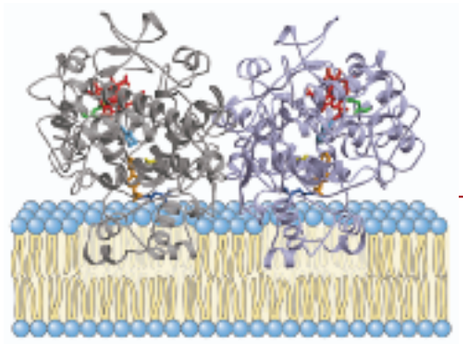


chapter 21

LIPID BIOSYNTHESIS

How the division of "spoils" came about I do not recall—it may have been by drawing lots. At any rate, David Shemin "drew" amino acid metabolism, which led to his classical work on heme biosynthesis. David Rittenburg was to continue his interest in protein synthesis and turnover, and lipids were to be my territory.

—Konrad Bloch, on how his career turned to problems of lipid metabolism after the death of his mentor, Rudolf Schoenheimer; article in Annual Review of Biochemistry, *1987*

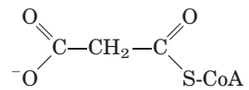
Lipids play a variety of cellular roles, some only recently recognized. They are the principal form of stored energy in most organisms and major constituents of cellular membranes. Specialized lipids serve as pigments (retinal, carotene), cofactors (vitamin K), detergents (bile salts), transporters (dolichols), hormones (vitamin D derivatives, sex hormones), extracellular and intracellular messengers (eicosanoids, phosphatidylinositol derivatives), and anchors for membrane proteins (covalently attached fatty acids, prenyl groups, and phosphatidylinositol). The ability to synthesize a variety of lipids is essential to all organisms. This chapter describes the biosynthetic pathways for some of the most common cellular lipids, illustrating the strategies employed in assembling these water-insoluble products from water-soluble precursors such as acetate. Like other biosynthetic pathways, these reaction sequences are endergonic and reductive. They use ATP as a source of metabolic energy and a reduced electron carrier (usually NADPH) as reductant.

We first describe the biosynthesis of fatty acids, the primary components of both triacylglycerols and phospholipids, then examine the assembly of fatty acids into triacylglycerols and the simpler membrane phospholipids. Finally, we consider the synthesis of cholesterol, a component of some membranes and the precursor of steroids such as the bile acids, sex hormones, and adrenocortical hormones.

21.1 Biosynthesis of Fatty Acids and Eicosanoids

After the discovery that fatty acid oxidation takes place by the oxidative removal of successive two-carbon (acetyl-CoA) units (see Fig. 17–8), biochemists thought the biosynthesis of fatty acids might proceed by a simple reversal of the same enzymatic steps. However, as they were to find out, fatty acid biosynthesis and breakdown occur by different pathways, are catalyzed by different sets of enzymes, and take place in different parts of the cell. Moreover, biosynthesis requires the participation of a three-carbon intermediate, **malonyl-CoA,** that is not involved in fatty acid breakdown.

We focus first on the pathway of fatty acid synthesis, then turn our attention to regulation of the pathway and to the biosynthesis of longer-chain fatty acids, unsaturated fatty acids, and their eicosanoid derivatives.



Malonyl-CoA

Malonyl-CoA Is Formed from Acetyl-CoA and Bicarbonate

The formation of malonyl-CoA from acetyl-CoA is an irreversible process, catalyzed by **acetyl-CoA carboxylase.** The bacterial enzyme has three separate polypeptide subunits (Fig. 21–1); in animal cells, all three

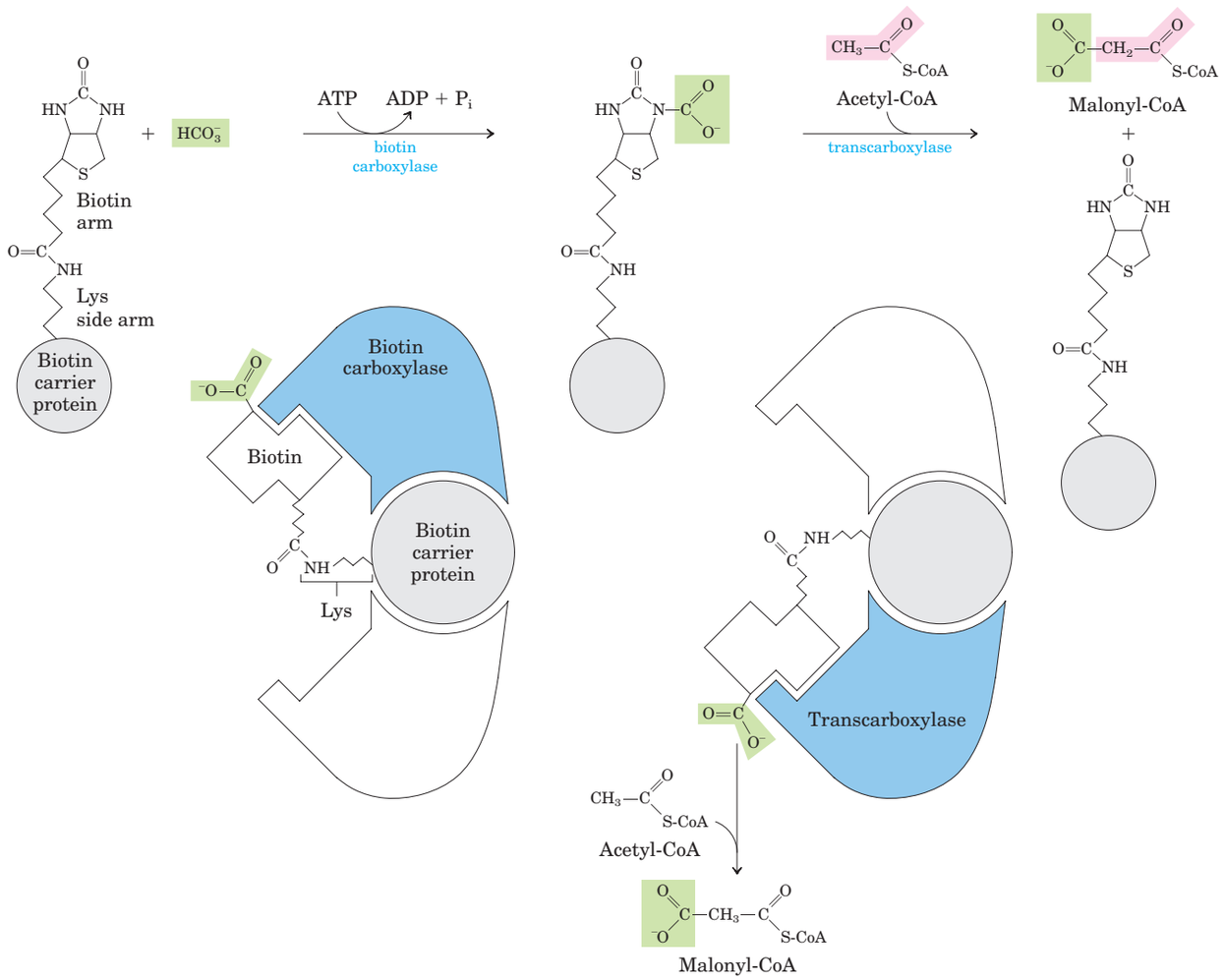


FIGURE 21–1 The acetyl-CoA carboxylase reaction. Acetyl-CoA carboxylase has three functional regions: biotin carrier protein (gray); biotin carboxylase, which activates CO_2 by attaching it to a nitrogen in the biotin ring in an ATP-dependent reaction (see Fig. 16–16); and transcarboxylase, which transfers activated CO_2 (shaded green) from biotin to acetyl-CoA, producing malonyl-CoA. The long, flexible biotin arm carries the activated CO_2 from the biotin carboxylase region to the transcarboxylase active site, as shown in the diagrams below the reaction arrows. The active enzyme in each step is shaded blue.

activities are part of a single multifunctional polypeptide. Plant cells contain both types of acetyl-CoA carboxylase. In all cases, the enzyme contains a biotin prosthetic group covalently bound in amide linkage to the ε-amino group of a Lys residue in one of the three polypeptides or domains of the enzyme molecule. The two-step reaction catalyzed by this enzyme is very similar to other biotin-dependent carboxylation reactions, such as those catalyzed by pyruvate carboxylase (see Fig. 16–16) and propionyl-CoA carboxylase (see Fig. 17–11). The carboxyl group, derived from bicarbonate (HCO_3^-), is first transferred to biotin in an ATP-dependent reaction. The biotinyl group serves as a temporary carrier of CO_2, transferring it to acetyl-CoA in the second step to yield malonyl-CoA.

Fatty Acid Synthesis Proceeds in a Repeating Reaction Sequence

The long carbon chains of fatty acids are assembled in a repeating four-step sequence (Fig. 21–2). A saturated acyl group produced by this set of reactions becomes the substrate for subsequent condensation with an activated malonyl group. With each passage through the cycle, the fatty acyl chain is extended by two carbons. When the chain length reaches 16 carbons, the product

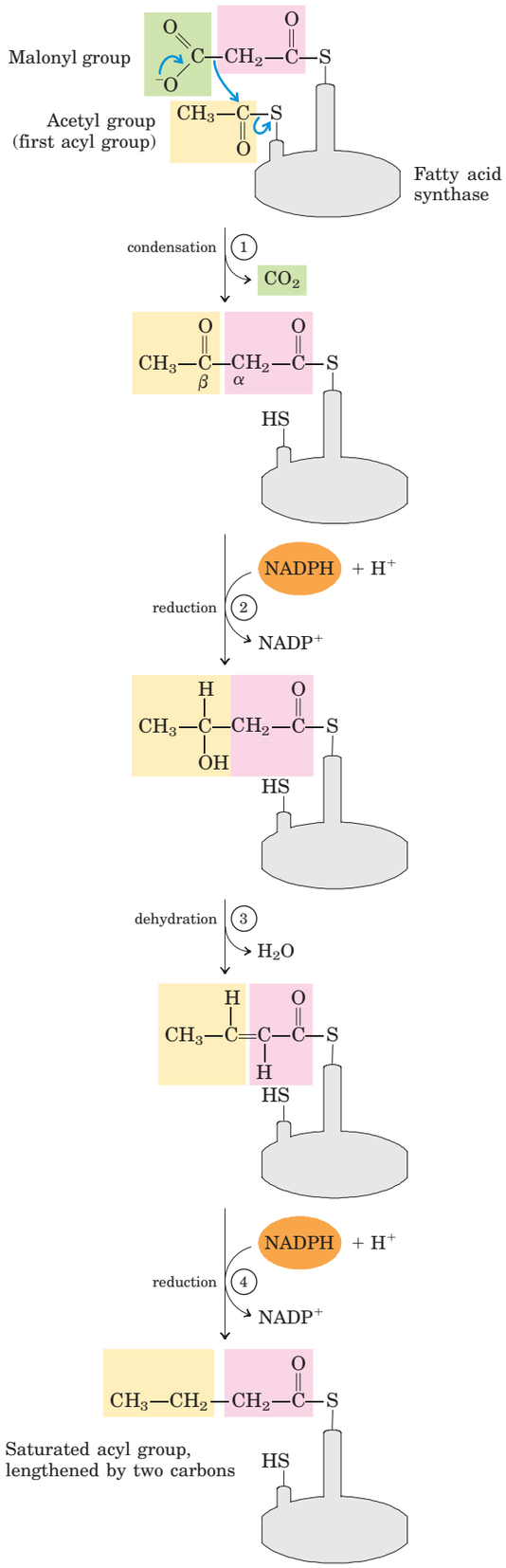


FIGURE 21–2 Addition of two carbons to a growing fatty acyl chain: a four-step sequence. Each malonyl group and acetyl (or longer acyl) group is activated by a thioester that links it to fatty acid synthase, a multienzyme complex described later in the text. (1) Condensation of an activated acyl group (an acetyl group from acetyl-CoA is the first 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. The mechanism of the first step of this reaction is given to illustrate the role of decarboxylation in facilitating condensation. The β-keto product of this condensation is then reduced in three more steps nearly identical to the reactions of β oxidation, but in the reverse sequence: (2) the β-keto group is reduced to an alcohol, (3) elimination of H_2O creates a double bond, and (4) the double bond is reduced to form the corresponding saturated fatty acyl group.

(palmitate, 16:0; see Table 10–1) leaves the cycle. Carbons C-16 and C-15 of the palmitate are derived from the methyl and carboxyl carbon atoms, respectively, of an acetyl-CoA used directly to prime the system at the outset (Fig. 21–3); the rest of the carbon atoms in the chain are derived from acetyl-CoA via malonyl-CoA.

Both the electron-carrying cofactor and the activating groups in the reductive anabolic sequence differ from those in the oxidative catabolic process. Recall that in β oxidation, NAD^+ and FAD serve as electron acceptors and the activating group is the thiol (—SH) group of coenzyme A (see Fig. 17–8). By contrast, the reducing agent in the synthetic sequence is NADPH and the activating groups are two different enzyme-bound —SH groups, as described below.

All the reactions in the synthetic process are catalyzed by a multienzyme complex, **fatty acid synthase.** Although the details of enzyme structure differ in prokaryotes such as *Escherichia coli* and in eukaryotes, the four-step process of fatty acid synthesis is the same in all organisms. We first describe the process as it occurs in *E. coli,* then consider differences in enzyme structure in other organisms.

The Fatty Acid Synthase Complex Has Seven Different Active Sites

The core of the *E. coli* fatty acid synthase system consists of seven separate polypeptides (Table 21–1), and at least three others act at some stage of the process. The proteins act together to catalyze the formation of fatty acids from acetyl-CoA and malonyl-CoA. Throughout the process, the intermediates remain covalently attached as thioesters to one of two thiol groups of the synthase complex. One point of attachment is the —SH group of a Cys residue in one of the seven synthase proteins (β-ketoacyl-ACP synthase); the other is the —SH group of acyl carrier protein.

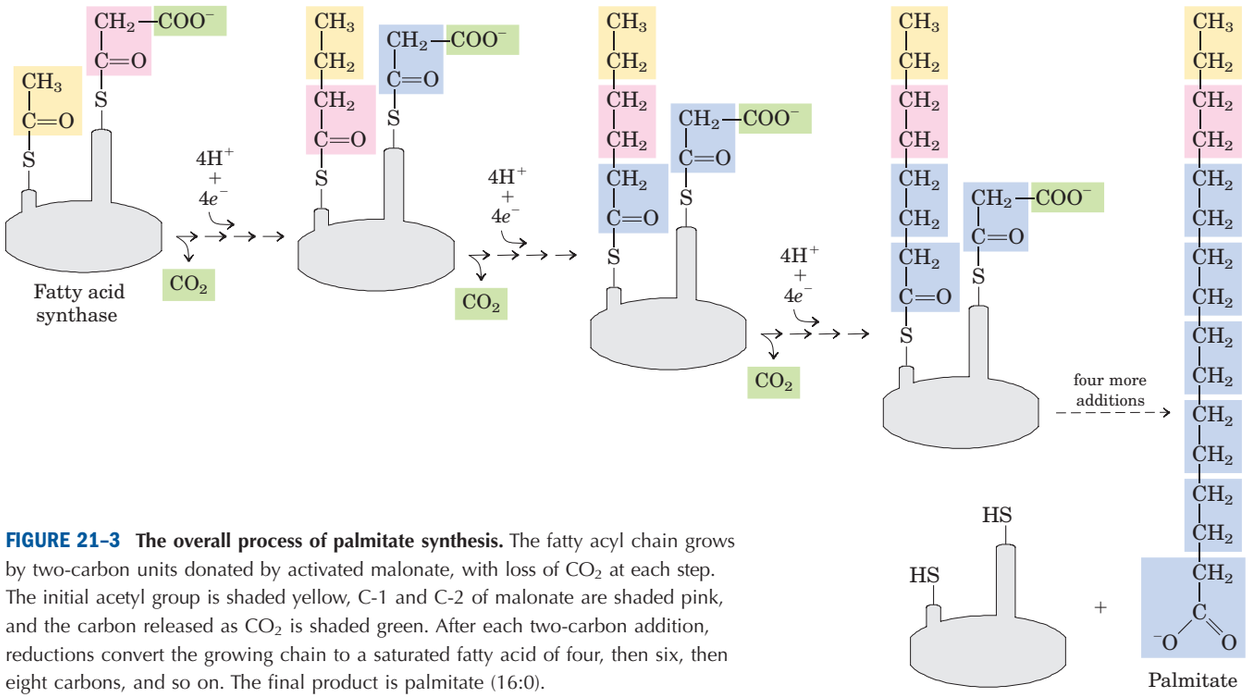


FIGURE 21–3 The overall process of palmitate synthesis. The fatty acyl chain grows by two-carbon units donated by activated malonate, with loss of CO_2 at each step. The initial acetyl group is shaded yellow, C-1 and C-2 of malonate are shaded pink, and the carbon released as CO_2 is shaded green. After each two-carbon addition, reductions convert the growing chain to a saturated fatty acid of four, then six, then eight carbons, and so on. The final product is palmitate (16:0).

Acyl carrier protein (ACP) of *E. coli* is a small protein (M_r 8,860) containing the prosthetic group **4′-phosphopantetheine** (Fig. 21–4; compare this with the panthothenic acid and β-mercaptoethylamine moiety of coenzyme A in Fig. 8–41). Hydrolysis of thioesters is highly exergonic, and the energy released helps to make two different steps (① and ⑤ in Fig. 21–5) in fatty acid synthesis (condensation) thermodynamically favorable. The 4′-phosphopante-theine prosthetic group of ACP is believed to serve as a flexible arm, tethering the growing fatty acyl chain to the surface of the fatty acid synthase complex while carrying the reaction intermediates from one enzyme active site to the next.

Fatty Acid Synthase Receives the Acetyl and Malonyl Groups

Before the condensation reactions that build up the fatty acid chain can begin, the two thiol groups on the enzyme complex must be charged with the correct acyl groups (Fig. 21–5, top). First, the acetyl group of acetyl-CoA is transferred to the Cys —SH group of the β-ketoacyl-ACP synthase. This reaction is catalyzed by **acetyl-CoA–ACP transacetylase** (AT in Fig. 21–5). The second reaction, transfer of the malonyl group from malonyl-CoA to the —SH group of ACP, is catalyzed by **malonyl-CoA–ACP transferase** (MT), also part of the complex. In the charged synthase complex, the acetyl

TABLE 21–1 Proteins of the Fatty Acid Synthase Complex of *E. coli*

Component	*Function*
Acyl carrier protein (ACP)	Carries acyl groups in thioester linkage
Acetyl-CoA–ACP transacetylase (AT)	Transfers acyl group from CoA to Cys residue of KS
β-Ketoacyl-ACP synthase (KS)	Condenses acyl and malonyl groups (KS has at least three isozymes)
Malonyl-CoA–ACP transferase (MT)	Transfers malonyl group from CoA to ACP
β-Ketoacyl-ACP reductase (KR)	Reduces β-keto group to β-hydroxyl group
β-Hydroxyacyl-ACP dehydratase (HD)	Removes H_2O from β-hydroxyacyl-ACP, creating double bond
Enoyl-ACP reductase (ER)	Reduces double bond, forming saturated acyl-ACP

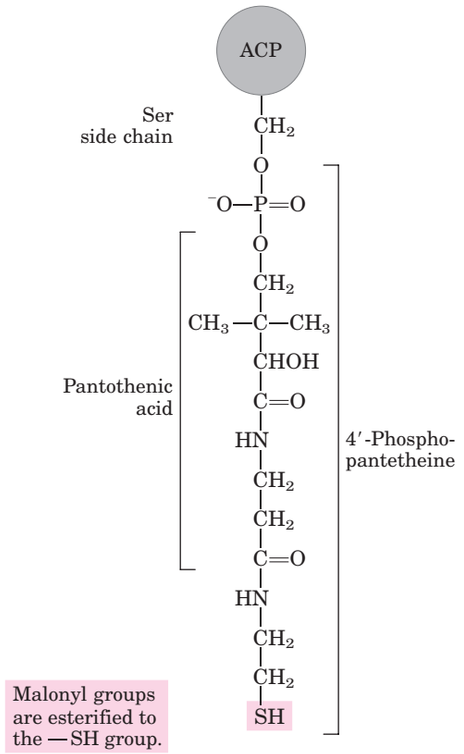


FIGURE 21–4 Acyl carrier protein (ACP). The prosthetic group is 4′-phosphopantetheine, which is covalently attached to the hydroxyl group of a Ser residue in ACP. Phosphopantetheine contains the B vitamin pantothenic acid, also found in the coenzyme A molecule. Its —SH group is the site of entry of malonyl groups during fatty acid synthesis.

and malonyl groups are very close to each other and are activated for the chain-lengthening process. The first four steps of this process are now considered in some detail; all step numbers refer to Figure 21–5.

