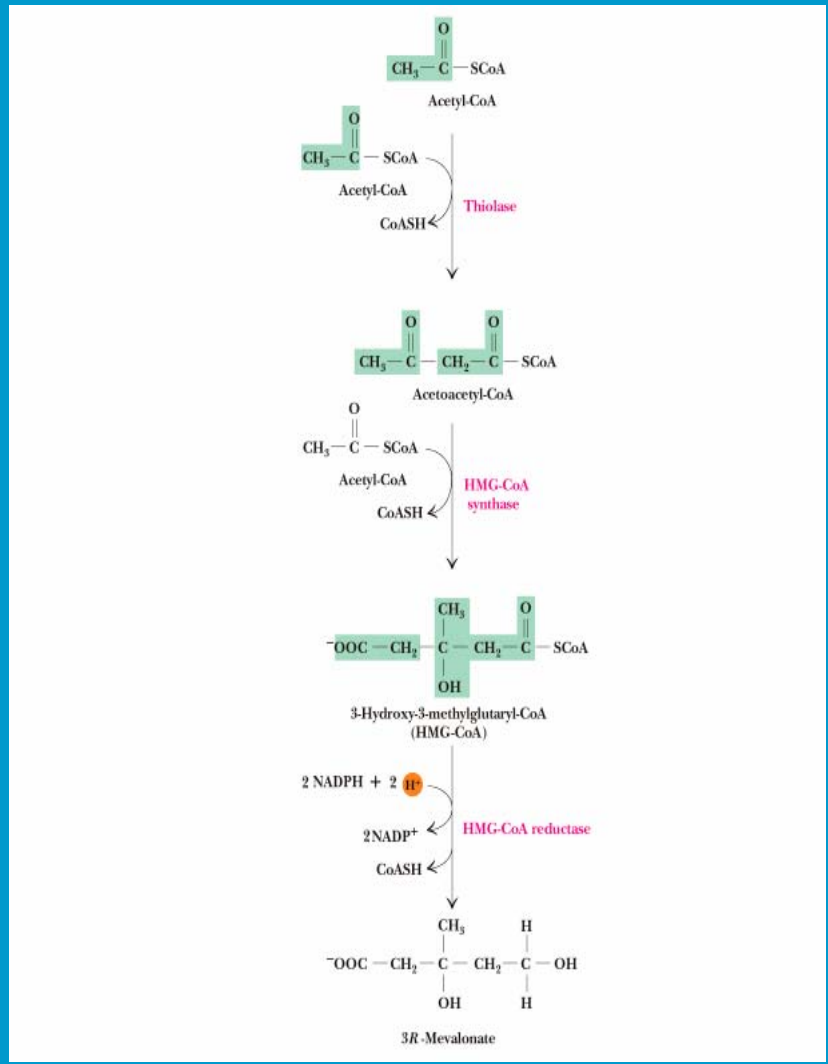
Cholesterol Biosynthesis

Occurs primarily in the liver

- Biosynthesis begins in the cytosol with the synthesis of mevalonate from acetyl-CoA
- First step is a thiolase reaction
- Second step makes HMG-CoA
- Third step - HMG-CoA reductase - is the rate-limiting step in cholesterol biosynthesis
- HMG-CoA reductase is site of action of cholesterol-lowering drugs



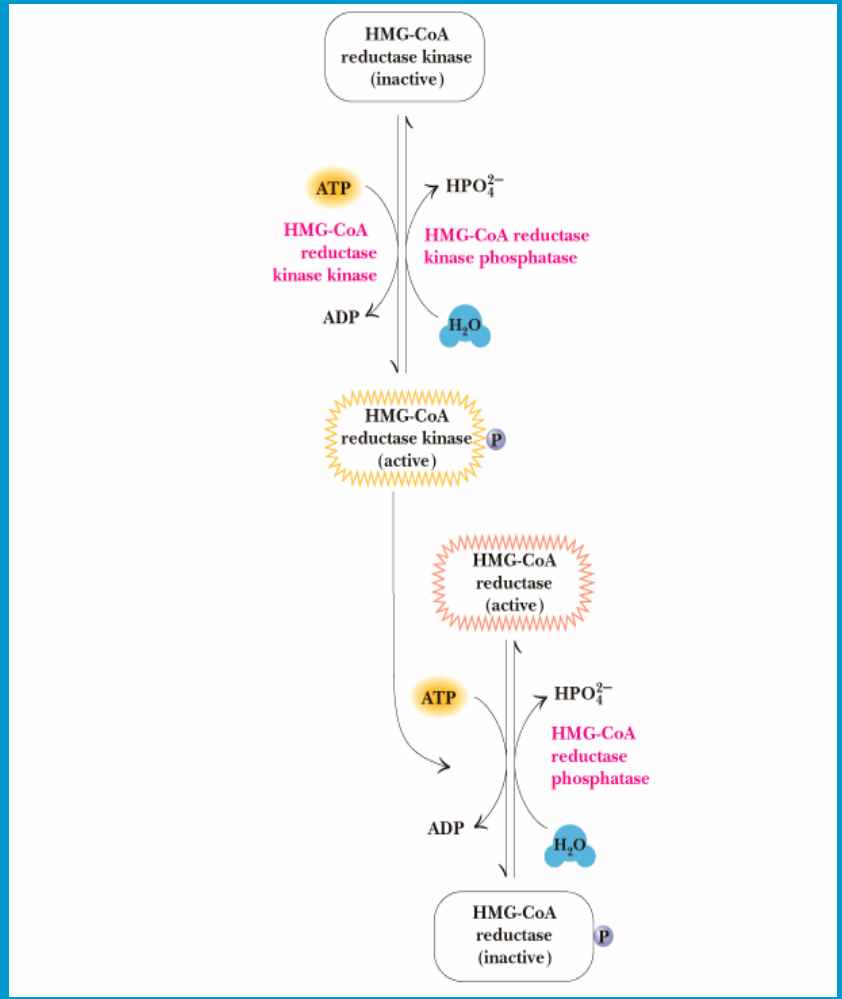
Regulation of HMG-CoA Reductase

It is 97-kD glycoprotein that spans the ER membrane with its active site facing the cytosol. As rate-limiting step, it is the principal site of regulation in cholesterol synthesis