Step* (1) *Condensation The first reaction in the formation of a fatty acid chain is condensation of the activated acetyl and malonyl groups to form **acetoacetyl-ACP,** an acetoacetyl group bound to ACP through the phosphopantetheine —SH group; simultaneously, a molecule of CO_2 is produced. In this reaction, catalyzed by ***β*-ketoacyl-ACP synthase** (KS), the acetyl group is transferred from the Cys —SH group of the enzyme to the malonyl group on the —SH of ACP, becoming the methyl-terminal two-carbon unit of the new acetoacetyl group.

The carbon atom of the CO_2 formed in this reaction is the same carbon originally introduced into malonyl-CoA from HCO_3^- by the acetyl-CoA carboxylase reaction (Fig. 21–1). Thus CO_2 is only transiently in covalent linkage during fatty acid biosynthesis; it is removed as each two-carbon unit is added.

Why do cells go to the trouble of adding CO_2 to make a malonyl group from an acetyl group, only to lose the CO_2 during the formation of acetoacetate? Recall that in the β oxidation of fatty acids (see Fig. 17–8), cleavage of the bond between two acyl groups (cleavage of an acetyl unit from the acyl chain) is highly exergonic, so the simple condensation of two acyl groups (two acetyl-CoA molecules, for example) is highly endergonic. The use of activated malonyl groups rather than acetyl groups is what makes the condensation reactions thermodynamically favorable. The methylene carbon (C-2) of the malonyl group, sandwiched between carbonyl and carboxyl carbons, is chemically situated to act as a good nucleophile. In the condensation step (step (1)), decarboxylation of the malonyl group facilitates the nucleophilic attack of the methylene carbon on the thioester linking the acetyl group to β-ketoacyl-ACP synthase, displacing the enzyme's —SH group. Coupling the condensation to the decarboxylation of the malonyl group renders the overall process highly exergonic. A similar carboxylation-decarboxylation sequence facilitates the formation of phosphoenolpyruvate from pyruvate in gluconeogenesis (see Fig. 14–17).

By using activated malonyl groups in the synthesis of fatty acids and activated acetate in their degradation, the cell makes both processes energetically favorable, although one is effectively the reversal of the other. The extra energy required to make fatty acid synthesis favorable is provided by the ATP used to synthesize malonyl-CoA from acetyl-CoA and HCO_3^- (Fig. 21–1).

Step* (2) *Reduction of the Carbonyl Group The acetoacetyl-ACP formed in the condensation step now undergoes reduction of the carbonyl group at C-3 to form D-*β*-hydroxybutyryl-ACP. This reaction is catalyzed by ***β*-ketoacyl-ACP reductase** (KR) and the electron donor is NADPH. Notice that the D-*β*-hydroxybutyryl group does not have the same stereoisomeric form as the L-*β*-hydroxyacyl intermediate in fatty acid oxidation (see Fig. 17–8).

Step* (3) *Dehydration The elements of water are now removed from C-2 and C-3 of D-*β*-hydroxybutyryl-ACP to yield a double bond in the product, ***trans*-Δ^2- butenoyl-ACP.** The enzyme that catalyzes this dehydration is ***β*-hydroxyacyl-ACP dehydratase** (HD).

Step* (4) *Reduction of the Double Bond Finally, the double bond of *trans*-Δ^2-butenoyl-ACP is reduced (saturated) to form **butyryl-ACP** by the action of **enoyl-ACP reductase** (ER); again, NADPH is the electron donor.

The Fatty Acid Synthase Reactions Are Repeated to Form Palmitate

Production of the four-carbon, saturated fatty acyl–ACP completes one pass through the fatty acid synthase

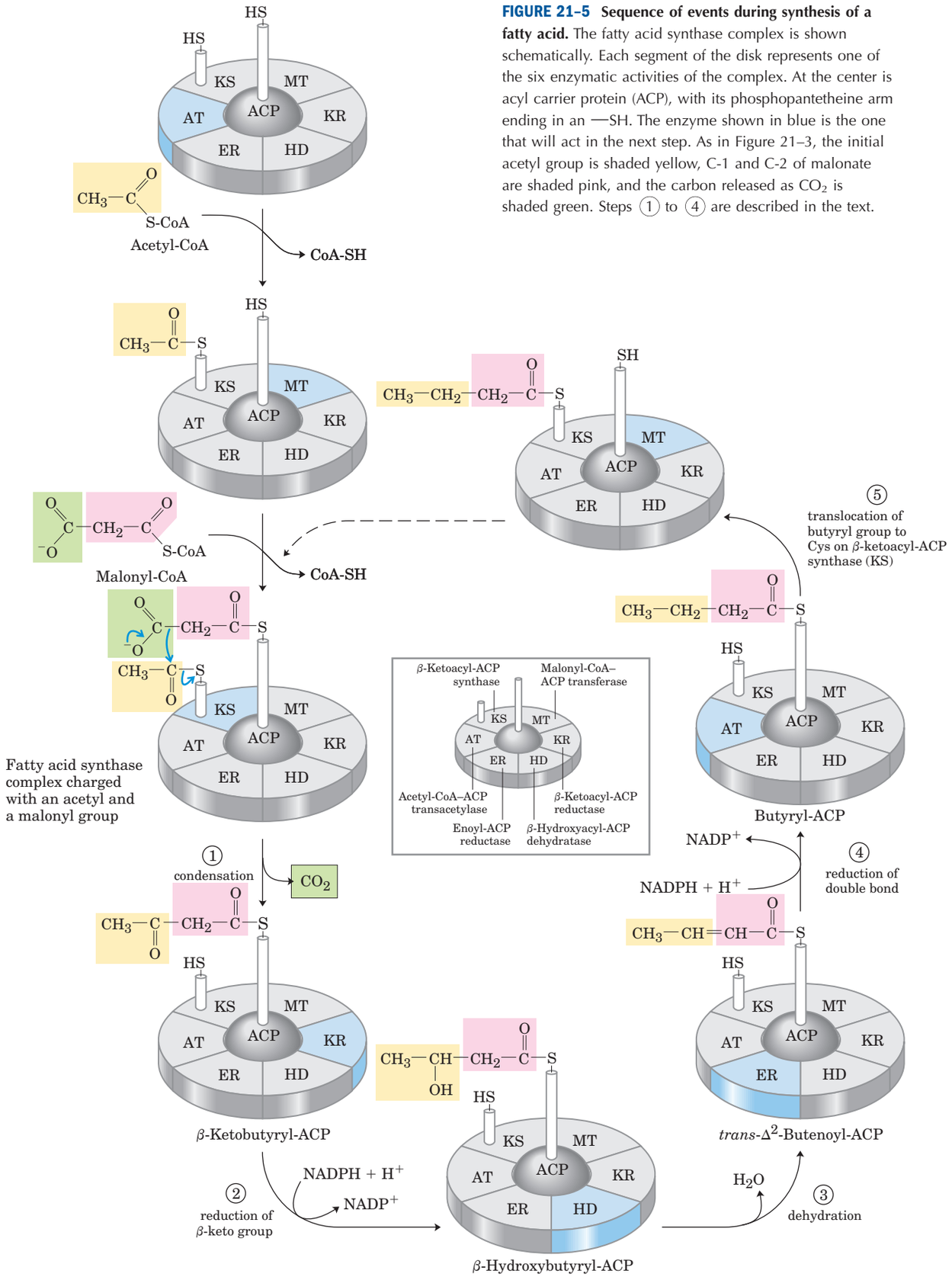


FIGURE 21–5 Sequence of events during synthesis of a fatty acid. The fatty acid synthase complex is shown schematically. Each segment of the disk represents one of the six enzymatic activities of the complex. At the center is acyl carrier protein (ACP), with its phosphopantetheine arm ending in an —SH. The enzyme shown in blue is the one that will act in the next step. As in Figure 21–3, the initial acetyl group is shaded yellow, C-1 and C-2 of malonate are shaded pink, and the carbon released as CO_2 is shaded green. Steps (1) to (4) are described in the text.

complex. The butyryl group is now transferred from the phosphopantetheine —SH group of ACP to the Cys —SH group of β-ketoacyl-ACP synthase, which initially bore the acetyl group (Fig. 21–5). To start the next cycle of four reactions that lengthens the chain by two more carbons, another malonyl group is linked to the now unoccupied phosphopantetheine —SH group of ACP (Fig. 21–6). Condensation occurs as the butyryl group, acting like the acetyl group in the first cycle, is linked to two carbons of the malonyl-ACP group with concurrent loss of CO_2. The product of this condensation is a six-carbon acyl group, covalently bound to the phosphopantetheine —SH group. Its β-keto group is reduced in the next three steps of the synthase cycle to yield the saturated acyl group, exactly as in the first round of reactions—in this case forming the six-carbon product.

Seven cycles of condensation and reduction produce the 16-carbon saturated palmitoyl group, still bound to ACP. For reasons not well understood, chain elongation by the synthase complex generally stops at this point and free palmitate is released from the ACP by a hydrolytic activity in the complex. Small amounts of longer fatty acids such as stearate (18:0) are also formed. In certain plants (coconut and palm, for example) chain termination occurs earlier; up to 90% of the fatty acids in the oils of these plants are between 8 and 14 carbons long.

We can consider the overall reaction for the synthesis of palmitate from acetyl-CoA in two parts. First, the formation of seven malonyl-CoA molecules:

$$7\ \text{Acetyl-CoA} + 7CO_2 + 7\text{ATP} \rightarrow 7\ \text{malonyl-CoA} + 7\text{ADP} + 7P_i \qquad (21\text{–}1)$$

then seven cycles of condensation and reduction:

$$\text{Acetyl-CoA} + 7\ \text{malonyl-CoA} + 14\text{NADPH} + 14H^+ \rightarrow \text{palmitate} + 7CO_2 + 8\ \text{CoA} + 14\text{NADP}^+ + 6H_2O \qquad (21\text{–}2)$$

The overall process (the sum of Eqns 21–1 and 21–2) is

$$8\ \text{Acetyl-CoA} + 7\text{ATP} + 14\text{NADPH} + 14H^+ \rightarrow \text{palmitate} + 8\ \text{CoA} + 7\text{ADP} + 7P_i + 14\text{NADP}^+ + 6H_2O \qquad (21\text{–}3)$$

The biosynthesis of fatty acids such as palmitate thus requires acetyl-CoA and the input of chemical energy in two forms: the group transfer potential of ATP and the reducing power of NADPH. The ATP is required to attach CO_2 to acetyl-CoA to make malonyl-CoA; the NADPH is required to reduce the double bonds. We return to the sources of acetyl-CoA and NADPH soon, but first let's consider the structure of the remarkable enzyme complex that catalyzes the synthesis of fatty acids.

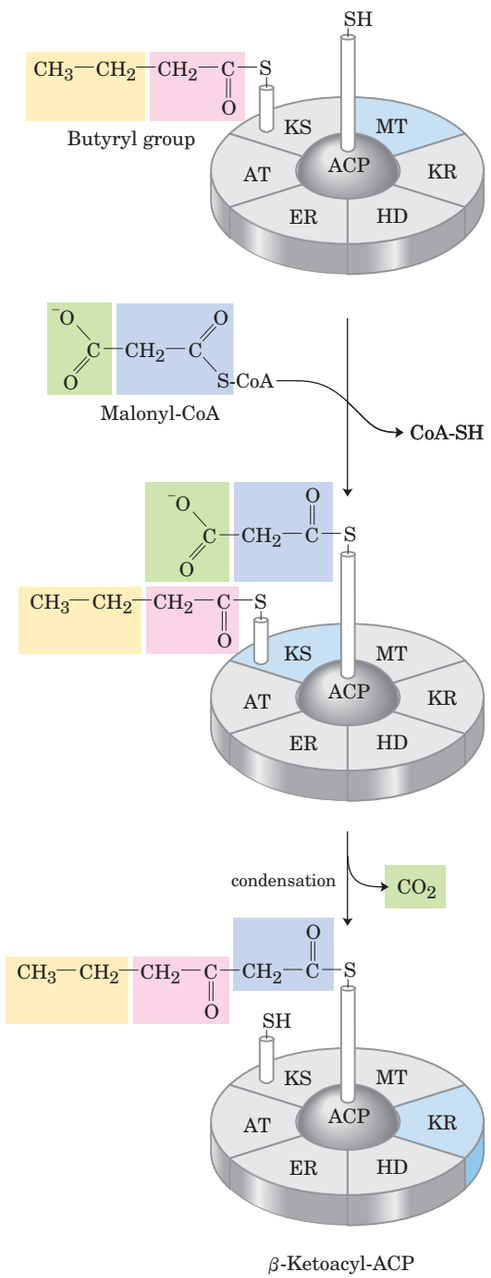


FIGURE 21–6 Beginning of the second round of the fatty acid synthesis cycle. The butyryl group is on the Cys —SH group. The incoming malonyl group is first attached to the phosphopantetheine —SH group. Then, in the condensation step, the entire butyryl group on the Cys —SH is exchanged for the carboxyl group of the malonyl residue, which is lost as CO_2 (green). This step is analogous to step (1) in Figure 21–5. The product, a six-carbon β-ketoacyl group, now contains four carbons derived from malonyl-CoA and two derived from the acetyl-CoA that started the reaction. The β-ketoacyl group then undergoes steps (2) through (4), as in Figure 21–5.

The Fatty Acid Synthase of Some Organisms Consists of Multifunctional Proteins

In *E. coli* and some plants, the seven active sites for fatty acid synthesis (six enzymes and ACP) reside in seven separate polypeptides (Fig. 21–7, top). In these complexes, each enzyme is positioned with its active site near that of the preceding and succeeding enzymes of the sequence. The flexible pantetheine arm of ACP can reach all the active sites, and it carries the growing fatty acyl chain from one site to the next; intermediates are not released from the enzyme complex until it has formed the finished product. As we have seen in earlier chapters, this channeling of intermediates from one active site to the next increases the efficiency of the overall process.

The fatty acid synthases of yeast and of vertebrates are also multienzyme complexes, and their integration is even more complete than in *E. coli* and plants. In yeast, the seven distinct active sites reside in two large, multifunctional polypeptides, with three activities on the α subunit and four on the β subunit. In vertebrates, a single large polypeptide (M_r 240,000) contains all seven enzymatic activities as well as a hydrolytic activity that cleaves the finished fatty acid from the ACP-like part of the enzyme complex. The vertebrate enzyme functions as a dimer (M_r 480,000) in which the two identical subunits lie head-to-tail. The subunits appear to function independently. When all the active sites in one subunit are inactivated by mutation, palmitate synthesis is only modestly reduced.

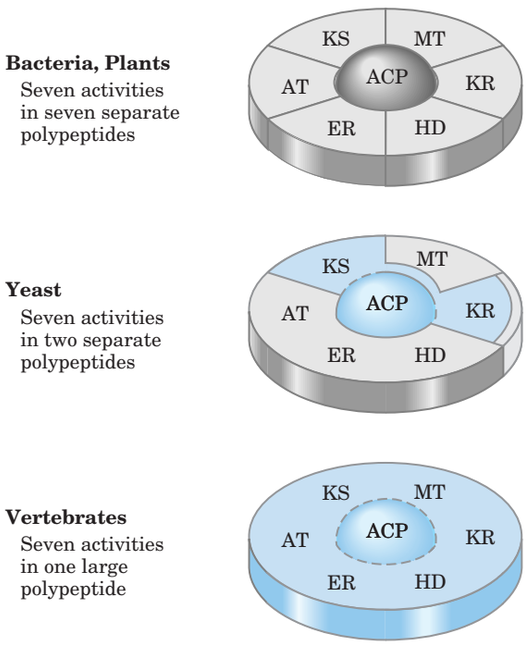


FIGURE 21–7 Structure of fatty acid synthases. The fatty acid synthase of bacteria and plants is a complex of at least seven different polypeptides. In yeast, all seven activities reside in only two polypeptides; the vertebrate enzyme is a single large polypeptide.

Fatty Acid Synthesis Occurs in the Cytosol of Many Organisms but in the Chloroplasts of Plants

In most higher eukaryotes, the fatty acid synthase complex is found exclusively in the cytosol (Fig. 21–8), as are the biosynthetic enzymes for nucleotides, amino acids, and glucose. This location segregates synthetic processes from degradative reactions, many of which take place in the mitochondrial matrix. There is a corresponding segregation of the electron-carrying cofactors used in anabolism (generally a reductive process) and those used in catabolism (generally oxidative).

Usually, NADPH is the electron carrier for anabolic reactions, and NAD^+ serves in catabolic reactions. In hepatocytes, the [NADPH]/[$NADP^+$] ratio is very high (about 75) in the cytosol, furnishing a strongly reducing environment for the reductive synthesis of fatty acids and other biomolecules. The cytosolic [NADH]/[NAD^+] ratio is much smaller (only about 8×10^{-4}), so the NAD^+-dependent oxidative catabolism of glucose can take place in the same compartment, and at the same time, as fatty acid synthesis. The [NADH]/[NAD^+] ratio in the mitochondrion is much higher than in the cytosol, because of the flow of electrons to NAD^+ from the oxidation of fatty acids, amino acids, pyruvate, and acetyl-CoA. This high mitochondrial [NADH]/[NAD^+] ratio favors the reduction of oxygen via the respiratory chain.

In hepatocytes and adipocytes, cytosolic NADPH is largely generated by the pentose phosphate pathway (see Fig. 14–21) and by **malic enzyme** (Fig. 21–9a). The NADP-linked malic enzyme that operates in the carbon-assimilation pathway of C_4 plants (see Fig. 20–23) is unrelated in function. The pyruvate produced in the reaction shown in Figure 21–9a reenters the mitochondrion. In hepatocytes and in the mammary gland of lactating animals, the NADPH required for fatty acid biosynthesis is supplied primarily by the pentose phosphate pathway (Fig. 21–9b).