1) **Phosphorylation by cAMP-** dependent kinases inactivates the reductase

3) **Degradation of HMG-CoA reductase** - half-life is 3 hrs and depends on cholesterol level [high cholesterol means short half-life]

3) **Gene expression** (mRNA production) is controlled by cholesterol levels [If Cho is high, levels of mRNA coding for the reductase are reduced. If Cho is low more mRNA is made



The thiolase brainteaser...

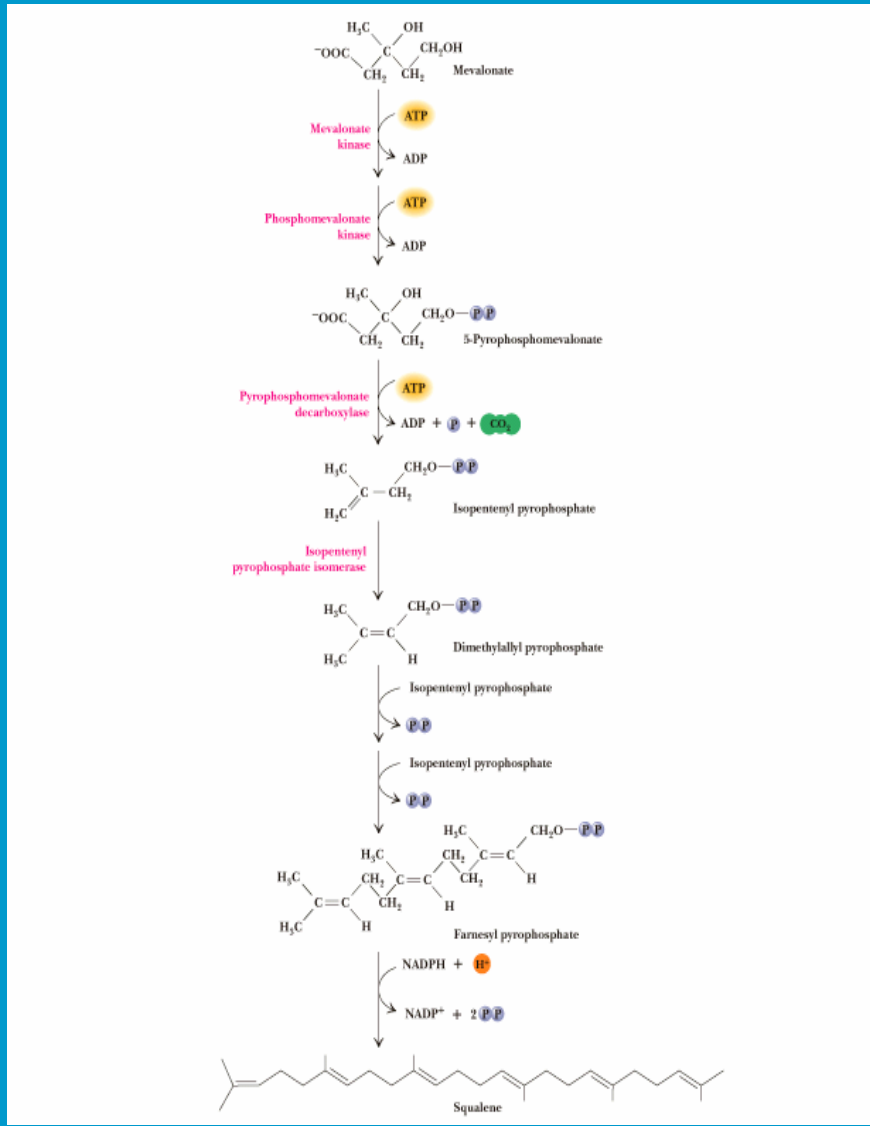
An important puzzle

- If acetate units can be condensed by thiolase to give acetoacetate in the 1st step of cholesterol biosynthesis, why not also use thiolase for FA synthesis, avoiding complexity of FA synthase?
- Solution: Subsequent reactions drive cholesterol synthesis, but eight successive thiolase reactions would be very unfavorable energetically for FA synthesis

Squalene from Mevalonate

Driven by ATP hydrolysis, decarboxylation and PP_i hydrolysis

- Six-carbon mevalonate makes five carbon isopentenyl PP_i and dimethylallyl PP_i
- Condensation of 3 of these yields farnesyl PP_i
- Two farnesyl PP_i s link to form squalene
- In 1952, **Bloch and Langdon** were first to show that squalene is derived from acetate units and that cholesterol is derived from squalene