In the photosynthetic cells of plants, fatty acid synthesis occurs not in the cytosol but in the chloroplast stroma (Fig. 21–8). This makes sense, given that NADPH is produced in chloroplasts by the light reactions of photosynthesis:

$$H_2O + NADP^+ \xrightarrow{\text{light}} \tfrac{1}{2}O_2 + NADPH + H^+$$

Again, the resulting high [NADPH]/[$NADP^+$] ratio provides the reducing environment that favors reductive anabolic processes such as fatty acid synthesis.

Acetate Is Shuttled out of Mitochondria as Citrate

In nonphotosynthetic eukaryotes, nearly all the acetyl-CoA used in fatty acid synthesis is formed in mito-

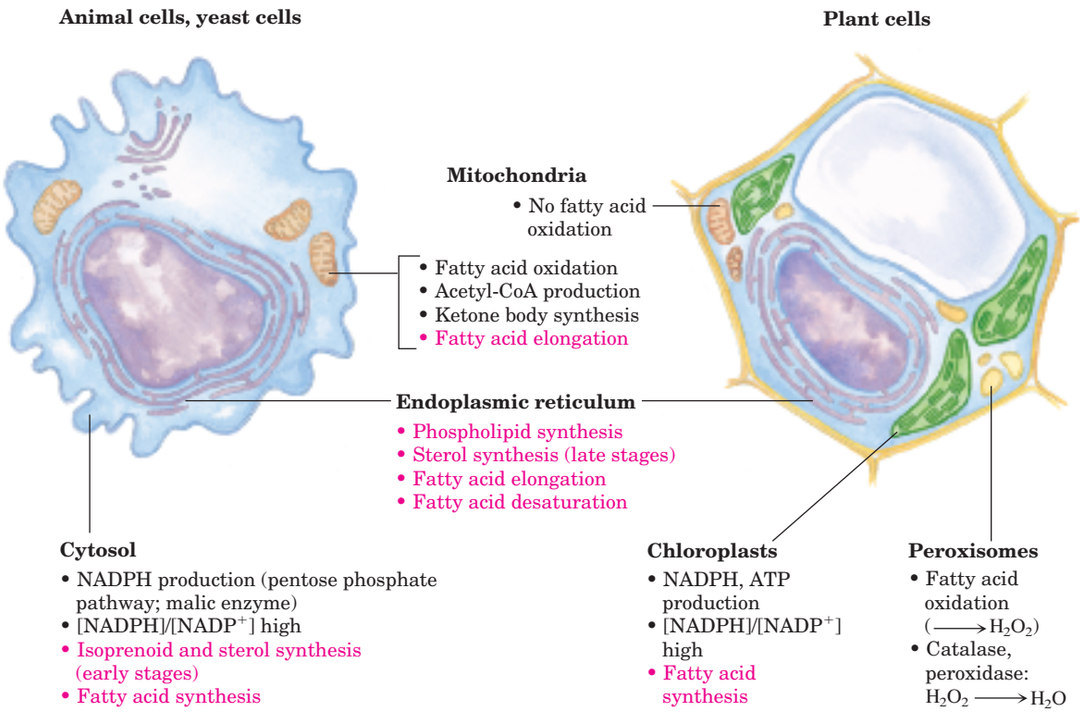


FIGURE 21–8 Subcellular localization of lipid metabolism. Yeast and vertebrate cells differ from higher plant cells in the compartmentation of lipid metabolism. Fatty acid synthesis takes place in the compartment in which NADPH is available for reductive synthesis (i.e., where the [NADPH]/[$NADP^+$] ratio is high). Processes in red type are covered in this chapter.

chondria from pyruvate oxidation and from the catabolism of the carbon skeletons of amino acids. Acetyl-CoA arising from the oxidation of fatty acids is not a significant source of acetyl-CoA for fatty acid biosynthesis in animals, because the two pathways are reciprocally regulated, as described below.

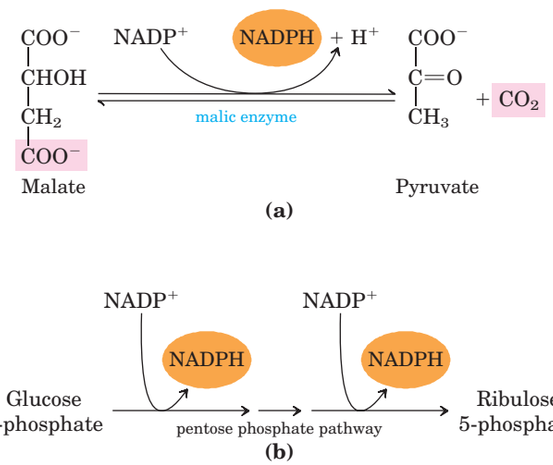


FIGURE 21–9 Production of NADPH. Two routes to NADPH, catalyzed by **(a)** malic enzyme and **(b)** the pentose phosphate pathway.

The mitochondrial inner membrane is impermeable to acetyl-CoA, so an indirect shuttle transfers acetyl group equivalents across the inner membrane (Fig. 21–10). Intramitochondrial acetyl-CoA first reacts with oxaloacetate to form citrate, in the citric acid cycle reaction catalyzed by **citrate synthase** (see Fig. 16–7). Citrate then passes through the inner membrane on the **citrate transporter.** In the cytosol, citrate cleavage by **citrate lyase** regenerates acetyl-CoA in an ATP-dependent reaction. Oxaloacetate cannot return to the mitochondrial matrix directly, as there is no oxaloacetate transporter. Instead, cytosolic malate dehydrogenase reduces the oxaloacetate to malate, which returns to the mitochondrial matrix on the malate–α-ketoglutarate transporter in exchange for citrate. In the matrix, malate is reoxidized to oxaloacetate to complete the shuttle. Alternatively, the malate produced in the cytosol is used to generate cytosolic NADPH through the activity of malic enzyme (Fig. 21–9a).

Fatty Acid Biosynthesis Is Tightly Regulated

When a cell or organism has more than enough metabolic fuel to meet its energy needs, the excess is generally converted to fatty acids and stored as lipids such as triacylglycerols. The reaction catalyzed by acetyl-CoA

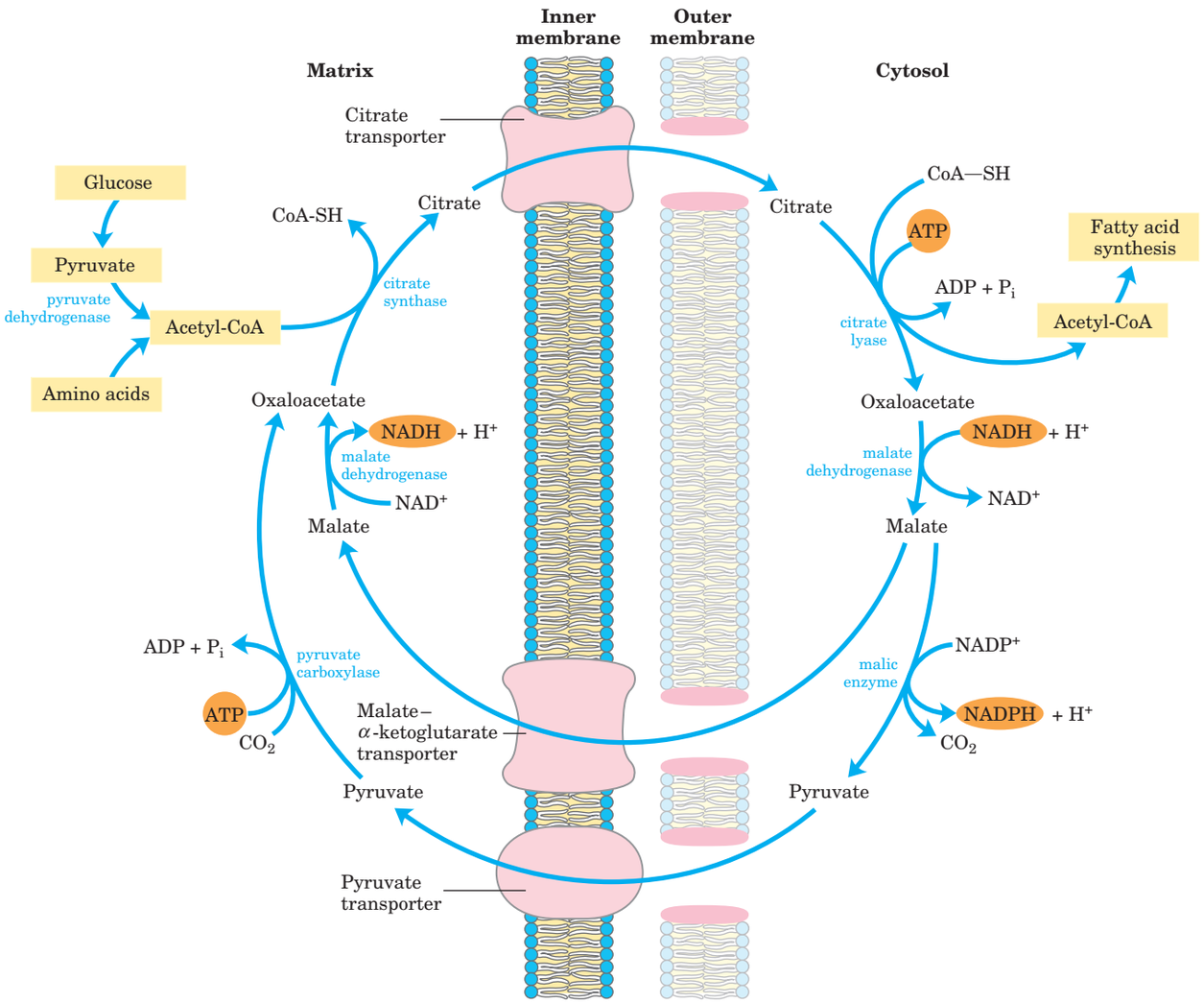


FIGURE 21–10 Shuttle for transfer of acetyl groups from mitochondria to the cytosol. The mitochondrial outer membrane is freely permeable to all these compounds. Pyruvate derived from amino acid catabolism in the mitochondrial matrix, or from glucose by glycolysis in the cytosol, is converted to acetyl-CoA in the matrix. Acetyl groups pass out of the mitochondrion as citrate; in the cytosol they are delivered as acetyl-CoA for fatty acid synthesis. Oxaloacetate is reduced to malate, which returns to the mitochondrial matrix and is converted to oxaloacetate. An alternative fate for cytosolic malate is oxidation by malic enzyme to generate cytosolic NADPH; the pyruvate produced returns to the mitochondrial matrix.

carboxylase is the rate-limiting step in the biosynthesis of fatty acids, and this enzyme is an important site of regulation. In vertebrates, palmitoyl-CoA, the principal product of fatty acid synthesis, is a feedback inhibitor of the enzyme; citrate is an allosteric activator (Fig. 21–11a), increasing V_{max}. Citrate plays a central role in diverting cellular metabolism from the consumption (oxidation) of metabolic fuel to the storage of fuel as fatty acids. When the concentrations of mitochondrial acetyl-CoA and ATP increase, citrate is transported out of mitochondria; it then becomes both the precursor of cytosolic acetyl-CoA and an allosteric signal for the activation of acetyl-CoA carboxylase. At the same time, citrate inhibits the activity of phosphofructokinase-1 (see Fig. 15–18), reducing the flow of carbon through glycolysis.

Acetyl-CoA carboxylase is also regulated by covalent modification. Phosphorylation, triggered by the hormones glucagon and epinephrine, inactivates the enzyme and reduces its sensitivity to activation by citrate, thereby slowing fatty acid synthesis. In its active (dephosphorylated) form, acetyl-CoA carboxylase polymerizes into long filaments (Fig. 21–11b); phosphorylation is accompanied by dissociation into monomeric subunits and loss of activity.

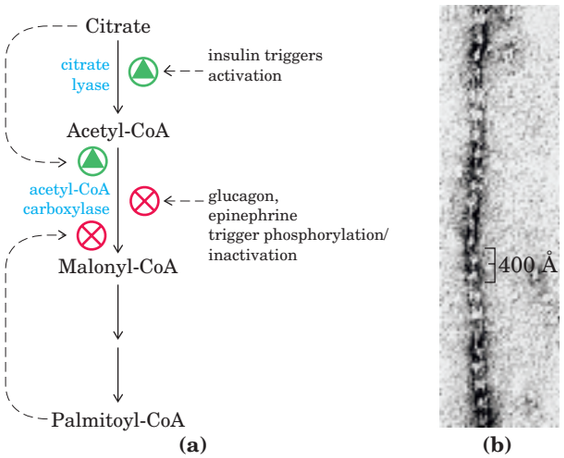


FIGURE 21–11 **Regulation of fatty acid synthesis. (a)** In the cells of vertebrates, both allosteric regulation and hormone-dependent covalent modification influence the flow of precursors into malonyl-CoA. In plants, acetyl-CoA carboxylase is activated by the changes in $[Mg^{2+}]$ and pH that accompany illumination (not shown here). **(b)** Filaments of acetyl-CoA carboxylase (the active, dephosphorylated form) as seen with the electron microscope.

The acetyl-CoA carboxylase of plants and bacteria is not regulated by citrate or by a phosphorylation-dephosphorylation cycle. The plant enzyme is activated by an increase in stromal pH and $[Mg^{2+}]$, which occurs on illumination of the plant (see Fig. 20–18). Bacteria do not use triacylglycerols as energy stores. In *E. coli,* the primary role of fatty acid synthesis is to provide precursors for membrane lipids; the regulation of this process is complex, involving guanine nucleotides (such as ppGpp) that coordinate cell growth with membrane formation (see Figs 8–42, 28–24).

In addition to the moment-by-moment regulation of enzymatic activity, these pathways are regulated at the level of gene expression. For example, when animals ingest an excess of certain polyunsaturated fatty acids, the expression of genes encoding a wide range of lipogenic enzymes in the liver is suppressed. The detailed mechanism by which these genes are regulated is not yet clear.

If fatty acid synthesis and β oxidation were to proceed simultaneously, the two processes would constitute a futile cycle, wasting energy. We noted earlier (see Fig. 17–12) that β oxidation is blocked by malonyl-CoA, which inhibits carnitine acyltransferase I. Thus during fatty acid synthesis, the production of the first intermediate, malonyl-CoA, shuts down β oxidation at the level of a transport system in the mitochondrial inner membrane. This control mechanism illustrates another advantage of segregating synthetic and degradative pathways in different cellular compartments.

Long-Chain Saturated Fatty Acids Are Synthesized from Palmitate

Palmitate, the principal product of the fatty acid synthase system in animal cells, is the precursor of other long-chain fatty acids (Fig. 21–12). It may be lengthened to form stearate (18:0) or even longer saturated fatty acids by further additions of acetyl groups, through the action of **fatty acid elongation systems** present in the smooth endoplasmic reticulum and in mitochondria. The more active elongation system of the ER extends the 16-carbon chain of palmitoyl-CoA by two carbons, forming stearoyl-CoA. Although different enzyme systems are involved, and coenzyme A rather than ACP is the acyl carrier in the reaction, the mechanism of elongation in the ER is otherwise identical to that in palmitate synthesis: donation of two carbons by malonyl-CoA, followed by reduction, dehydration, and reduction to the saturated 18-carbon product, stearoyl-CoA.

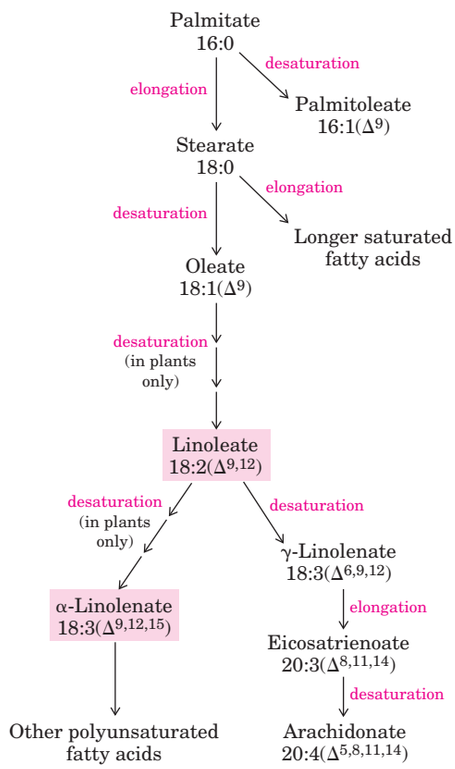


FIGURE 21–12 **Routes of synthesis of other fatty acids.** Palmitate is the precursor of stearate and longer-chain saturated fatty acids, as well as the monounsaturated acids palmitoleate and oleate. Mammals cannot convert oleate to linoleate or α-linolenate (shaded pink), which are therefore required in the diet as essential fatty acids. Conversion of linoleate to other polyunsaturated fatty acids and eicosanoids is outlined. Unsaturated fatty acids are symbolized by indicating the number of carbons and the number and position of the double bonds, as in Table 10–1.

Desaturation of Fatty Acids Requires a Mixed-Function Oxidase

Palmitate and stearate serve as precursors of the two most common monounsaturated fatty acids of animal tissues: palmitoleate, 16:1(Δ^9), and oleate, 18:1(Δ^9); both of these fatty acids have a single cis double bond between C-9 and C-10 (see Table 10–1). The double bond is introduced into the fatty acid chain by an oxidative reaction catalyzed by **fatty acyl–CoA desaturase** (Fig. 21–13), a **mixed-function oxidase** (Box 21–1). Two different substrates, the fatty acid and NADH or NADPH, simultaneously undergo two-electron oxidations. The path of electron flow includes a cytochrome (cytochrome b_5) and a flavoprotein (cytochrome b_5 reductase), both of which, like fatty acyl–CoA desaturase, are in the smooth ER. Bacteria have two cytochrome b_5 reductases, one NADH-dependent and the other NADPH-dependent; which of these is the main electron donor in vivo is unclear. In plants, oleate is produced by a stearoyl-ACP desaturase in the chloroplast stroma that uses reduced ferredoxin as the electron donor.

Mammalian hepatocytes can readily introduce double bonds at the Δ^9 position of fatty acids but cannot introduce additional double bonds between C-10 and the

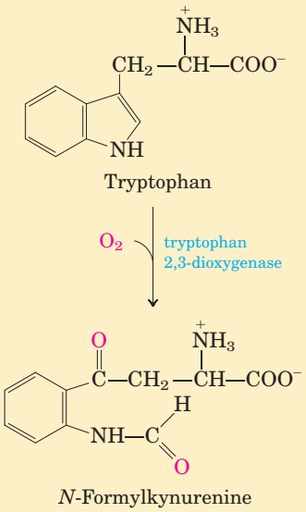
BOX 21–1 THE WORLD OF BIOCHEMISTRY

Mixed-Function Oxidases, Oxygenases, and Cytochrome P-450

In this chapter we encounter several enzymes that carry out oxidation-reduction reactions in which molecular oxygen is a participant. The reaction that introduces a double bond into a fatty acyl chain (see Fig. 21–13) is one such reaction.

The nomenclature for enzymes that catalyze reactions of this general type is often confusing to students, as is the mechanism of the reactions. **Oxidase** is the general name for enzymes that catalyze oxidations in which molecular oxygen is the electron acceptor but oxygen atoms do not appear in the oxidized product (however, there is an exception to this "rule," as we shall see!). The enzyme that creates a double bond in fatty acyl–CoA during the oxidation of fatty acids in peroxisomes (see Fig. 17–13) is an oxidase of this type; a second example is the cytochrome oxidase of the mitochondrial electron-transfer chain (see Fig. 19–14). In the first case, the transfer of two electrons to H_2O produces hydrogen peroxide, H_2O_2; in the second, two electrons reduce $\frac{1}{2}O_2$ to H_2O. Many, but not all, oxidases are flavoproteins.

Oxygenases catalyze oxidative reactions in which oxygen atoms *are* directly incorporated into the substrate molecule, forming a new hydroxyl or carboxyl group, for example. **Dioxygenases** catalyze reactions in which both oxygen atoms of O_2 are incorporated into the organic substrate molecule. An example of a dioxygenase is tryptophan 2, 3-dioxygenase, which catalyzes the opening of the five-membered ring of tryptophan in the catabolism of this amino acid. When this reaction takes place in the presence of $^{18}O_2$, the isotopic oxygen atoms are found in the two carbonyl groups of the product (shown in red).



Monooxygenases, more abundant and more complex in their action, catalyze reactions in which only one of the two oxygen atoms of O_2 is incorporated into the organic substrate, the other being reduced to H_2O. Monooxygenases require two substrates to serve as reductants of the two oxygen atoms of O_2. The main substrate accepts one of the two oxygen atoms, and a cosubstrate furnishes hydrogen atoms to reduce the other oxygen atom to H_2O. The general reaction equation for monooxygenases is

$$AH + BH_2 + O{-}O \longrightarrow A{-}OH + B + H_2O$$

where AH is the main substrate and BH_2 the cosubstrate. Because most monooxygenases catalyze reactions in which the main substrate becomes hydroxylated, they are also called **hydroxylases.** They are

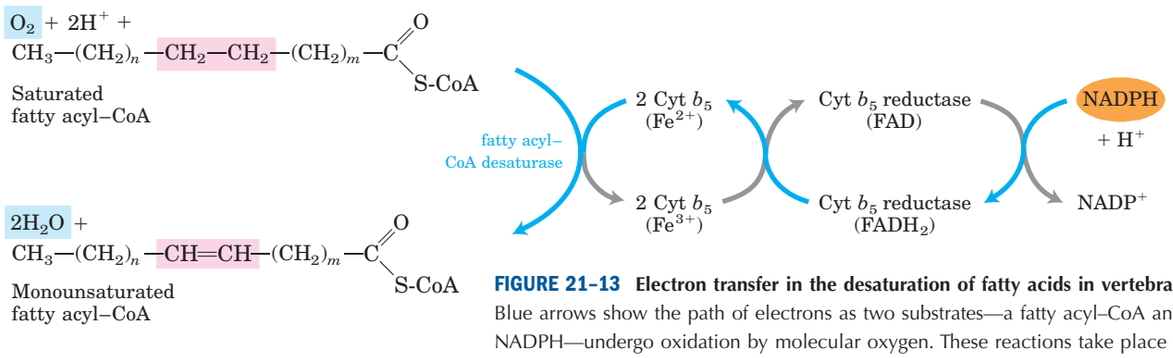


FIGURE 21–13 Electron transfer in the desaturation of fatty acids in vertebrates. Blue arrows show the path of electrons as two substrates—a fatty acyl–CoA and NADPH—undergo oxidation by molecular oxygen. These reactions take place on the lumenal face of the smooth ER. A similar pathway, but with different electron carriers, occurs in plants.

also sometimes called **mixed-function oxidases** or **mixed-function oxygenases,** to indicate that they oxidize two different substrates simultaneously. (Note here the use of "oxidase"—a deviation from the general meaning of this term noted above.)

There are different classes of monooxygenases, depending on the nature of the cosubstrate. Some use reduced flavin nucleotides ($FMNH_2$ or $FADH_2$), others use NADH or NADPH, and still others use α-ketoglutarate as the cosubstrate. The enzyme that hydroxylates the phenyl ring of phenylalanine to form tyrosine is a monooxygenase for which tetrahydrobiopterin serves as cosubstrate (see Fig. 18–23). This is the enzyme that is defective in the human genetic disease phenylketonuria.

The most numerous and most complex monooxygenation reactions are those employing a type of heme protein called **cytochrome P-450.** This cytochrome is usually present in the smooth ER rather than the mitochondria. Like mitochondrial cytochrome oxidase, cytochrome P-450 can react with O_2 and bind carbon monoxide, but it can be differentiated from cytochrome oxidase because the carbon monoxide complex of its reduced form absorbs light strongly at 450 nm—thus the name P-450.

Cytochrome P-450 catalyzes hydroxylation reactions in which an organic substrate, RH, is hydroxylated to R—OH, incorporating one oxygen atom of O_2; the other oxygen atom is reduced to H_2O by reducing equivalents that are furnished by NADH or NADPH but are usually passed to cytochrome P-450 by an iron-sulfur protein. Figure 1 shows a simplified outline of the action of cytochrome P-450, which has intermediate steps not yet fully understood.

Cytochrome P-450 is actually a family of similar proteins; several hundred members of this protein family are known, each with a different substrate specificity. In the adrenal cortex, for example, a specific cytochrome P-450 participates in the hydroxylation of steroids to yield the adrenocortical hormones (see Fig. 21–47). Cytochrome P-450 is also important in the hydroxylation of many different drugs, such as barbiturates and other xenobiotics (substances foreign to the organism), particularly if they are hydrophobic and relatively insoluble. The environmental carcinogen benzo[*a*]pyrene (found in cigarette smoke) undergoes cytochrome P-450–dependent hydroxylation during detoxification. Hydroxylation of xenobiotics makes them more soluble in water and allows their excretion in the urine. Unfortunately, hydroxylation of some compounds converts them to toxic substances, subverting the detoxification system.

Reactions described in this chapter that are catalyzed by mixed-function oxidases are those involved in fatty acyl–CoA desaturation (Fig. 21–13), leukotriene synthesis (Fig. 21–16), plasmalogen synthesis (Fig. 21–30), conversion of squalene to cholesterol (Fig. 21–37), and steroid hormone synthesis (Fig. 21–47).

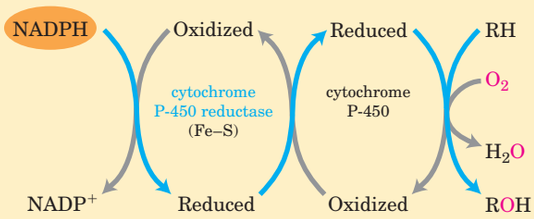


FIGURE 1

methyl-terminal end. Thus mammals cannot synthesize linoleate, 18:2($\Delta^{9,12}$), or α-linolenate, 18:3($\Delta^{9,12,15}$). Plants, however, can synthesize both; the desaturases that introduce double bonds at the Δ^{12} and Δ^{15} positions are located in the ER and the chloroplast. The ER enzymes act not on free fatty acids but on a phospholipid, phosphatidylcholine, that contains at least one oleate linked to the glycerol (Fig. 21–14). Both plants and bacteria must synthesize polyunsaturated fatty acids to ensure membrane fluidity at reduced temperatures.

Because they are necessary precursors for the synthesis of other products, linoleate and linolenate are **essential fatty acids** for mammals; they must be obtained from dietary plant material. Once ingested, linoleate may be converted to certain other polyunsaturated acids, particularly γ-linolenate, eicosatrienoate, and arachidonate (eicosatetraenoate), all of which can be made only from linoleate (Fig. 21–12). Arachidonate, 20:4($\Delta^{5,8,11,14}$), is an essential precursor of regulatory lipids, the eicosanoids. The 20-carbon fatty acids are synthesized from linoleate (and linolenate) by fatty acid elongation reactions analogous to those described on page 797.

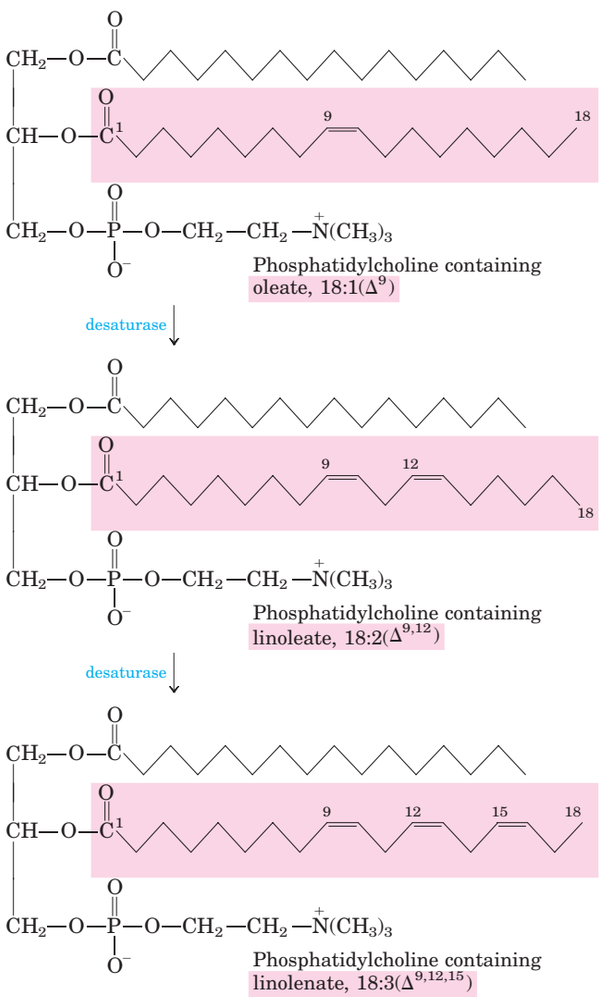


FIGURE 21–14 Action of plant desaturases. Desaturases in plants oxidize phosphatidylcholine-bound oleate to polyunsaturated fatty acids. Some of the products are released from the phosphatidylcholine by hydrolysis.

Eicosanoids Are Formed from 20-Carbon Polyunsaturated Fatty Acids

Eicosanoids are a family of very potent biological signaling molecules that act as short-range messengers, affecting tissues near the cells that produce them. In response to hormonal or other stimuli, phospholipase A_2, present in most types of mammalian cells, attacks membrane phospholipids, releasing arachidonate from the middle carbon of glycerol. Enzymes of the smooth ER then convert arachidonate to **prostaglandins,** beginning with the formation of prostaglandin H_2 (PGH_2), the immediate precursor of many other prostaglandins and of thromboxanes (Fig. 21–15a). The two reactions that lead to PGH_2 are catalyzed by a bifunctional enzyme, **cyclooxygenase (COX),** also called **prostaglandin H_2 synthase.** In the first of two steps, the cyclooxygenase activity introduces molecular oxygen to convert arachidonate to PGG_2. The second step, catalyzed by the peroxidase activity of COX, converts PGG_2 to PGH_2.

Aspirin (acetylsalicylate; Fig. 21–15b) irreversibly inactivates the cyclooxygenase activity of COX by acetylating a Ser residue and blocking the enzyme's active site, thus inhibiting the synthesis of prostaglandins and thromboxanes. Ibuprofen, a widely used nonsteroidal antiinflammatory drug (NSAID; Fig. 21–15c), inhibits the same enzyme. The recent discovery that there are two isozymes of COX has led to the development of more precisely targeted NSAIDs with fewer undesirable side effects (Box 21–2).

Thromboxane synthase, present in blood platelets (thrombocytes), converts PGH_2 to thromboxane A_2, from which other **thromboxanes** are derived (Fig. 21–15a). Thromboxanes induce constriction of blood vessels and platelet aggregation, early steps in blood clotting. Low doses of aspirin, taken regularly, reduce the probability of heart attacks and strokes by reducing thromboxane production. ■

Thromboxanes, like prostaglandins, contain a ring of five or six atoms; the pathway from arachidonate to these two classes of compounds is sometimes called the "cyclic" pathway, to distinguish it from the "linear" pathway that leads from arachidonate to the **leukotrienes,** which are linear compounds (Fig. 21–16). Leukotriene synthesis begins with the action of several lipoxygenases that catalyze the incorporation of molecular oxygen into arachidonate. These enzymes, found in leukocytes and in heart, brain, lung, and spleen, are mixed-function oxidases that use cytochrome P-450 (Box 21–1). The various leukotrienes differ in the position of the peroxide

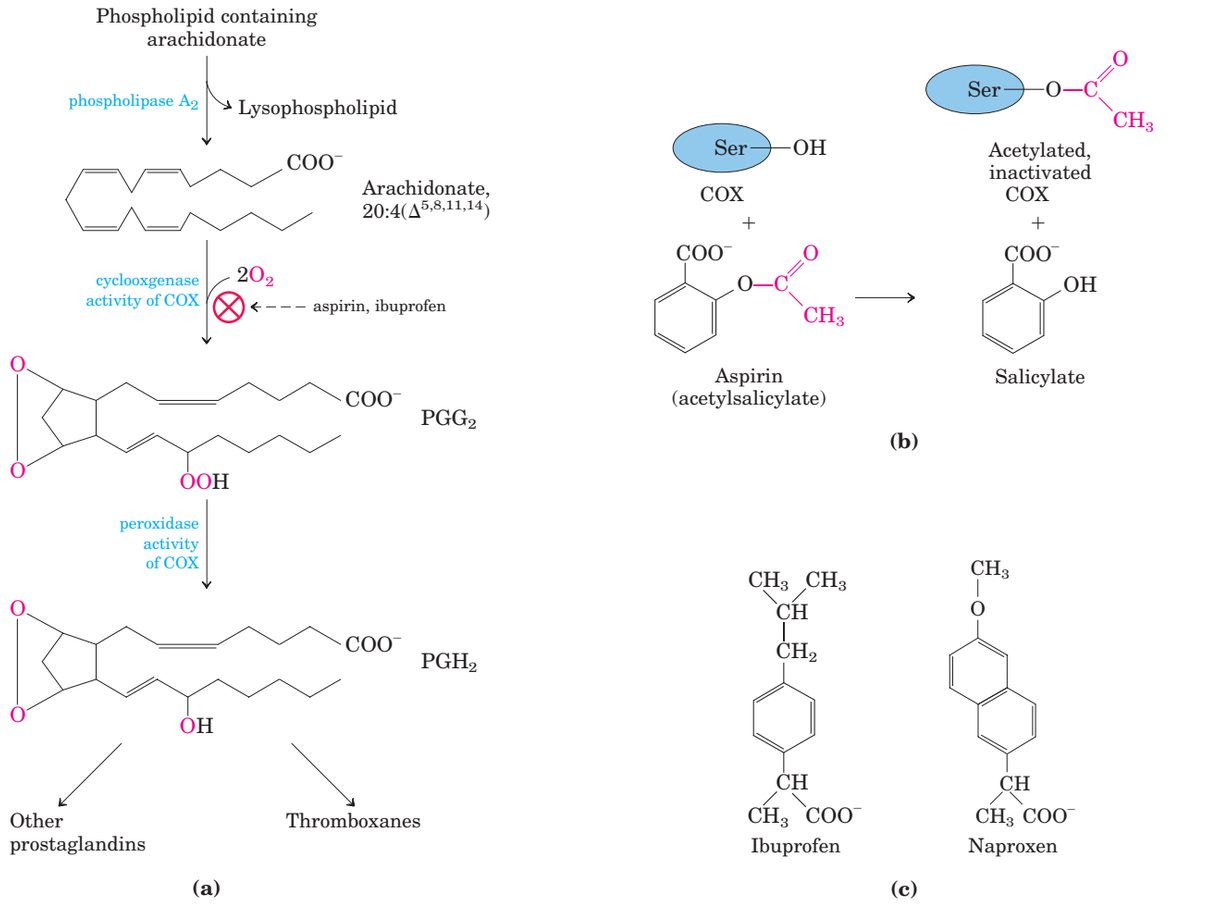


FIGURE 21–15 The "cyclic" pathway from arachidonate to prostaglandins and thromboxanes. (a) After arachidonate is released from phospholipids by the action of phospholipase A_2, the cyclooxygenase and peroxidase activities of COX (also called prostaglandin H_2 synthase) catalyze the production of PGH_2, the precursor of other prostaglandins and thromboxanes. **(b)** Aspirin inhibits the first reaction by acetylating an essential Ser residue on the enzyme. **(c)** Ibuprofen and naproxen inhibit the same step, probably by mimicking the structure of the substrate or an intermediate in the reaction.

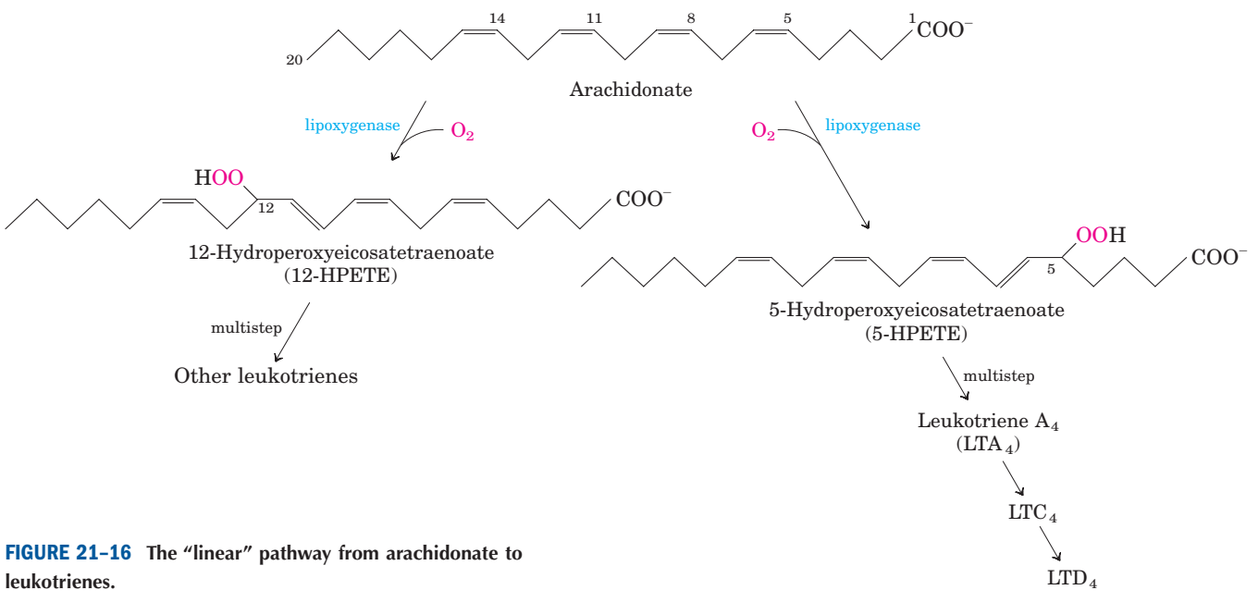


FIGURE 21–16 The "linear" pathway from arachidonate to leukotrienes.

BOX 21–2 BIOCHEMISTRY IN MEDICINE

Relief Is in (the Active) Site: Cyclooxygenase Isozymes and the Search for a Better Aspirin

Each year, several *thousand tons* of aspirin (acetylsalicylate) are consumed around the world for the relief of headaches, sore muscles, inflamed joints, and fever. Because aspirin inhibits platelet aggregation and blood clotting, it is also used in low doses to treat patients at risk of heart attacks. The medicinal properties of the compounds known as salicylates, including aspirin, were first described by western science in 1763, when Edmund Stone of England noted that bark of the willow tree *Salix alba* was effective against fevers, aches, and pains. By the 1830s, German chemists had purified the active components from willow and from another plant rich in salicylates, the meadowsweet, *Spiraea ulmaria.* However, salicylate itself was bitter-tasting and its use had some unpleasant side effects, including severe stomach irritation in some cases. To address these problems, Felix Hoffmann and Arthur Eichengrun synthesized acetylsalicylate at the Bayer company in Germany in 1897. The new compound, with fewer side effects than salicylate, was marketed in 1899 under the trade name Aspirin (from *a* for acetyl and *spir* for *Spirsaüre,* the German word for the acid prepared from *Spiraea*). Within a few years, aspirin was in widespread use.