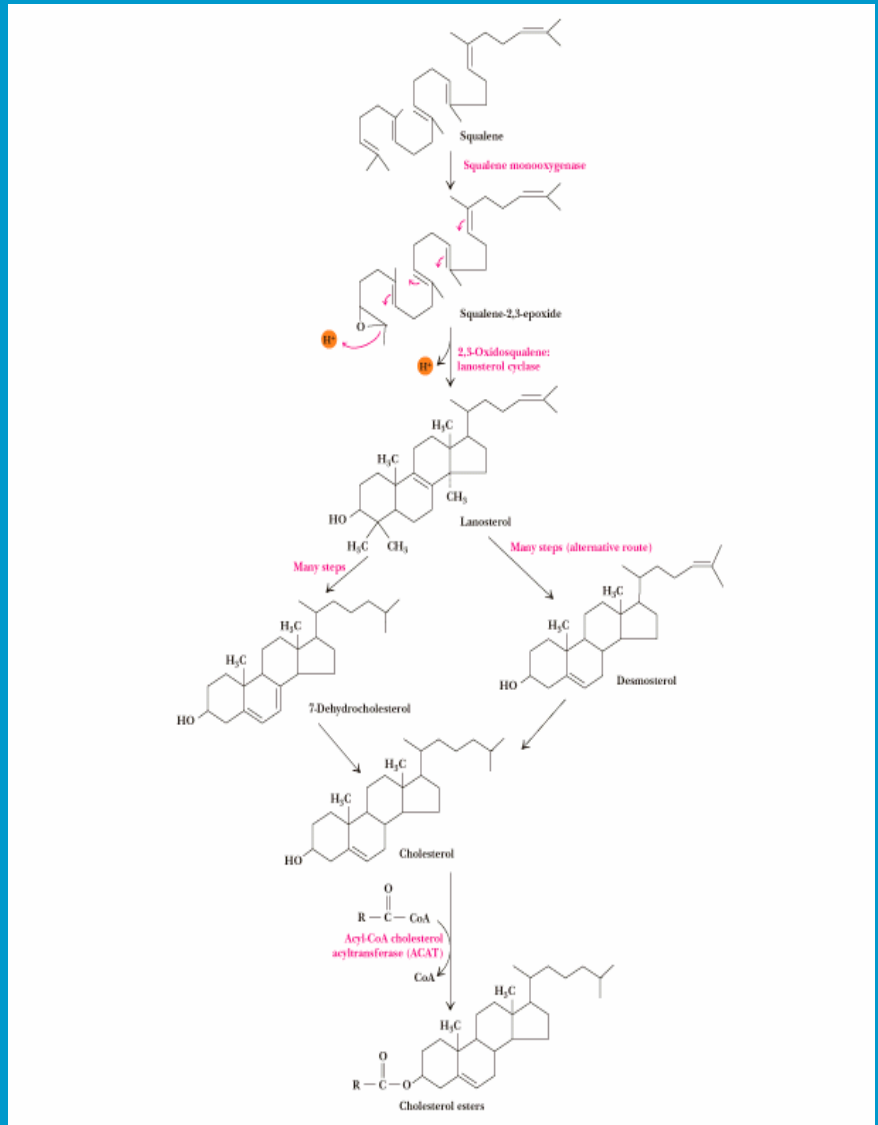


Cholesterol from Squalene

At the endoplasmic reticulum membrane

- Squalene monooxygenase converts squalene to squalene-2,3-epoxide
- A cyclase converts the epoxide to lanosterol
- Though lanosterol looks like cholesterol, 20 more steps are required to form cholesterol!

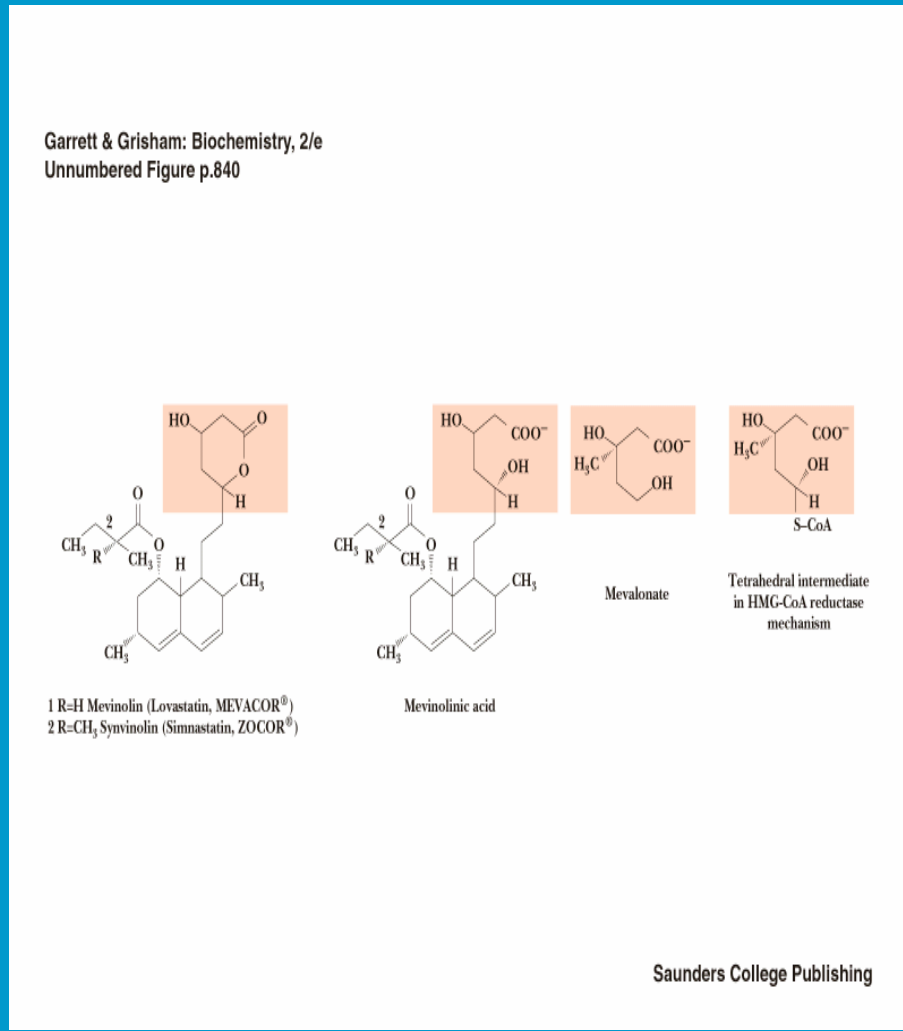
  All in the endoplasmic reticulum membrane