Aspirin (now a generic name) is one of a number of nonsteroidal antiinflammatory drugs (NSAIDs); others include ibuprofen and naproxen (see Fig. 21–15), all now sold over the counter. Unfortunately, aspirin reduces but does not eliminate the side effects of salicylates. In some patients, aspirin itself can produce stomach bleeding, kidney failure, and, in extreme cases, death. New NSAIDs with the beneficial effects of aspirin but without its side effects would be medically valuable.

Aspirin and other NSAIDs inhibit the cyclooxygenase activity of prostaglandin H_2 synthase (also called COX, for cyclooxygenase), which adds molecular oxygen to arachidonate to initiate prostaglandin synthesis (see Fig. 21–15a). Prostaglandins regulate many physiological processes, including platelet aggregation, uterine contractions, pain, inflammation, and the secretion of mucins that protect the gastric mucosa from acid and proteolytic enzymes in the stomach. The stomach irritation that is a common side effect of aspirin use results from the drug's interference with the secretion of gastric mucin.

Mammals have two isozymes of prostaglandin H_2 synthase, COX-1 and COX-2. These have different functions but closely similar amino acid sequences (60% to 65% sequence identity) and similar reaction mechanisms at both of their catalytic centers. COX-1 is responsible for the synthesis of the prostaglandins that regulate the secretion of gastric mucin, and COX-2 for the prostaglandins that mediate inflammation, pain, and fever. Aspirin inhibits both isozymes about equally, so a dose sufficient to reduce inflammation also risks stomach irritation. Much research is aimed at developing new NSAIDs that inhibit COX-2 specifically, and several such drugs have become available.

The development of COX-2–specific inhibitors has been helped immensely by knowledge of the detailed three-dimensional structures of COX-1 and COX-2 (Fig. 1). Both proteins are homodimers. Each monomer (M_r 70,000) has an amphipathic domain that penetrates but does not span the ER; this anchors the enzyme on the lumenal side of the ER (a very unusual topology—generally the hydrophobic regions of integral membrane proteins span the entire bilayer). Both catalytic sites are on the globular domain protruding into the ER lumen.

COX-1 and COX-2 have virtually identical tertiary and quaternary structures, but they differ subtly in a long, thin hydrophobic channel extending from the membrane interior to the lumenal surface. The channel includes both catalytic sites and is presumed to be the binding site for the hydrophobic substrate, arachidonate. Both COX-1 and COX-2 have been crystallized in the presence of several different bound NSAID compounds, defining the NSAID-binding site (Fig. 1). The bound drugs block the hydrophobic channel and prevent arachidonate entry. The subtle differences between the channels of COX-1 and COX-2 have guided

group introduced by the lipoxygenases. This linear pathway from arachidonate, unlike the cyclic pathway, is not inhibited by aspirin or other NSAIDs.

Plants also derive important signaling molecules from fatty acids. As in animals, a key step in the initiation of signaling involves activation of a specific phospholipase. In plants, the fatty acid substrate that is released is α-linolenate. A lipoxygenase then catalyzes the first step in a pathway that converts linolenate to jasmonate, a substance known to have signaling roles in insect defense, resistance to fungal pathogens, and pollen maturation. Jasmonate (see Fig. 12–28) also affects seed germination, root growth, and fruit and seed development.

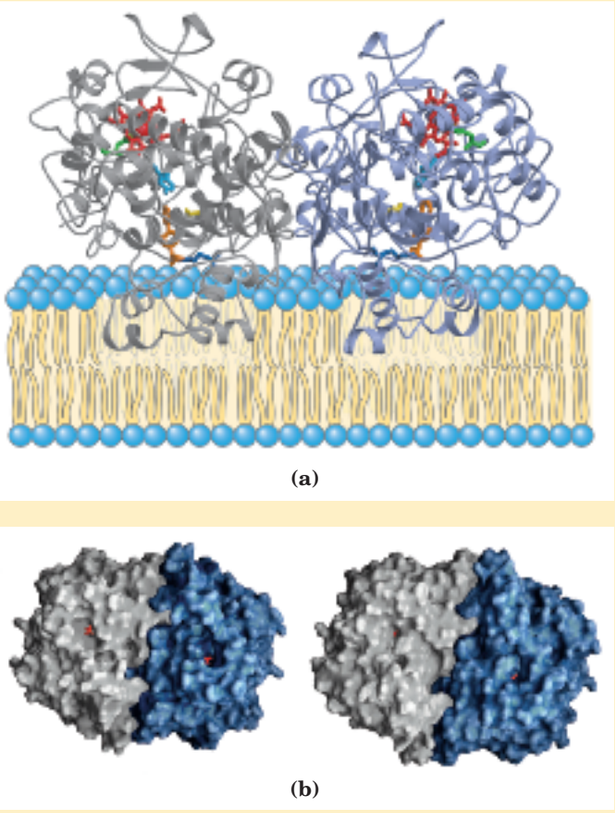
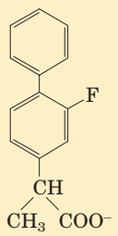


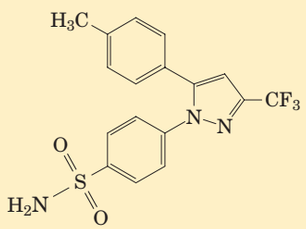
FIGURE 1 Structures of COX-1 and COX-2. (a) COX-1 with an NSAID inhibitor (flurbiprofen, orange) bound (PDB ID 3PGH). The enzyme consists of two identical monomers (gray and blue) each with three domains: a membrane anchor consisting of four amphipathic helices; a second domain that somewhat resembles a domain of the epidermal growth factor; and the catalytic domain, which contains the cyclooxygenase and peroxidase activities, as well as the hydrophobic channel in which the substrate (arachidonate) binds. The heme that is part of the peroxidase active sites is shown in red; Tyr^{385}, a key residue in the cyclooxygenase site, is turquoise. Other catalytically important residues include Arg^{120} (dark blue), His^{388} (green), and Ser^{530} (yellow). Flurbiprofen blocks access to the enzyme active site.



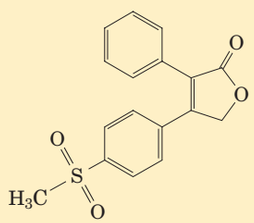
Flurbiprofen

(b) A look at COX-1 and COX-2 side by side. The COX-1 enzyme from sheep is shown at left, with bound ibuprofen (PDB ID 1EQG). The COX-2 enzyme from mouse is shown at right, with a similar inhibitor, Sc-558, bound (PDB ID 6COX). The inhibitors (red) are partially buried within the structures in these representations, which emphasize surface contours. COX-1 and COX-2 are very similar in structure, but enzymologists exploit the small differences in the structures of the active sites and the channels leading to them to design inhibitors specific for one enzyme or the other.

the design of NSAIDs that selectively fit COX-2 and therefore inhibit COX-2 more effectively than COX-1. Two of these drugs have been approved for use worldwide: celecoxib (Celebrex) for osteoarthritis and rheumatoid arthritis, and rofecoxib (Vioxx) for osteoarthritis and acute musculoskeletal pain (Fig. 2). In clinical trials, these drugs have proven effective while significantly reducing the stomach irritation and other side effects of aspirin and other NSAIDs. The use of precise structural information about an enzyme's active site is a powerful tool in the development of better, more specific drugs.



Celecoxib
(Celebrex)



Rofecoxib
(Vioxx)

FIGURE 2 Two COX-2–specific drugs that bind to COX-2 about 1,000 times better than to COX-1.

SUMMARY 21.1 Biosynthesis of Fatty Acids and Eicosanoids

- Long-chain saturated fatty acids are synthesized from acetyl-CoA by a cytosolic complex of six enzyme activities plus acyl carrier protein (ACP). The fatty acid synthase complex, which in some organisms consists of multifunctional polypeptides, contains two types of —SH groups (one furnished by the phosphopantetheine of ACP, the other by a Cys residue of β-ketoacyl-ACP synthase) that function as carriers of the fatty acyl intermediates.

- Malonyl-ACP, formed from acetyl-CoA (shuttled out of mitochondria) and CO_2, condenses with an acetyl bound to the Cys—SH to yield acetoacetyl-ACP, with release of CO_2. This is followed by reduction to the D-β-hydroxy derivative, dehydration to the *trans*-Δ^2-unsaturated acyl-ACP, and reduction to butyryl-ACP. NADPH is the electron donor for both reductions. Fatty acid synthesis is regulated at the level of malonyl-CoA formation.
- Six more molecules of malonyl-ACP react successively at the carboxyl end of the growing fatty acid chain to form palmitoyl-ACP—the end product of the fatty acid synthase reaction. Free palmitate is released by hydrolysis.
- Palmitate may be elongated to the 18-carbon stearate. Palmitate and stearate can be desaturated to yield palmitoleate and oleate, respectively, by the action of mixed-function oxidases.
- Mammals cannot make linoleate and must obtain it from plant sources; they convert exogenous linoleate to arachidonate, the parent compound of eicosanoids (prostaglandins, thromboxanes, and leukotrienes), a family of very potent signaling molecules.

21.2 Biosynthesis of Triacylglycerols

Most of the fatty acids synthesized or ingested by an organism have one of two fates: incorporation into triacylglycerols for the storage of metabolic energy or incorporation into the phospholipid components of membranes. The partitioning between these alternative fates depends on the organism's current needs. During rapid growth, synthesis of new membranes requires the production of membrane phospholipids; when an organism has a plentiful food supply but is not actively growing, it shunts most of its fatty acids into storage fats. Both pathways begin at the same point: the formation of fatty acyl esters of glycerol. In this section we examine the route to triacylglycerols and its regulation, and the production of glycerol 3-phosphate in the process of glyceroneogenesis.

Triacylglycerols and Glycerophospholipids Are Synthesized from the Same Precursors

Animals can synthesize and store large quantities of triacylglycerols, to be used later as fuel (see Box 17–1). Humans can store only a few hundred grams of glycogen in liver and muscle, barely enough to supply the body's energy needs for 12 hours. In contrast, the total amount of stored triacylglycerol in a 70-kg man of average build is about 15 kg, enough to support basal energy needs for as long as 12 weeks (see Table 23–5). Triacylglycerols have the highest energy content of all stored nutrients—more than 38 kJ/g. Whenever carbohydrate is ingested in excess of the organism's capacity to store glycogen, the excess is converted to triacylglycerols and stored in adipose tissue. Plants also manufacture triacylglycerols as an energy-rich fuel, mainly stored in fruits, nuts, and seeds.

In animal tissues, triacylglycerols and glycerophospholipids such as phosphatidylethanolamine share two precursors (fatty acyl–CoA and L-glycerol 3-phosphate) and several biosynthetic steps. The vast majority of the glycerol 3-phosphate is derived from the glycolytic intermediate dihydroxyacetone phosphate (DHAP) by the action of the cytosolic NAD-linked **glycerol 3-phosphate dehydrogenase;** in liver and kidney, a small amount of glycerol 3-phosphate is also formed from glycerol by the action of **glycerol kinase** (Fig. 21–17). The other precursors of triacylglycerols are fatty acyl–CoAs, formed from fatty acids by **acyl-CoA synthetases,** the same enzymes responsible for the activation of fatty acids for β oxidation (see Fig. 17–5).

The first stage in the biosynthesis of triacylglycerols is the acylation of the two free hydroxyl groups of L-glycerol 3-phosphate by two molecules of fatty acyl–CoA to yield **diacylglycerol 3-phosphate,** more commonly called **phosphatidic acid** or phosphatidate (Fig. 21–17). Phosphatidic acid is present in only trace amounts in cells but is a central intermediate in lipid biosynthesis; it can be converted either to a triacylglycerol or to a glycerophospholipid. In the pathway to triacylglycerols, phosphatidic acid is hydrolyzed by **phosphatidic acid phosphatase** to form a 1,2-diacylglycerol (Fig. 21–18). Diacylglycerols are then converted to triacylglycerols by transesterification with a third fatty acyl–CoA.

Triacylglycerol Biosynthesis in Animals Is Regulated by Hormones

In humans, the amount of body fat stays relatively constant over long periods, although there may be minor short-term changes as caloric intake fluctuates. Carbohydrate, fat, or protein consumed in excess of energy needs is stored in the form of triacylglycerols that can be drawn upon for energy, enabling the body to withstand periods of fasting.

Biosynthesis and degradation of triacylglycerols are regulated such that the favored path depends on the metabolic resources and requirements of the moment. The rate of triacylglycerol biosynthesis is profoundly altered by the action of several hormones. Insulin, for example, promotes the conversion of carbohydrate to triacylglycerols (Fig. 21–19). People with severe diabetes mellitus, due to failure of insulin secretion or action, not only are unable to use glucose properly but also fail to synthesize fatty acids from

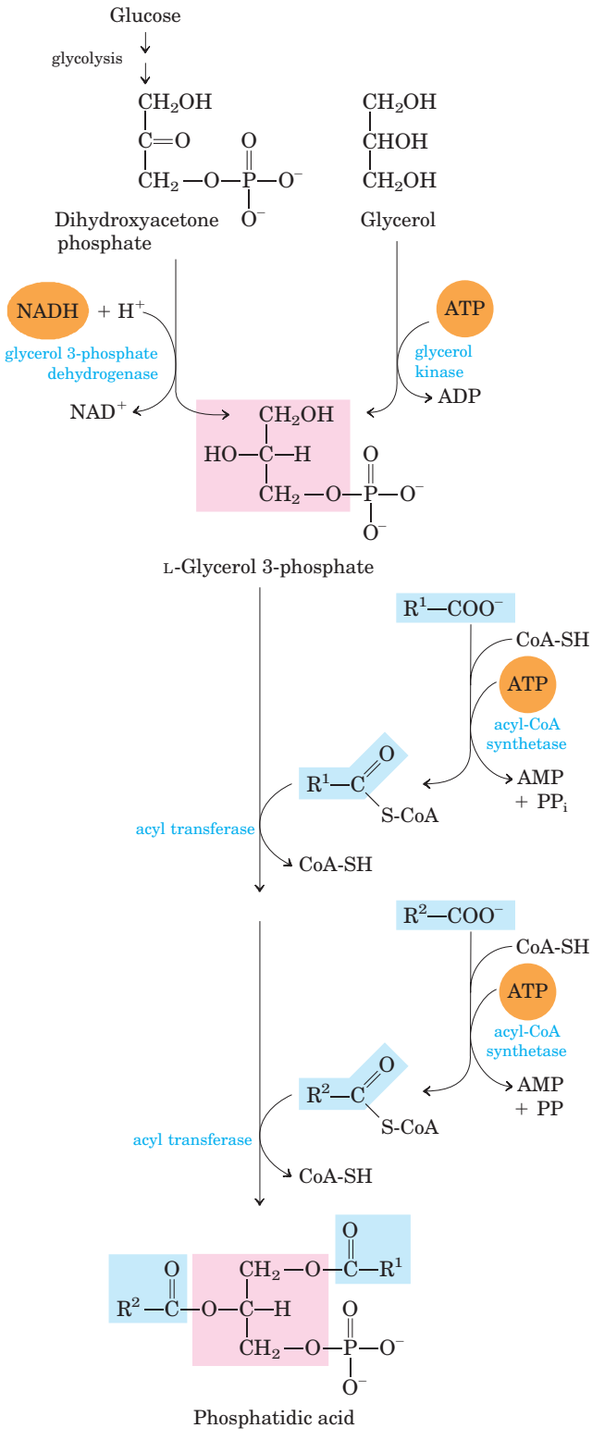


FIGURE 21–17 Biosynthesis of phosphatidic acid. A fatty acyl group is activated by formation of the fatty acyl–CoA, then transferred to ester linkage with L-glycerol 3-phosphate, formed in either of the two ways shown. Phosphatidic acid is shown here with the correct stereochemistry at C-2 of the glycerol molecule. To conserve space in subsequent figures (and in Fig. 21–14), both fatty acyl groups of glycerophospholipids, and all three acyl groups of triacylglycerols, are shown projecting to the right.

carbohydrates or amino acids. If the diabetes is untreated, these individuals have increased rates of fat oxidation and ketone body formation (Chapter 17) and therefore lose weight. ■

An additional factor in the balance between biosynthesis and degradation of triacylglycerols is that approximately 75% of all fatty acids released by lipolysis are reesterified to form triacylglycerols rather than used for fuel. This ratio persists even under starvation conditions, when energy metabolism is shunted from the use of carbohydrate to the oxidation of fatty acids. Some of this fatty acid recycling takes place in adipose tissue, with the reesterification occurring before release into the bloodstream; some takes place via a systemic cycle in which free fatty acids are transported to liver, recycled to triacylglycerol, exported again to the blood (transport of lipids in the blood is discussed in Section 21.4), and taken up again by adipose tissue after release from triacylglycerol by extracellular lipoprotein lipase

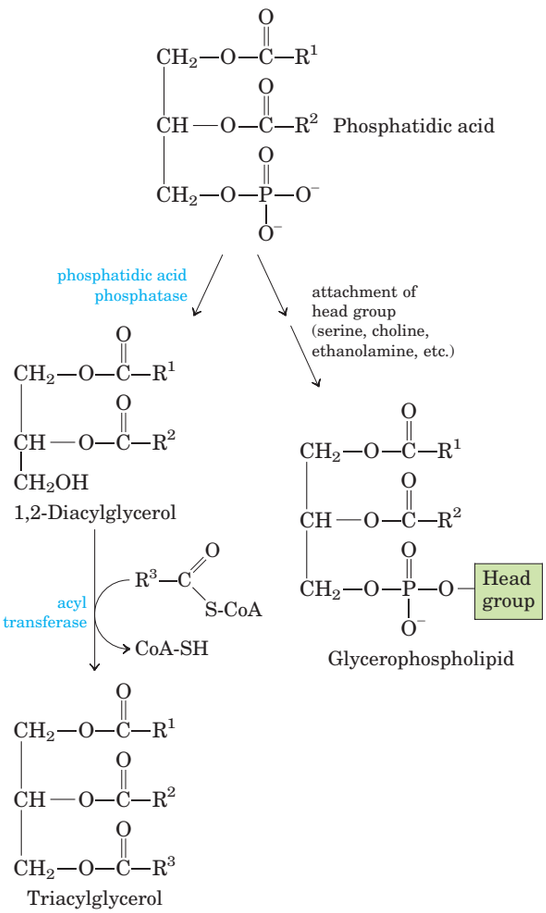


FIGURE 21–18 Phosphatidic acid in lipid biosynthesis. Phosphatidic acid is the precursor of both triacylglycerols and glycerophospholipids. The mechanisms for head-group attachment in phospholipid synthesis are described later in this section.

(Fig. 21–20; see also Fig. 17–1). Flux through this **triacylglycerol cycle** between adipose tissue and liver may be quite low when other fuels are available and the release of fatty acids from adipose tissue is limited, but as noted above, the proportion of released fatty acids that are reesterified remains roughly constant at 75% under all metabolic conditions. The level of free fatty acids in the blood thus reflects both the rate of release of fatty acids and the balance between the synthesis and breakdown of triacylglycerols in adipose tissue and liver.

When the mobilization of fatty acids is required to meet energy needs, release from adipose tissue is stimulated by the hormones glucagon and epinephrine (see Figs 17–3, 17–12). Simultaneously, these hormonal signals decrease the rate of glycolysis and increase the rate of gluconeogenesis in the liver (providing glucose for the brain, as further elaborated in Chapter 23). The released fatty acid is taken up by a number of tissues, including muscle, where it is oxidized to provide energy. Much of the fatty acid taken up by liver is not oxidized but is recycled to triacylglycerol and returned to adipose tissue.

The function of the apparently futile triacylglycerol cycle (futile cycles are discussed in Chapter 15) is not well understood. However, as we learn more about how the triacylglycerol cycle is sustained via metabolism in two separate organs and is coordinately regulated, some possibilities emerge. For example, the excess capacity in the triacylglycerol cycle (the fatty acid that is eventually reconverted to triacylglycerol rather than oxidized as fuel) could represent an energy reserve in the bloodstream during fasting, one that would be more rapidly mobilized in a "fight or flight" emergency than would stored triacylglycerol.

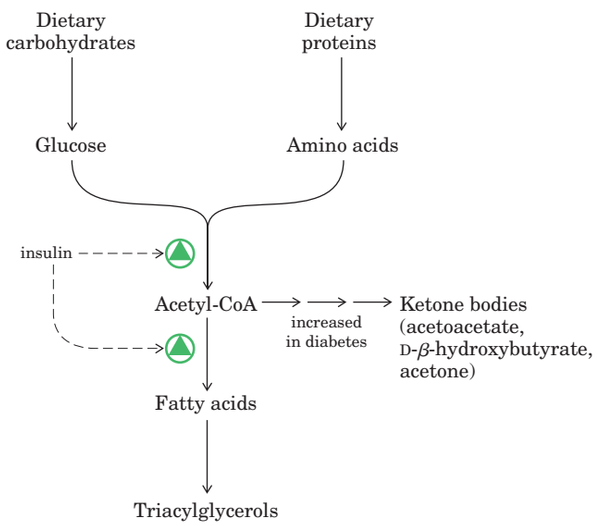


FIGURE 21–19 Regulation of triacylglycerol synthesis by insulin. Insulin stimulates conversion of dietary carbohydrates and proteins to fat. Individuals with diabetes mellitus lack insulin; in uncontrolled disease, this results in diminished fatty acid synthesis, and the acetyl-CoA arising from catabolism of carbohydrates and proteins is shunted instead to ketone body production. People in severe ketosis smell of acetone, so the condition is sometimes mistaken for drunkenness (p. 909).

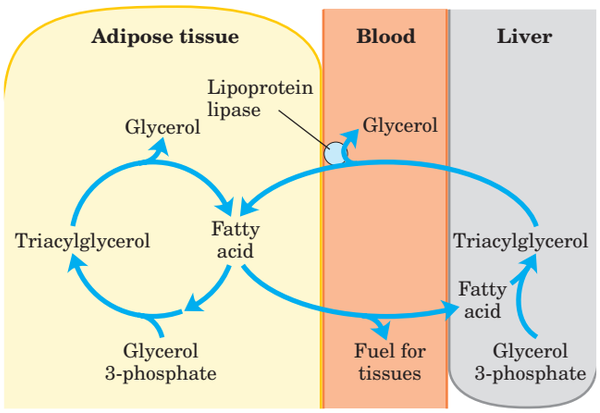


FIGURE 21–20 The triacylglycerol cycle. In mammals, triacylglycerol molecules are broken down and resynthesized in a triacylglycerol cycle during starvation. Some of the fatty acids released by lipolysis of triacylglycerol in adipose tissue pass into the bloodstream, and the remainder are used for resynthesis of triacylglycerol. Some of the fatty acids released into the blood are used for energy (in muscle, for example), and some are taken up by the liver and used in triacylglycerol synthesis. The triacylglycerol formed in the liver is transported in the blood back to adipose tissue, where the fatty acid is released by extracellular lipoprotein lipase, taken up by adipocytes, and reesterified into triacylglycerol.

The constant recycling of triacylglycerols in adipose tissue even during starvation raises a second question: what is the source of the glycerol 3-phosphate required for this process? As noted above, glycolysis is suppressed in these conditions by the action of glucagon and epinephrine, so little DHAP is available, and glycerol released during lipolysis cannot be converted directly to glycerol 3-phosphate in adipose tissue, because these cells lack glycerol kinase (Fig. 21–17). So, how is sufficient glycerol 3-phosphate produced? The answer lies in a pathway discovered more than three decades ago and given little attention until recently, a pathway intimately linked to the triacylglycerol cycle and, in a larger sense, to the balance between fatty acid and carbohydrate metabolism.

Adipose Tissue Generates Glycerol 3-phosphate by Glyceroneogenesis

Glyceroneogenesis is a shortened version of gluconeogenesis, from pyruvate to DHAP (see Fig. 14–16), followed by conversion of the DHAP to glycerol 3-phosphate by cytosolic NAD-linked glycerol 3-phosphate dehydrogenase (Fig. 21–21). Glycerol 3-phosphate is subsequently used in triacylglycerol synthesis. Glycero-

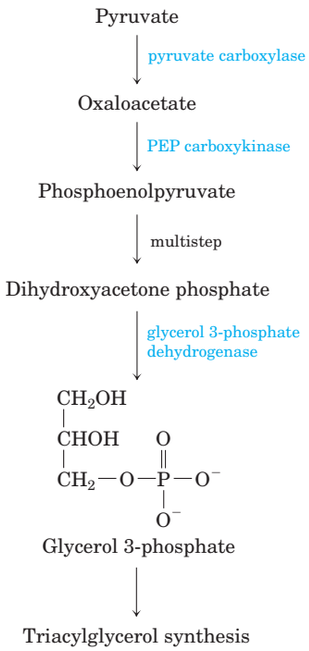
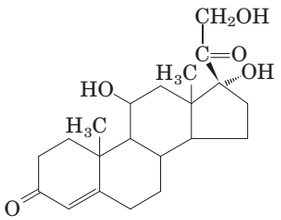


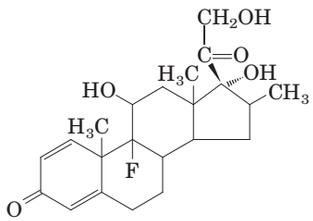
FIGURE 21–21 Glyceroneogenesis. The pathway is essentially an abbreviated version of gluconeogenesis, from pyruvate to dihydroxyacetone phosphate (DHAP), followed by conversion of DHAP to glycerol 3-phosphate, which is used for the synthesis of triacylglycerol.

neogenesis was discovered in the 1960s by Lea Reshef, Richard Hanson, and John Ballard, and simultaneously by Eleazar Shafrir and his coworkers, who were intrigued by the presence of two gluconeogenic enzymes, pyruvate carboxylase and phosphoenolpyruvate (PEP) carboxykinase, in adipose tissue, where glucose is not synthesized. After a long period of inattention, interest in this pathway has been renewed by the demonstration of a link between glyceroneogenesis and late-onset (type 2) diabetes, as we shall see.

Glyceroneogenesis has multiple roles. In adipose tissue, glyceroneogenesis coupled with reesterification of free fatty acids controls the rate of fatty acid release to the blood. In brown adipose tissue, the same pathway may control the rate at which free fatty acids are delivered to mitochondria for use in thermogenesis (see Fig. 19–30). And in fasting humans, glyceroneogenesis in the liver alone supports the synthesis of enough glycerol 3-phosphate to account for up to 65% of fatty acids reesterified to triacylglycerol.

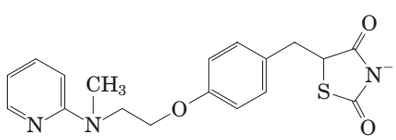
Flux through the triacylglycerol cycle between liver and adipose tissue is controlled to a large degree by the activity of PEP carboxykinase, which limits the rate of both gluconeogenesis and glyceroneogenesis. Glucocorticoid hormones such as cortisol (a biological steroid derived from cholesterol; see Fig. 21–46) and dexamethasone (a synthetic glucocorticoid) regulate the levels of PEP carboxykinase reciprocally in the liver and adipose tissue. Acting through the glucocorticoid receptor, these steroid hormones increase the expression of the gene encoding PEP carboxykinase in the liver, thus increasing gluconeogenesis and glyceroneogenesis (Fig. 21–22).

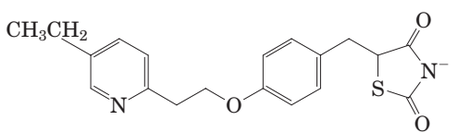

Cortisol


Dexamethasone

Stimulation of glyceroneogenesis leads to an increase in the synthesis of triacylglycerol molecules in the liver and their release into the blood. At the same time, glucocorticoids suppress the expression of the gene encoding PEP carboxykinase in adipose tissue. This results in a decrease in glyceroneogenesis in adipose tissue; recycling of fatty acids declines as a result, and more free fatty acids are released into the blood. Thus glyceroneogenesis is regulated reciprocally in the liver and adipose tissue, affecting lipid metabolism in opposite ways: a lower rate of glyceroneogenesis in adipose tissue leads to more fatty acid release (rather than recycling), whereas a higher rate in the liver leads to more synthesis and export of triacylglycerols. The net result is an increase in flux through the triacylglycerol cycle. When the glucocorticoids are no longer present, flux through the cycle declines as the expression of PEP carboxykinase increases in adipose tissue and decreases in the liver.

The recent attention given to glyceroneogenesis has arisen in part from the connection between this pathway and diabetes. High levels of free fatty acids in the blood interfere with glucose utilization in muscle and promote the insulin resistance that leads to type 2 diabetes. A new class of drugs called **thiazolidinediones** have been shown to reduce the levels of fatty acids circulating in the blood and increase sensitivity to


Rosiglitazone (Avandia)


Pioglitazone (Actos)

Thiazolidinediones

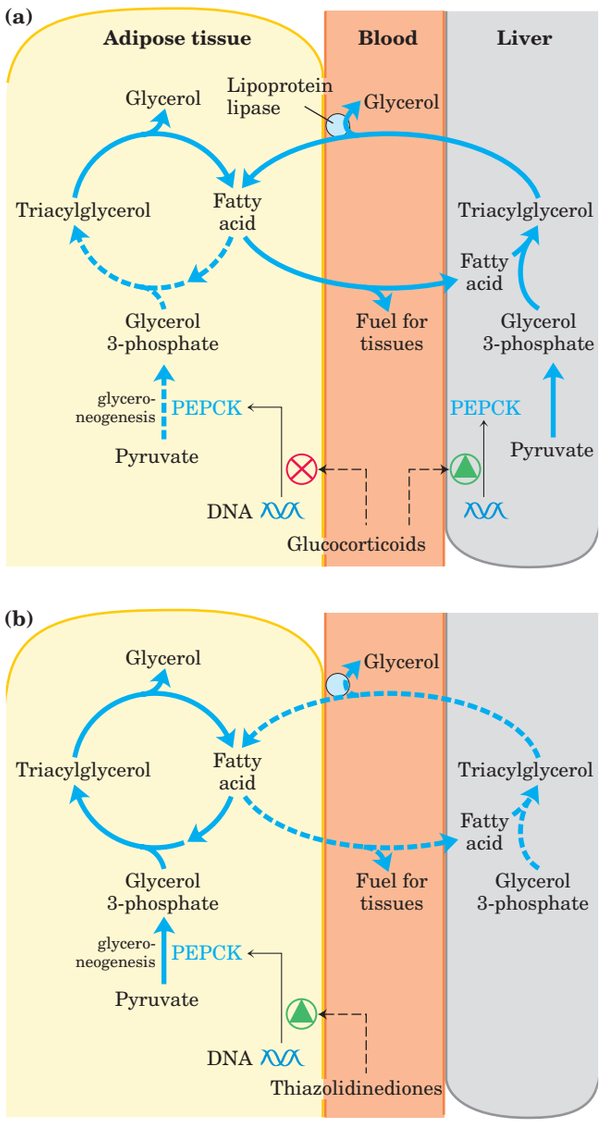


FIGURE 21–22 Regulation of glyceroneogenesis. (a) Glucocorticoid hormones stimulate glyceroneogenesis and gluconeogenesis in the liver, while suppressing glyceroneogenesis in the adipose tissue (by reciprocal regulation of the gene expressing PEP carboxykinase (PEPCK) in the two tissues); this increases the flux through the triacylglycerol cycle. The glycerol freed by the breakdown of triacylglycerol in adipose tissue is released to the blood and transported to the liver, where it is primarily converted to glucose, although some is converted to glycerol 3-phosphate by glycerol kinase.

(b) A class of drugs called thiazolidinediones are now used to treat type 2 diabetes. In this disease, high levels of free fatty acids in the blood interfere with glucose utilization in muscle and promote insulin resistance. Thiazolidinediones activate a nuclear receptor called peroxisome proliferator-activated receptor γ (PPARγ), which induces the activity of PEP carboxykinase. Therapeutically, thiazolidinediones increase the rate of glyceroneogenesis, thus increasing the resynthesis of triacylglycerol in adipose tissue and reducing the amount of free fatty acid in the blood.

insulin. Thiazolidinediones bind to and activate a nuclear hormone receptor called peroxisome proliferator-activated receptor γ (PPARγ), leading to the induction in adipose tissue of PEP carboxykinase (Fig. 21–22); a higher activity of PEP carboxykinase then leads to increased synthesis of the precursors of glyceroneogenesis. The therapeutic effect of thiazolidinediones is thus due, at least in part, to the increase in glyceroneogenesis, which in turn increases the resynthesis of triacylglycerol in adipose tissue and reduces the release of free fatty acid from adipose tissue into the blood. ■

SUMMARY 21.2 Biosynthesis of Triacylglycerols

- Triacylglycerols are formed by reaction of two molecules of fatty acyl–CoA with glycerol 3-phosphate to form phosphatidic acid; this product is dephosphorylated to a diacylglycerol, then acylated by a third molecule of fatty acyl–CoA to yield a triacylglycerol.
- The synthesis and degradation of triacylglycerol are hormonally regulated.
- Mobilization and recycling of triacylglycerol molecules results in a triacylglycerol cycle. Triacylglycerols are resynthesized from free fatty acids and glycerol 3-phosphate even during starvation. The dihydroxyacetone phosphate precursor of glycerol 3-phosphate is derived from pyruvate via glyceroneogenesis.

21.3 Biosynthesis of Membrane Phospholipids

In Chapter 10 we introduced two major classes of membrane phospholipids: glycerophospholipids and sphingolipids. Many different phospholipid species can be constructed by combining various fatty acids and polar head groups with the glycerol or sphingosine backbone (see Figs 10–8, 10–12). All the biosynthetic pathways follow a few basic patterns. In general, the assembly of phospholipids from simple precursors requires (1) synthesis of the backbone molecule (glycerol or sphingosine); (2) attachment of fatty acid(s) to the backbone through an ester or amide linkage; (3) addition of a hydrophilic head group to the backbone through a phosphodiester linkage; and, in some cases, (4) alteration or exchange of the head group to yield the final phospholipid product.

In eukaryotic cells, phospholipid synthesis occurs primarily on the surfaces of the smooth endoplasmic reticulum and the mitochondrial inner membrane. Some newly formed phospholipids remain at the site of synthesis, but most are destined for other cellular locations.

The process by which water-insoluble phospholipids move from the site of synthesis to the point of their eventual function is not fully understood, but we conclude this section by discussing some mechanisms that have emerged in recent years.

Cells Have Two Strategies for Attaching Phospholipid Head Groups

The first steps of glycerophospholipid synthesis are shared with the pathway to triacylglycerols (Fig. 21–17): two fatty acyl groups are esterified to C-1 and C-2 of L-glycerol 3-phosphate to form phosphatidic acid. Commonly but not invariably, the fatty acid at C-1 is saturated and that at C-2 is unsaturated. A second route to phosphatidic acid is the phosphorylation of a diacylglycerol by a specific kinase.

The polar head group of glycerophospholipids is attached through a phosphodiester bond, in which each of two alcohol hydroxyls (one on the polar head group and one on C-3 of glycerol) forms an ester with phosphoric acid (Fig. 21–23). In the biosynthetic process, one of the hydroxyls is first activated by attachment of a nucleotide, cytidine diphosphate (CDP). Cytidine monophosphate (CMP) is then displaced in a nucleophilic attack by the other hydroxyl (Fig. 21–24). The CDP is attached either to the diacylglycerol, forming the activated phosphatidic acid **CDP-diacylglycerol** (strategy 1), or to the hydroxyl of the head group (strategy 2). Eukaryotic cells employ both strategies, whereas prokaryotes use only the first. The central importance of cytidine nucleotides in lipid biosynthesis was discovered by Eugene P. Kennedy in the early 1960s.

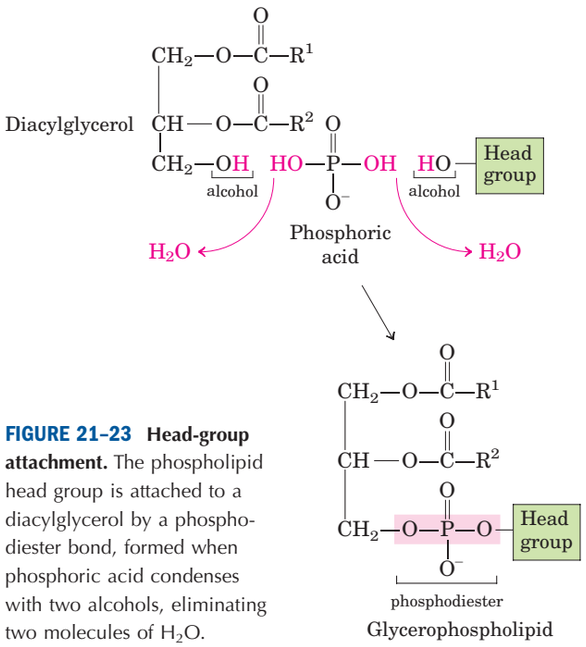


FIGURE 21–23 Head-group attachment. The phospholipid head group is attached to a diacylglycerol by a phosphodiester bond, formed when phosphoric acid condenses with two alcohols, eliminating two molecules of H_2O.