Inhibiting Cholesterol Synthesis

Merck and the Lovastatin story..

- HMG-CoA reductase is the key - the rate-limiting step in cholesterol biosynthesis
- Lovastatin (mevinolin) blocks HMG-CoA reductase and prevents synthesis of cholesterol
- Lovastatin is an (inactive) lactone (isolated from *Aspergillus terreus*)
- In the body, the lactone is hydrolyzed to mevinolinic acid, a competitive (Transition state analog) inhibitor of the reductase, K_i = 0.6 nM!



Dosages: 20 to 80 mg per day

Lipid Transport & Lipoproteins

Lipoproteins are the carriers of most lipids in the body

Lipoprotein - a cluster of lipids, often with a monolayer membrane, together with an apolipoprotein

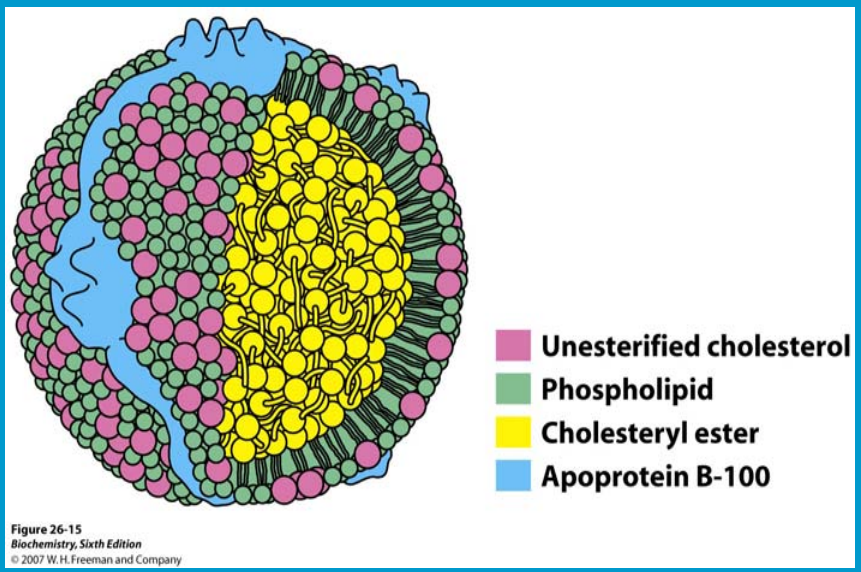


Figure 26-15
Biochemistry, Sixth Edition

TABLE 26.1 Properties of plasma lipoproteins

Plasma lipoproteins	Density (g ml^{-1})	Diameter (nm)	Apolipoprotein	Physiological role	Composition (%) TAG	CE	C	PL	P
Chylomicron	<0.95	75–1200	B48, C, E	Dietary fat transport	86	3	1	8	2
Very low density lipoprotein	0.95–1.006	30–80	B100, C, E	Endogenous fat transport	52	14	7	18	8
Intermediate-density lipoprotein	1.006–1.019	15–35	B100, E	LDL precursor	38	30	8	23	11
Low-density lipoprotein	1.019–1.063	18–25	B100	Cholesterol transport	10	38	8	22	21
High-density lipoprotein	1.063–1.21	7.5–20	A	Reverse cholesterol transport	5–10	14–21	3–7	19–29	33–57

Abbreviations: TAG, triacylglyerol; CE, cholesterol ester; C, free cholesterol; PL, phospholipid; P, protein.

Table 26-1
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Chylomicrons form in the intestines
HDL, VLDL assemble in the ER of liver cells
LDL not made directly, but evolves from VLDL

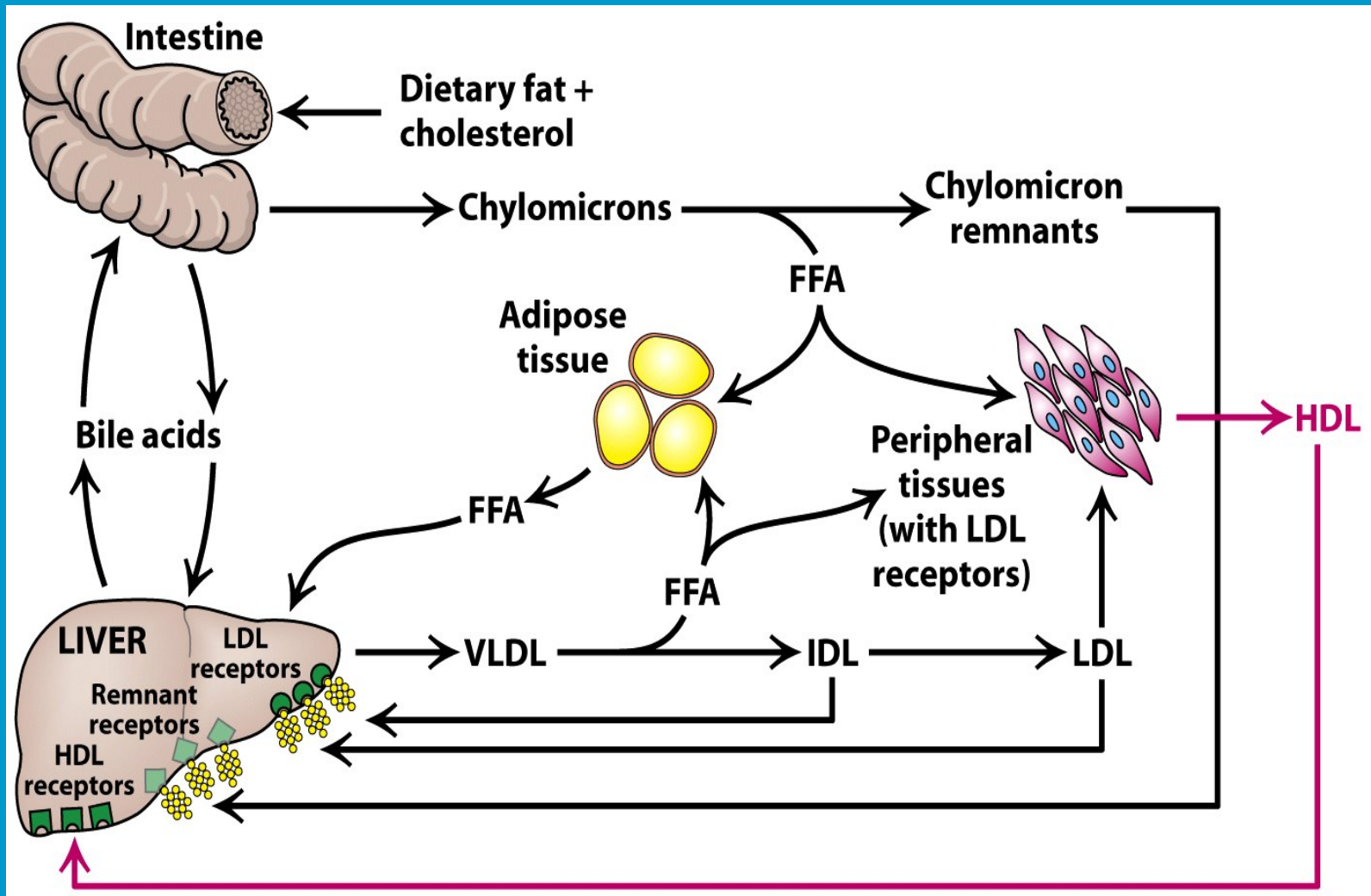

Figure 26-16
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Lipoproteins

The division of labor

- Chylomicrons' main task is to carry triglycerides
- LDLs are main carriers of cholesterol and cholesterol esters
- Relative amounts of HDL and LDL affect disposition of cholesterol and formation of arterial plaques

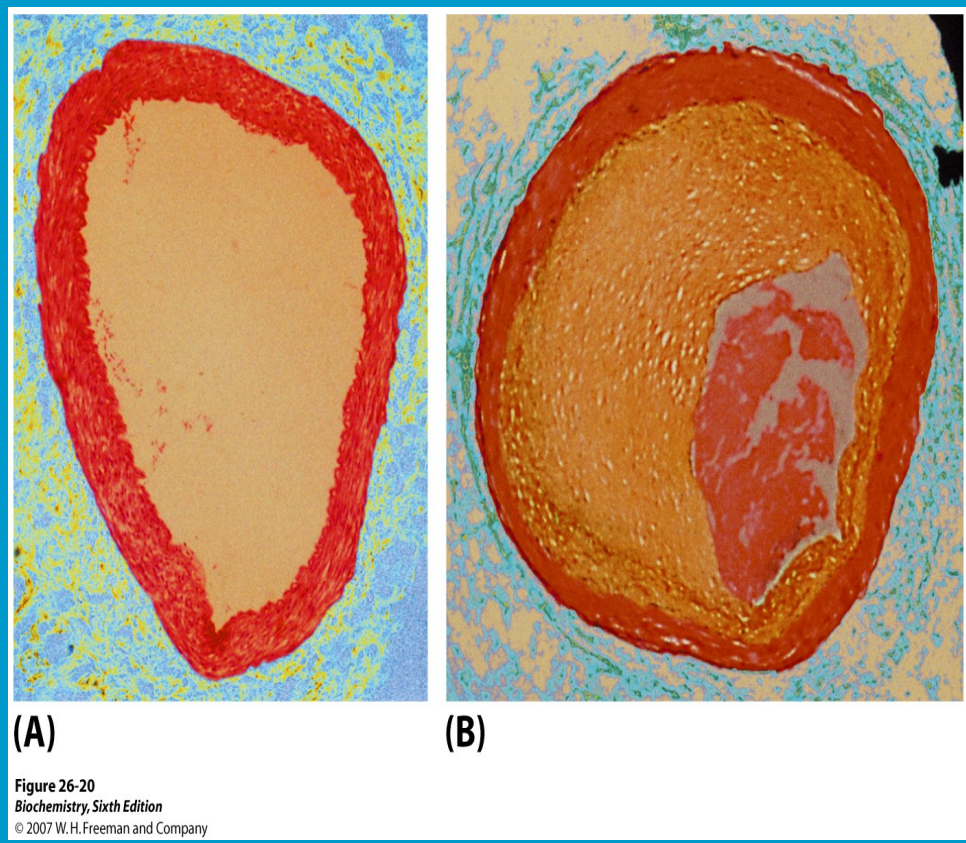

Figure 26-20
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Typical values for HDL, LDL