Eugene P. Kennedy

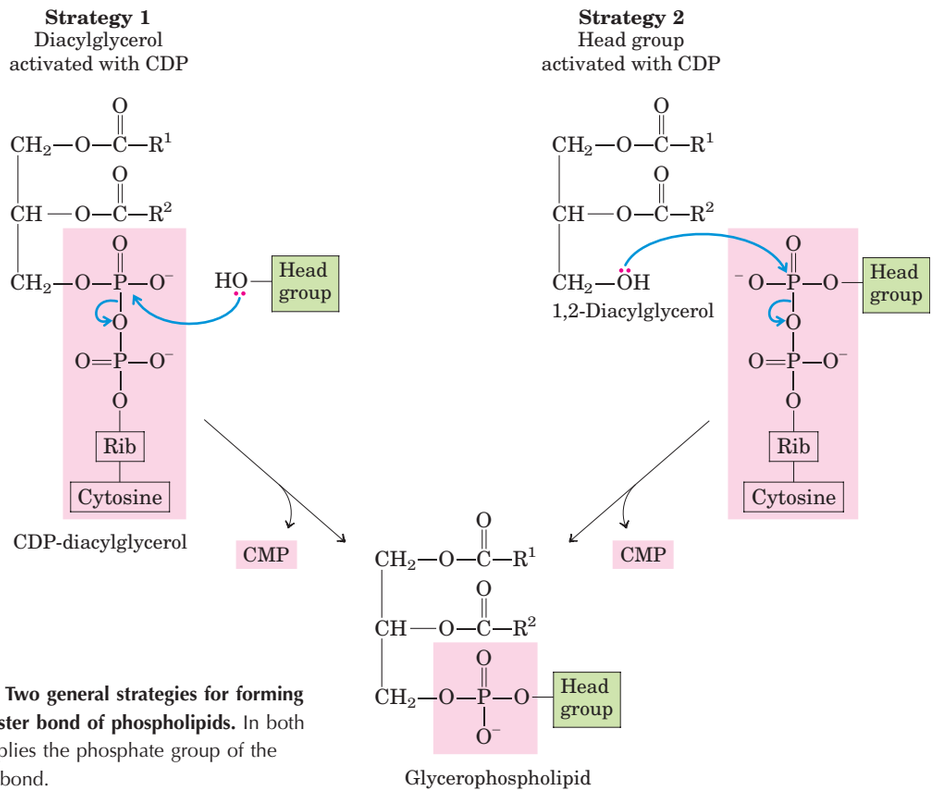


FIGURE 21–24 Two general strategies for forming the phosphodiester bond of phospholipids. In both cases, CDP supplies the phosphate group of the phosphodiester bond.

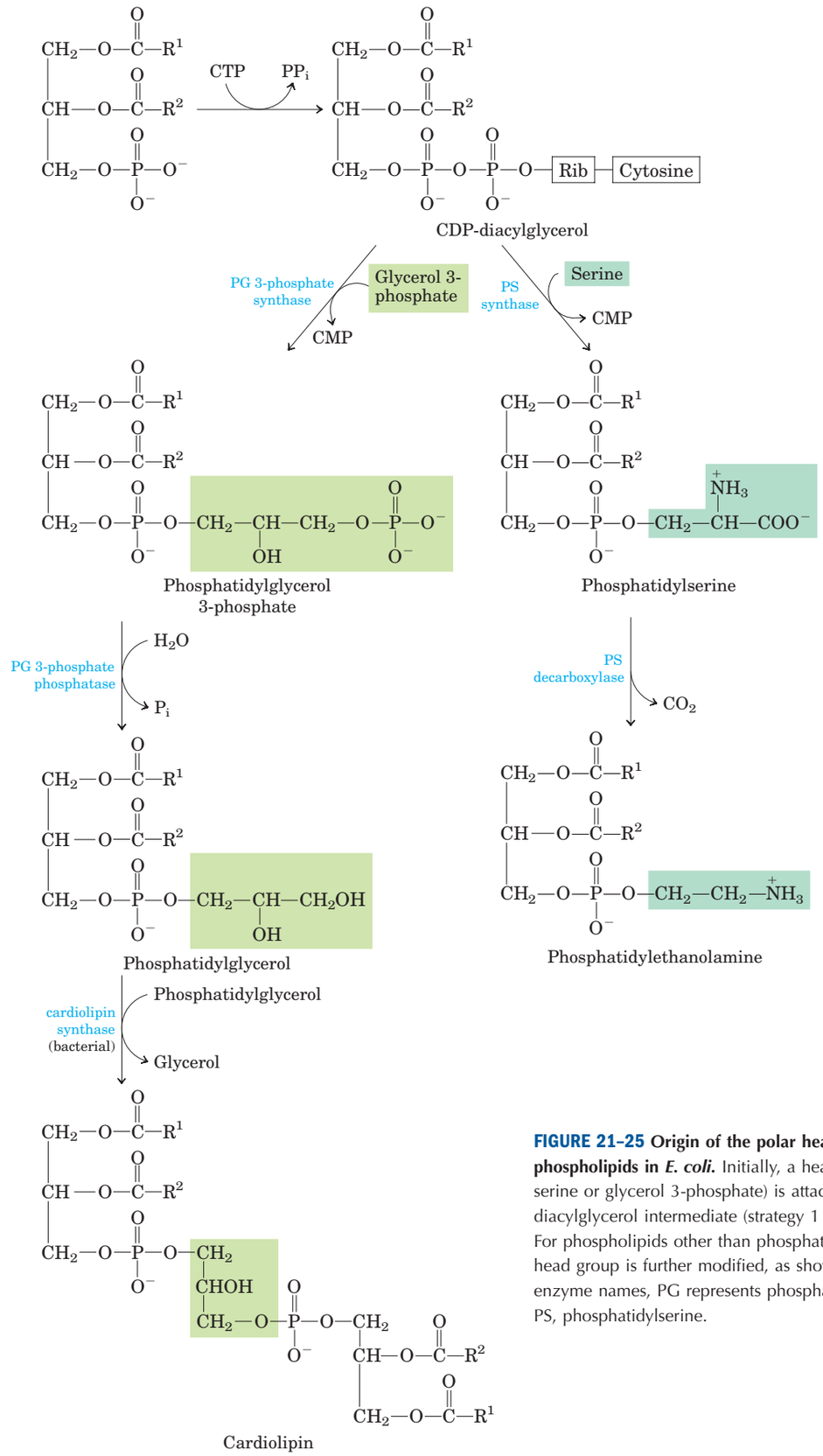


FIGURE 21–25 Origin of the polar head groups of phospholipids in *E. coli*. Initially, a head group (either serine or glycerol 3-phosphate) is attached via a CDP-diacylglycerol intermediate (strategy 1 in Fig. 21–24). For phospholipids other than phosphatidylserine, the head group is further modified, as shown here. In the enzyme names, PG represents phosphatidylglycerol; PS, phosphatidylserine.

Phospholipid Synthesis in *E. coli* Employs CDP-Diacylglycerol

The first strategy for head-group attachment is illustrated by the synthesis of phosphatidylserine, phosphatidylethanolamine, and phosphatidylglycerol in *E. coli.* The diacylglycerol is activated by condensation of phosphatidic acid with cytidine triphosphate (CTP) to form CDP-diacylglycerol, with the elimination of pyrophosphate (Fig. 21–25). Displacement of CMP through nucleophilic attack by the hydroxyl group of serine or by the C-1 hydroxyl of glycerol 3-phosphate yields **phosphatidylserine** or phosphatidylglycerol 3-phosphate, respectively. The latter is processed further by cleavage of the phosphate monoester (with release of P_i) to yield **phosphatidylglycerol.**

Phosphatidylserine and phosphatidylglycerol can serve as precursors of other membrane lipids in bacteria (Fig. 21–25). Decarboxylation of the serine moiety in phosphatidylserine, catalyzed by phosphatidylserine decarboxylase, yields **phosphatidylethanolamine.** In *E. coli,* condensation of two molecules of phosphatidylglycerol, with elimination of one glycerol, yields **cardiolipin,** in which two diacylglycerols are joined through a common head group.

Eukaryotes Synthesize Anionic Phospholipids from CDP-Diacylglycerol

In eukaryotes, phosphatidylglycerol, cardiolipin, and the phosphatidylinositols (all anionic phospholipids; see Fig. 10–8) are synthesized by the same strategy used for phospholipid synthesis in bacteria. Phosphatidylglycerol is made exactly as in bacteria. Cardiolipin synthesis in eukaryotes differs slightly: phosphatidylglycerol condenses with CDP-diacylglycerol (Fig. 21–26), not another molecule of phosphatidylglycerol as in *E. coli* (Fig. 21–25).

Phosphatidylinositol is synthesized by condensation of CDP-diacylglycerol with inositol (Fig. 21–26). Specific **phosphatidylinositol kinases** then convert phosphatidylinositol to its phosphorylated derivatives (see Fig. 10–17). Phosphatidylinositol and its phosphorylated products in the plasma membrane play a central role in signal transduction in eukaryotes (see Figs 12–8, 12–19).

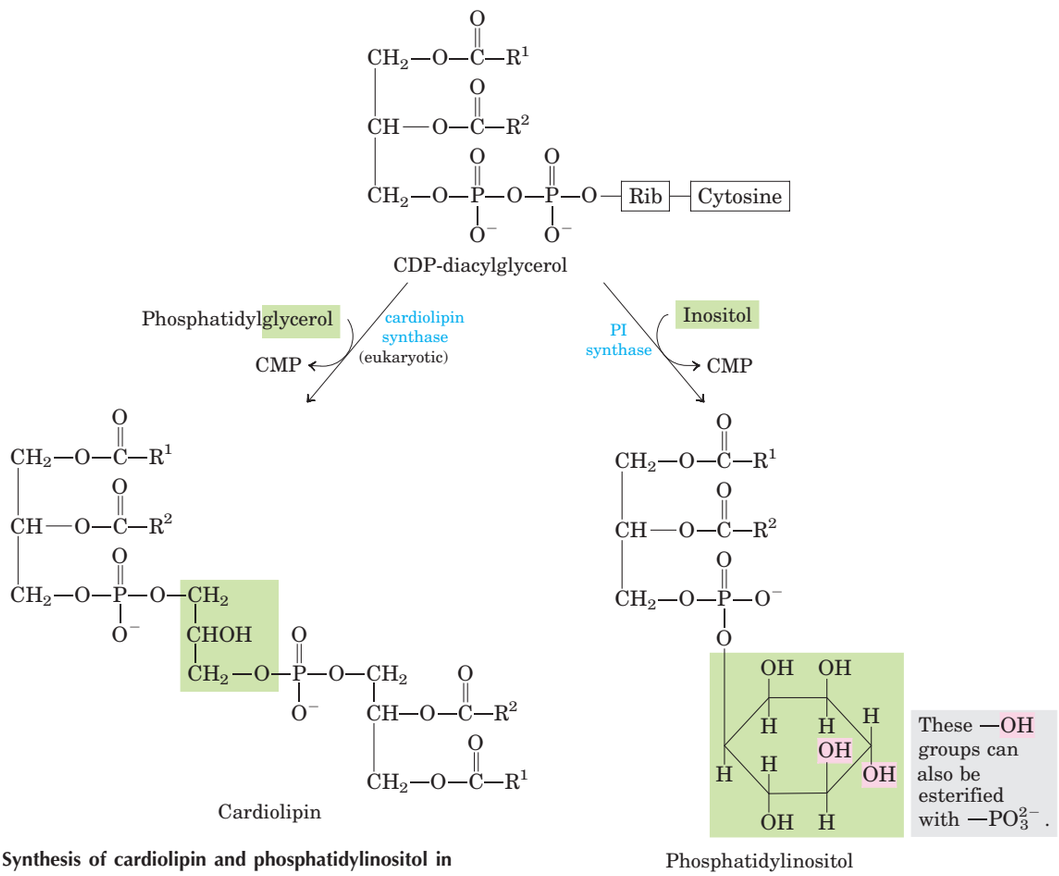


FIGURE 21–26 Synthesis of cardiolipin and phosphatidylinositol in eukaryotes. These glycerophospholipids are synthesized using strategy 1 in Figure 21-24. Phosphatidylglycerol is synthesized as in bacteria (see Fig. 21–25). PI represents phosphatidylinositol.

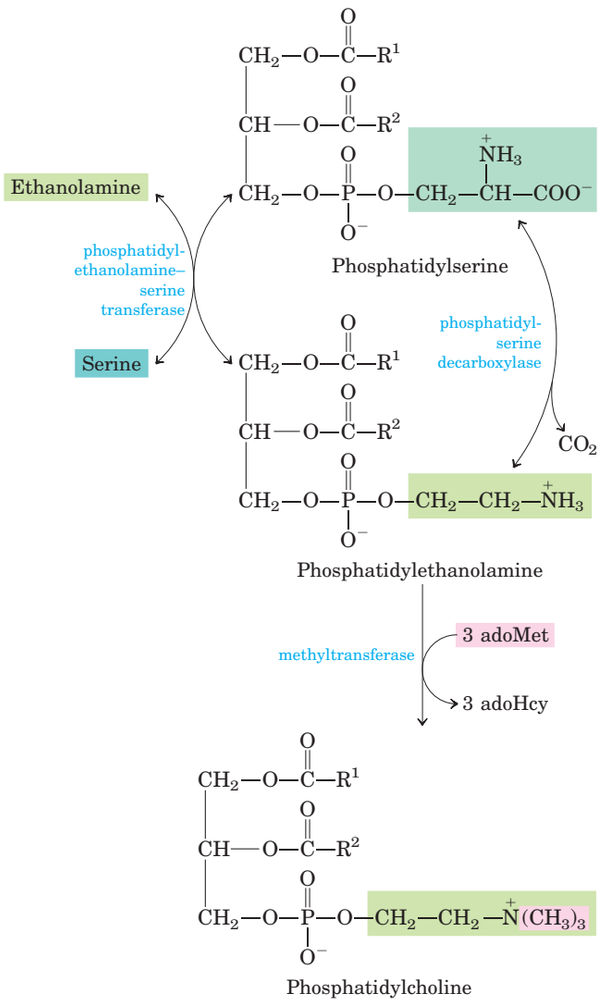


FIGURE 21–27 The "salvage" pathway from phosphatidylserine to phosphatidylethanolamine and phosphatidylcholine in yeast. Phosphatidylserine and phosphatidylethanolamine are interconverted by a reversible head-group exchange reaction. In mammals, phosphatidylserine is derived from phosphatidylethanolamine by a reversal of this reaction; adoMet is *S*-adenosylmethionine; adoHcy, *S*-adenosylhomocysteine.

Eukaryotic Pathways to Phosphatidylserine, Phosphatidylethanolamine, and Phosphatidylcholine Are Interrelated

Yeast, like bacteria, can produce phosphatidylserine by condensation of CDP-diacylglycerol and serine, and can synthesize phosphatidylethanolamine from phosphatidylserine in the reaction catalyzed by phosphatidylserine decarboxylase (Fig. 21–27). In mammalian cells, an alternative route to phosphatidylserine is a head-group exchange reaction, in which free serine displaces ethanolamine. Phosphatidylethanolamine may also be converted to **phosphatidylcholine** (lecithin) by the addition of three methyl groups to its amino group; *S*-adenosylmethionine is the methyl group donor (see Fig. 18–18) for all three methylation reactions.

In mammals, phosphatidylserine is not synthesized from CDP-diacylglycerol; instead, it is derived from phosphatidylethanolamine via the head-group exchange reaction (Fig. 21–27). Synthesis of phosphatidylethanolamine and phosphatidylcholine in mammals occurs by strategy 2 of Figure 21–24: phosphorylation and activation of the head group, followed by condensation with

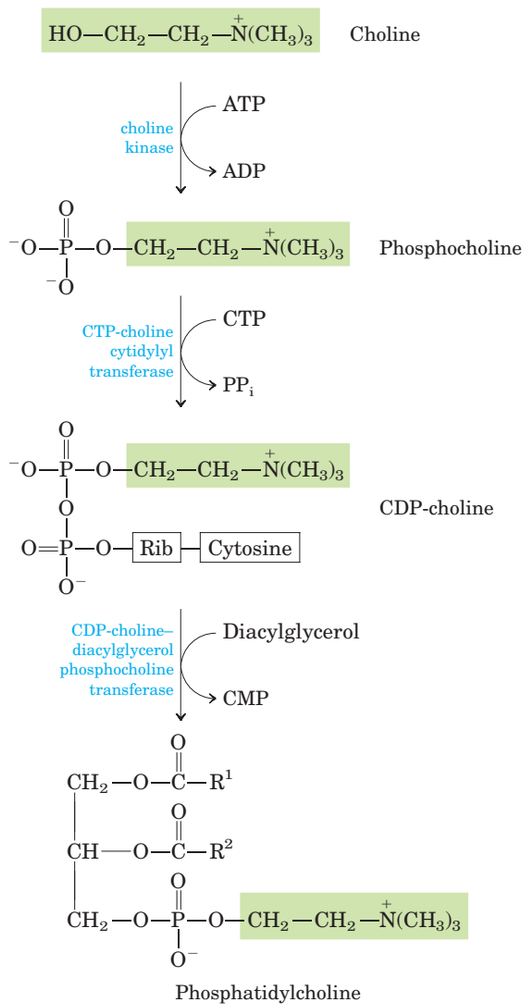


FIGURE 21–28 Pathway for phosphatidylcholine synthesis from choline in mammals. The same strategy shown here (strategy 2 in Fig. 21–24) is also used for salvaging ethanolamine in phosphatidylethanolamine synthesis.

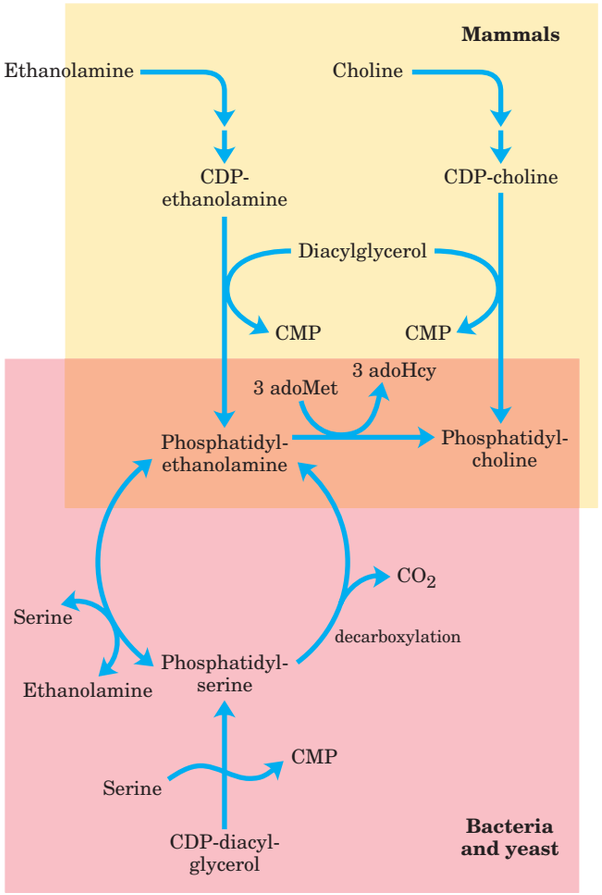


FIGURE 21–29 Summary of the pathways to phosphatidylcholine and phosphatidylethanolamine. Conversion of phosphatidylethanolamine to phosphatidylcholine in mammals takes place only in the liver.

diacylglycerol. For example, choline is reused ("salvaged") by being phosphorylated then converted to CDP-choline by condensation with CTP. A diacylglycerol displaces CMP from CDP-choline, producing phosphatidylcholine (Fig. 21–28). An analogous salvage pathway converts ethanolamine obtained in the diet to phosphatidylethanolamine. In the liver, phosphatidylcholine is also produced by methylation of phosphatidylethanolamine (with *S*-adenosylmethionine, as described above), but in all other tissues phosphatidylcholine is produced only by condensation of diacylglycerol and CDP-choline. The pathways to phosphatidylcholine and phosphatidylethanolamine in various organisms are summarized in Figure 21–29.

Although the role of lipid composition in membrane function is not entirely understood, changes in composition can produce dramatic effects. Researchers have isolated fruit flies with mutations in the gene that encodes ethanolamine kinase (analogous to choline kinase; Fig. 21–28). Lack of this enzyme eliminates one pathway for phosphatidylethanolamine synthesis, thereby reducing the amount of this lipid in cellular membranes. Flies with this mutation—those with the genotype *easily shocked*—exhibit transient paralysis following electrical stimulation or mechanical shock that would not affect wild-type flies.