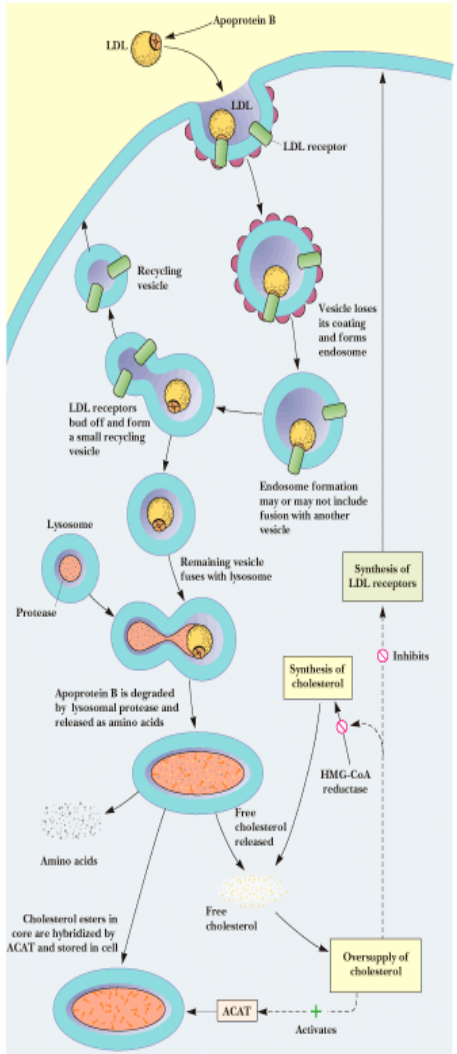
for males, females 15-29

- Cholesterol: females - 157-167, males - 150-174
- HDL: females - 52-55, males 45
- LDL: females - 100-106, males 97-116
- **The cholesterol/HDL ratio is key: greater than 4.5 is a risk factor for heart disease**
- However, with age, total cholesterol rises,and HDLs may fall, so exercise and diet become keys
- Regular, vigorous exercise raises HDLs and a low fat diet that avoids red meat reduces serum cholesterol levels

Lipoproteins in Circulation

Progressive degradation by lipases

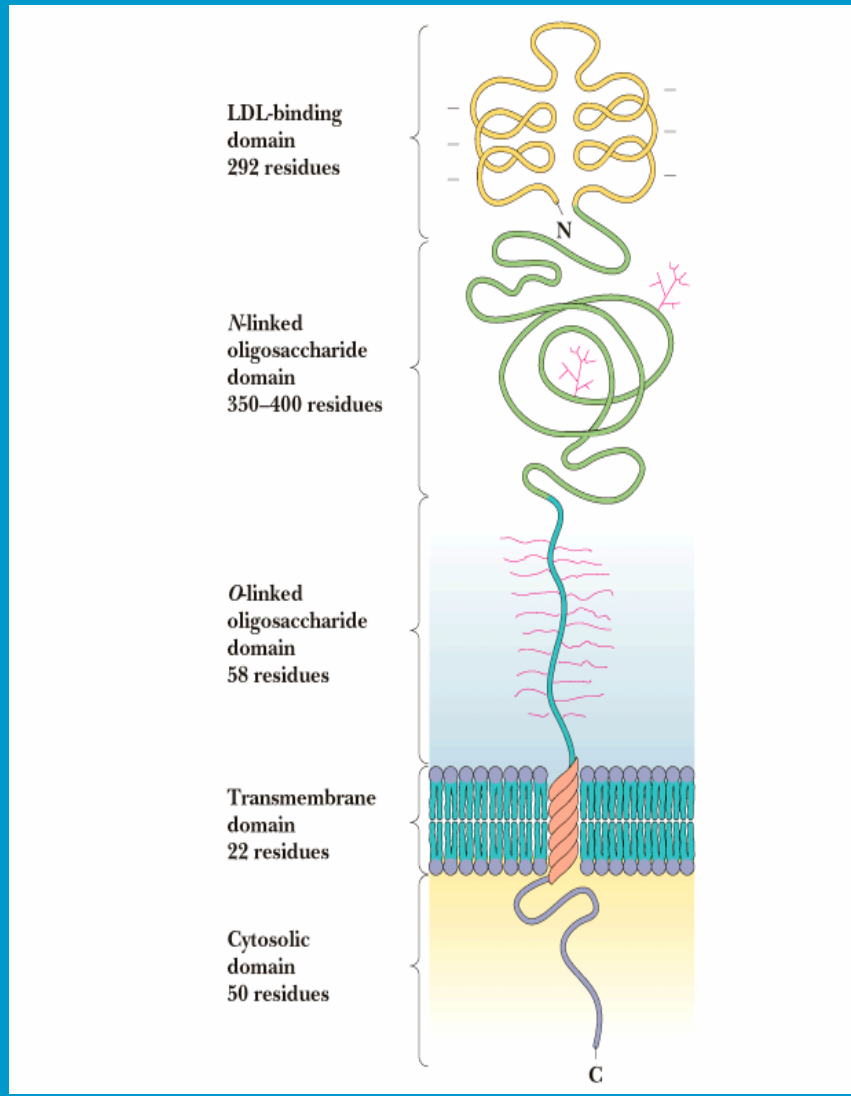
- Mostly in the capillaries of muscle and adipose cells, lipoprotein lipases hydrolyze triglycerides from lipoproteins, making the lipoproteins smaller and raising their density
- Thus chylomicrons and VLDLs are progressively converted to IDL and then LDL, which either return to the liver for reprocessing or are redirected to adipose tissues and adrenal glands



The LDL Receptor

A complex plasma membrane protein

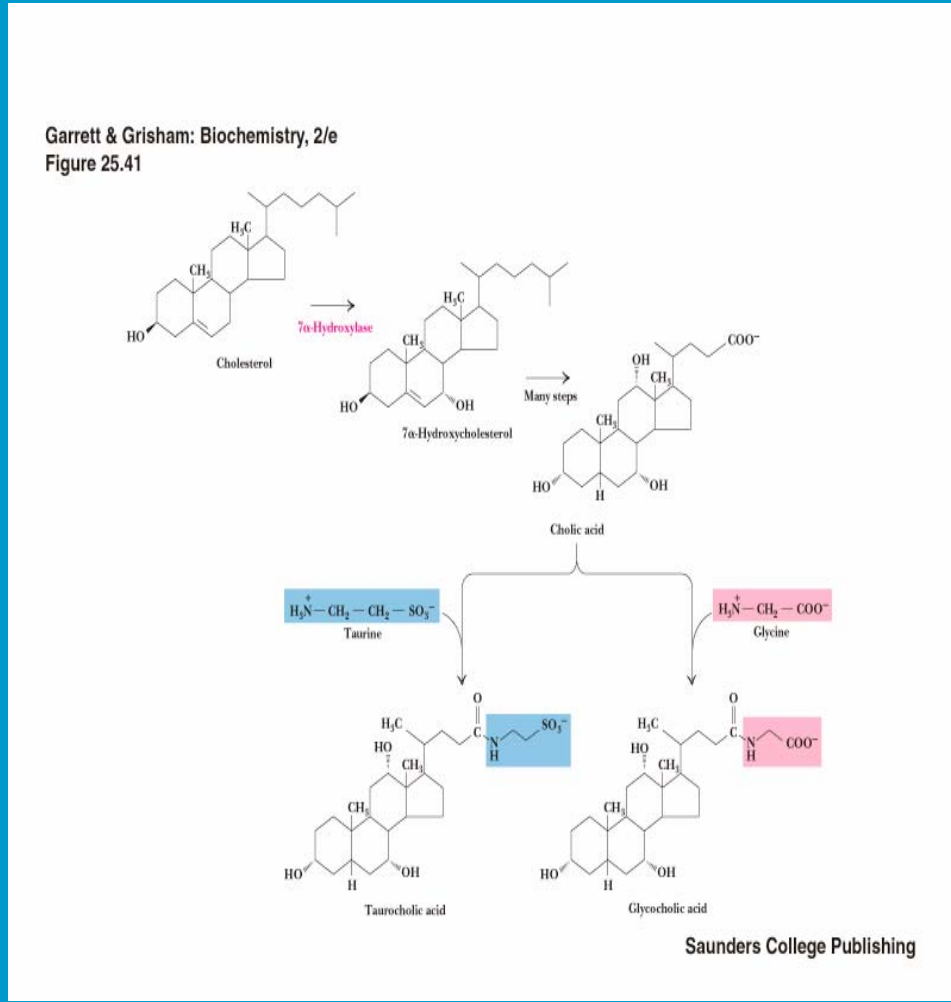
- LDL binding domain on N-terminus
- N-linked and O-linked oligosaccharide domains
- A single TMS
- A cytosolic domain essential to aggregation of receptors in the membrane during endocytosis
- Dysfunctions in or absence of LDL receptors lead to **familial hypercholesterolemia**



Biosynthesis of Bile Acids

Carboxylic acid derivatives of cholesterol

- Essential for the digestion of food, especially for solubilization of ingested fats
- Synthesized from cholesterol
- Cholic acid conjugates with taurine and glycine to form taurocholic and glycocholic acids
- First step is oxidation of cholesterol by a mixed-function oxidase



Steroid Hormone Synthesis

Desmolase (in mitochondria) forms pregnenolone, precursor to all others

- **Pregnenolone migrates from mitochondria to ER where progesterone is formed**
- **Progesterone is a branch point - it produces sex steroids (testosterone and estradiol), and corticosteroids (cortisol and aldosterone)**
- **Anabolic steroids are illegal and dangerous**
- **Recall the Ben Johnson story (stanozolol) and the recent years baseball scandals (androstenedione)….**

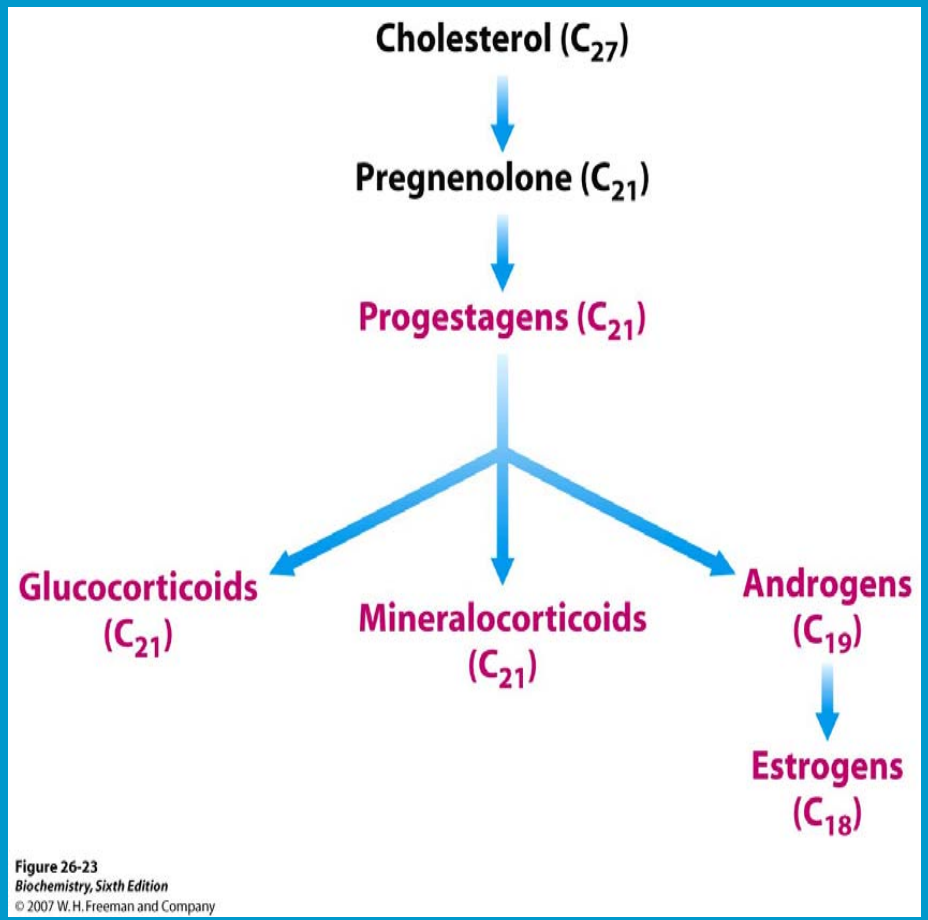


Figure 26-23
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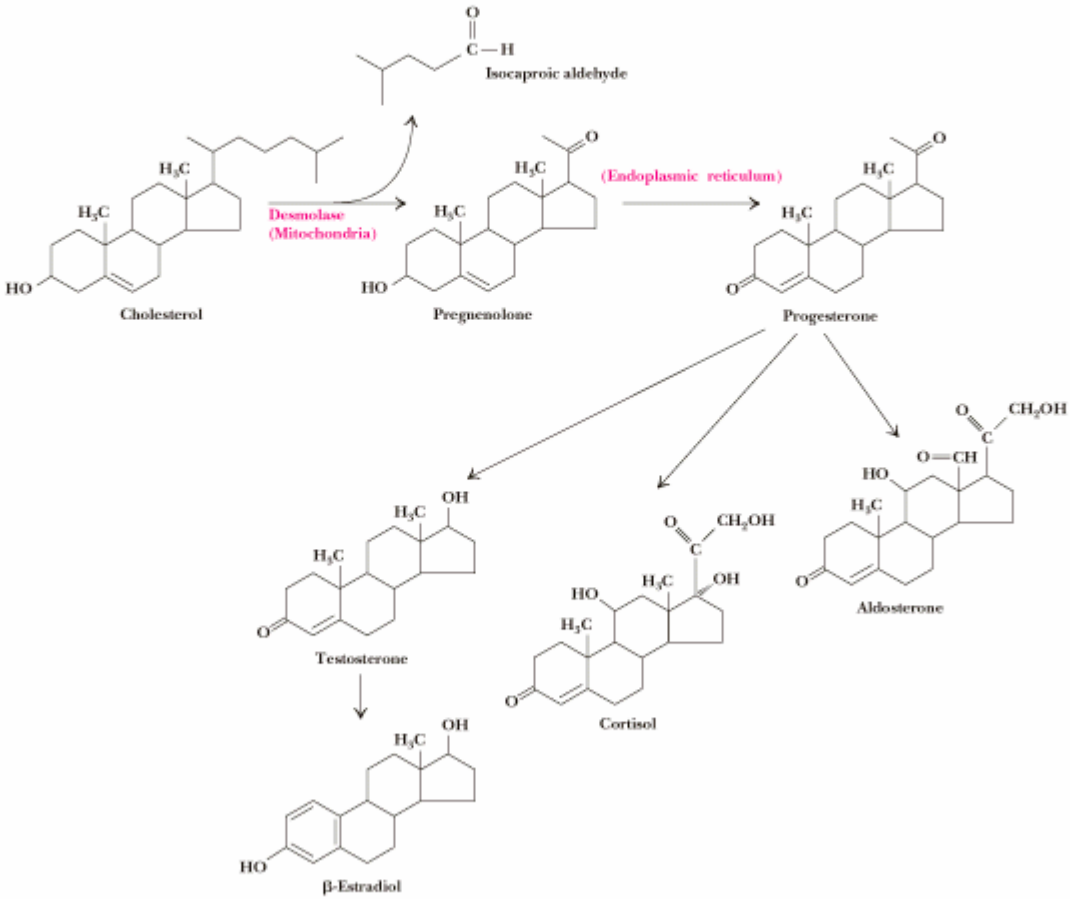
Figure 25.43



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