Plasmalogen Synthesis Requires Formation of an Ether-Linked Fatty Alcohol

The biosynthetic pathway to ether lipids, including **plasmalogens** and the **platelet-activating factor** (see Fig. 10–9), involves the displacement of an esterified fatty acyl group by a long-chain alcohol to form the ether linkage (Fig. 21–30). Head-group attachment follows, by mechanisms essentially like those used in formation of the common ester-linked phospholipids. Finally, the characteristic double bond of plasmalogens (shaded blue in Fig. 21–30) is introduced by the action of a mixed-function oxidase similar to that responsible for desaturation of fatty acids (Fig. 21–13). The peroxisome is the primary site of plasmalogen synthesis.

Sphingolipid and Glycerophospholipid Synthesis Share Precursors and Some Mechanisms

The biosynthesis of sphingolipids takes place in four stages: (1) synthesis of the 18-carbon amine **sphinganine** from palmitoyl-CoA and serine; (2) attachment of a fatty acid in amide linkage to yield ***N*-acylsphinganine;** (3) desaturation of the sphinganine moiety to form ***N*-acylsphingosine** (ceramide); and (4) attachment of a head group to produce a sphingolipid such as a **cerebroside** or **sphingomyelin** (Fig. 21–31). The pathway shares several features with the pathways leading to glycerophospholipids: NADPH provides reducing power, and fatty acids enter as their activated CoA derivatives. In cerebroside formation, sugars enter as their activated nucleotide derivatives. Head-group attachment in sphingolipid synthesis has several novel aspects. Phosphatidylcholine, rather than CDP-choline, serves as the donor of phosphocholine in the synthesis of sphingomyelin.

In glycolipids, the cerebrosides and **gangliosides** (see Fig. 10–12), the head-group sugar is attached directly to the C-1 hydroxyl of sphingosine in glycosidic linkage rather than through a phosphodiester bond. The sugar donor is a UDP-sugar (UDP-glucose or UDP-galactose).

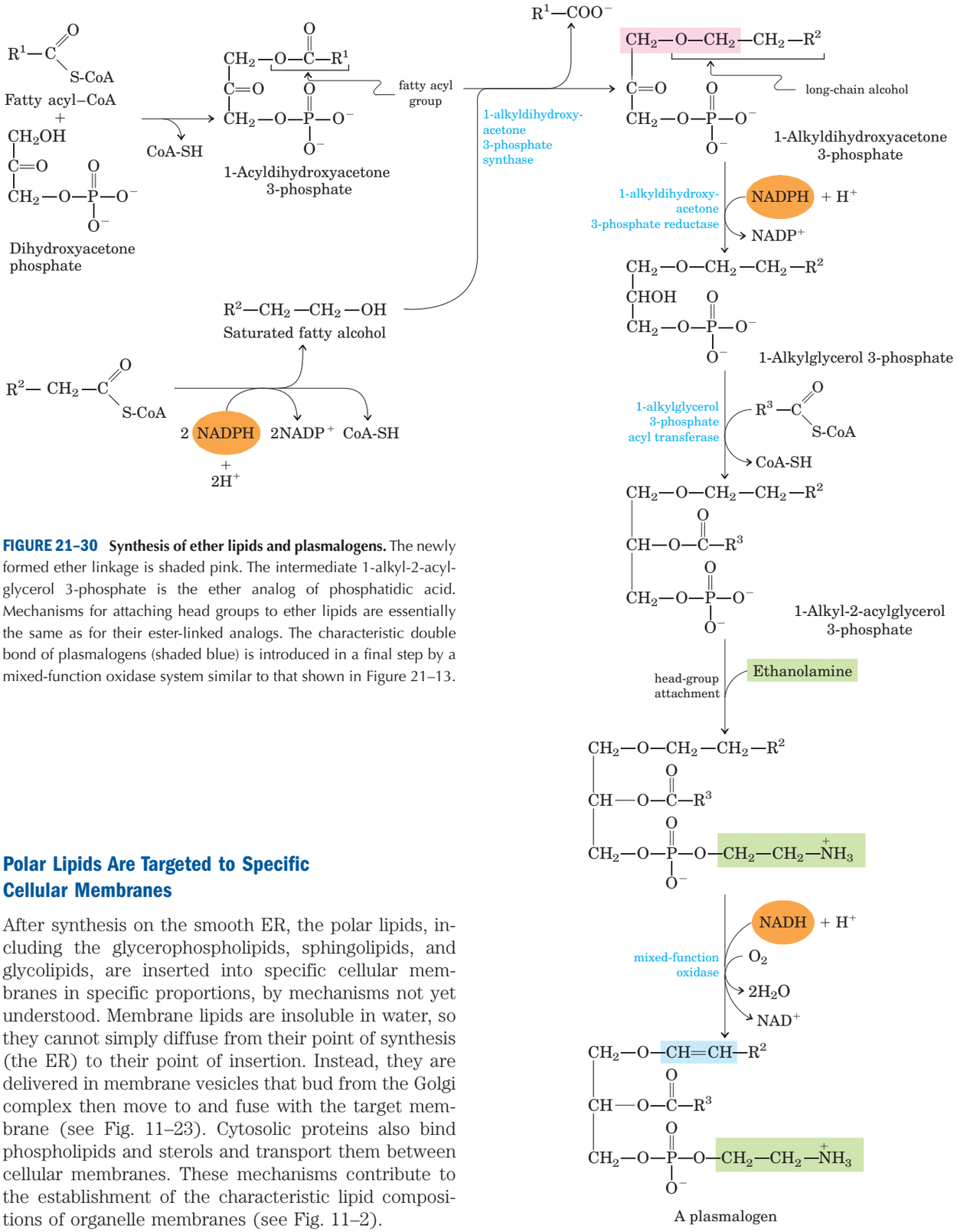
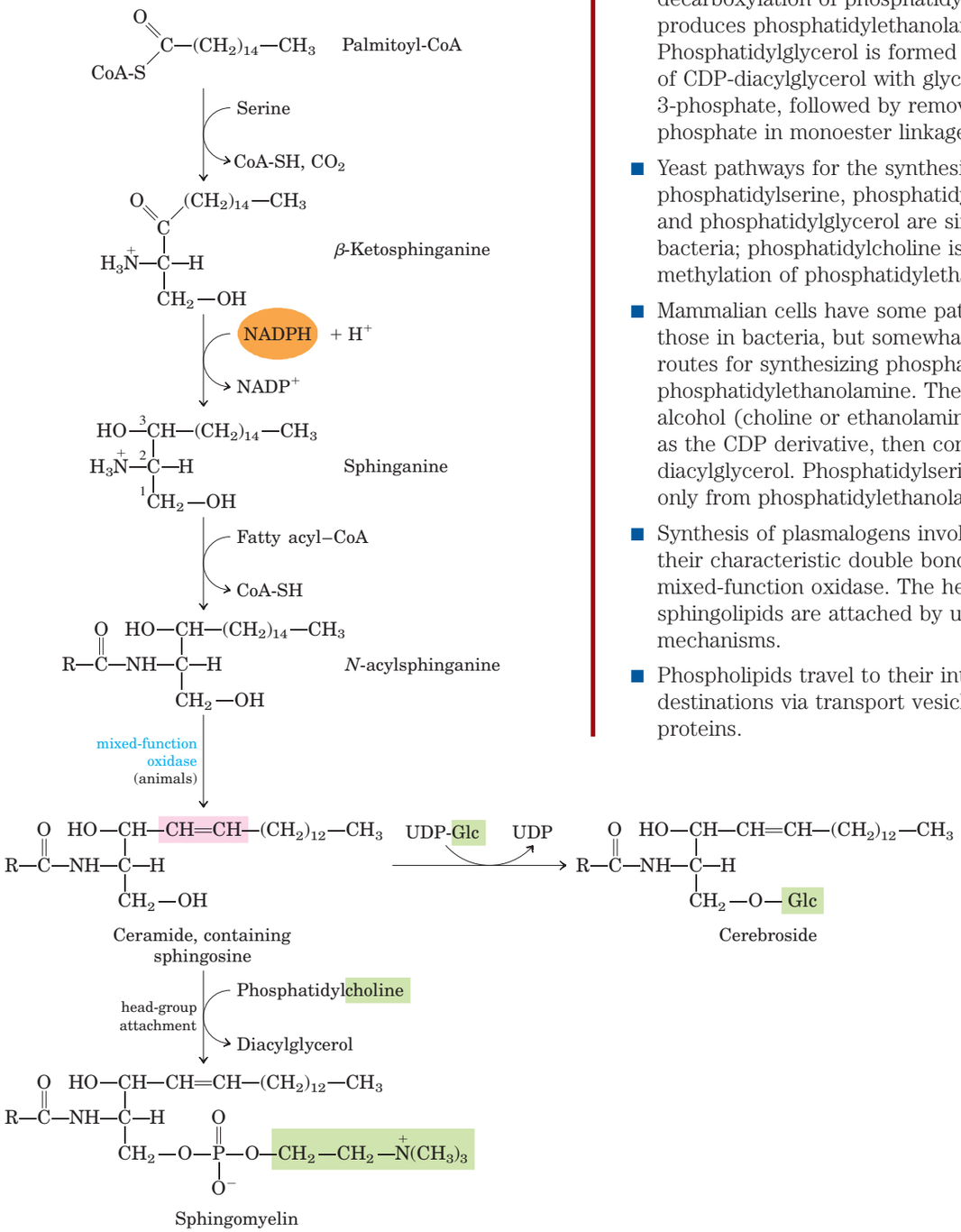


FIGURE 21–30 Synthesis of ether lipids and plasmalogens. The newly formed ether linkage is shaded pink. The intermediate 1-alkyl-2-acylglycerol 3-phosphate is the ether analog of phosphatidic acid. Mechanisms for attaching head groups to ether lipids are essentially the same as for their ester-linked analogs. The characteristic double bond of plasmalogens (shaded blue) is introduced in a final step by a mixed-function oxidase system similar to that shown in Figure 21–13.

Polar Lipids Are Targeted to Specific Cellular Membranes

After synthesis on the smooth ER, the polar lipids, including the glycerophospholipids, sphingolipids, and glycolipids, are inserted into specific cellular membranes in specific proportions, by mechanisms not yet understood. Membrane lipids are insoluble in water, so they cannot simply diffuse from their point of synthesis (the ER) to their point of insertion. Instead, they are delivered in membrane vesicles that bud from the Golgi complex then move to and fuse with the target membrane (see Fig. 11–23). Cytosolic proteins also bind phospholipids and sterols and transport them between cellular membranes. These mechanisms contribute to the establishment of the characteristic lipid compositions of organelle membranes (see Fig. 11–2).

FIGURE 21–31 Biosynthesis of sphingolipids. Condensation of palmitoyl-CoA and serine followed by reduction with NADPH yields sphinganine, which is then acylated to *N*-acylsphinganine (a ceramide). In animals, a double bond (shaded pink) is created by a mixed-function oxidase, before the final addition of a head group: phosphatidylcholine, to form sphingomyelin; glucose, to form a cerebroside.



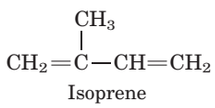
SUMMARY 21.3 Biosynthesis of Membrane Phospholipids

- Diacylglycerols are the principal precursors of glycerophospholipids.
- In bacteria, phosphatidylserine is formed by the condensation of serine with CDP-diacylglycerol; decarboxylation of phosphatidylserine produces phosphatidylethanolamine. Phosphatidylglycerol is formed by condensation of CDP-diacylglycerol with glycerol 3-phosphate, followed by removal of the phosphate in monoester linkage.
- Yeast pathways for the synthesis of phosphatidylserine, phosphatidylethanolamine, and phosphatidylglycerol are similar to those in bacteria; phosphatidylcholine is formed by methylation of phosphatidylethanolamine.
- Mammalian cells have some pathways similar to those in bacteria, but somewhat different routes for synthesizing phosphatidylcholine and phosphatidylethanolamine. The head-group alcohol (choline or ethanolamine) is activated as the CDP derivative, then condensed with diacylglycerol. Phosphatidylserine is derived only from phosphatidylethanolamine.
- Synthesis of plasmalogens involves formation of their characteristic double bond by a mixed-function oxidase. The head groups of sphingolipids are attached by unique mechanisms.
- Phospholipids travel to their intracellular destinations via transport vesicles or specific proteins.

21.4 Biosynthesis of Cholesterol, Steroids, and Isoprenoids

Cholesterol is doubtless the most publicized lipid, notorious because of the strong correlation between high levels of cholesterol in the blood and the incidence of human cardiovascular diseases. Less well advertised is cholesterol's crucial role as a component of cellular membranes and as a precursor of steroid hormones and bile acids. Cholesterol is an essential molecule in many animals, including humans, but is not required in the mammalian diet—all cells can synthesize it from simple precursors.

The structure of this 27-carbon compound suggests a complex biosynthetic pathway, but all of its carbon atoms are provided by a single precursor—acetate (Fig. 21–32). The **isoprene** units that are the essential intermediates in the pathway from acetate to cholesterol are also precursors to many other natural lipids, and the mechanisms by which isoprene units are polymerized are similar in all these pathways.



We begin with an account of the main steps in the biosynthesis of cholesterol from acetate, then discuss the transport of cholesterol in the blood, its uptake by cells, the normal regulation of cholesterol synthesis, and its regulation in those with defects in cholesterol uptake or transport. We next consider other cellular components derived from cholesterol, such as bile acids and steroid hormones. Finally, an outline of the biosynthetic pathways to some of the many compounds derived from isoprene units, which share early steps with the pathway to cholesterol, illustrates the extraordinary versatility of isoprenoid condensations in biosynthesis.

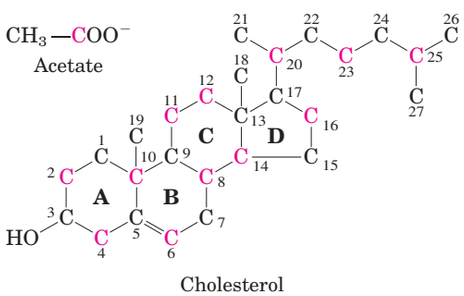


FIGURE 21–32 **Origin of the carbon atoms of cholesterol.** This can be deduced from tracer experiments with acetate labeled in the methyl carbon (black) or the carboxyl carbon (red). The individual rings in the fused-ring system are designated A through D.

Cholesterol Is Made from Acetyl-CoA in Four Stages

Cholesterol, like long-chain fatty acids, is made from acetyl-CoA, but the assembly plan is quite different. In early experiments, animals were fed acetate labeled with ^{14}C in either the methyl carbon or the carboxyl carbon. The pattern of labeling in the cholesterol isolated from the two groups of animals (Fig. 21–32) provided the blueprint for working out the enzymatic steps in cholesterol biosynthesis.

Synthesis takes place in four stages, as shown in Figure 21–33: ① condensation of three acetate units to form a six-carbon intermediate, mevalonate; ② conversion of mevalonate to activated isoprene units; ③ polymerization of six 5-carbon isoprene units to form the 30-carbon linear squalene; and ④ cyclization of squalene to form the four rings of the steroid nucleus, with a further series of changes (oxidations, removal or migration of methyl groups) to produce cholesterol.

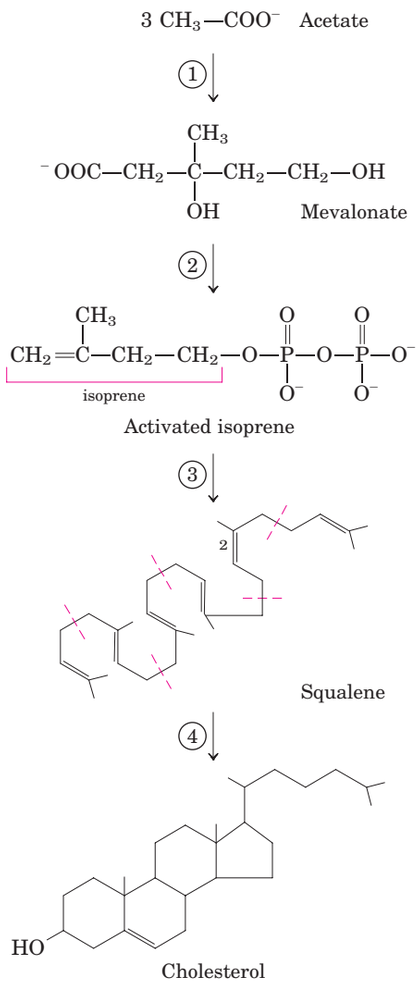


FIGURE 21–33 **Summary of cholesterol biosynthesis.** The four stages are discussed in the text. Isoprene units in squalene are set off by red dashed lines.

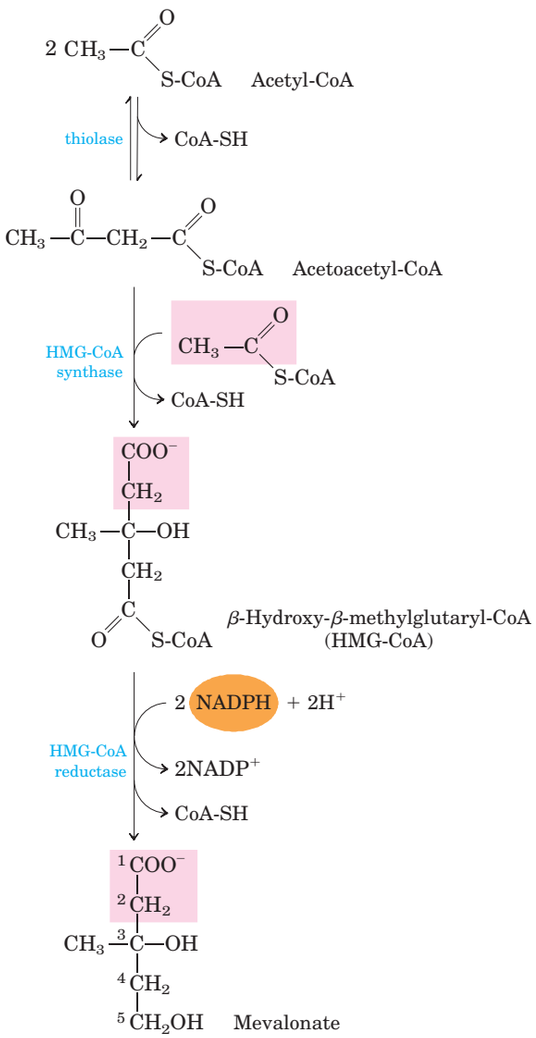


FIGURE 21–34 **Formation of mevalonate from acetyl-CoA.** The origin of C-1 and C-2 of mevalonate from acetyl-CoA is shown in pink.

Stage (1) Synthesis of Mevalonate from Acetate The first stage in cholesterol biosynthesis leads to the intermediate **mevalonate** (Fig. 21–34). Two molecules of acetyl-CoA condense to form acetoacetyl-CoA, which condenses with a third molecule of acetyl-CoA to yield the six-carbon compound ***β*-hydroxy-*β*-methylglutaryl-CoA (HMG-CoA).** These first two reactions are catalyzed by **thiolase** and **HMG-CoA synthase,** respectively. The cytosolic HMG-CoA synthase in this pathway is distinct from the mitochondrial isozyme that catalyzes HMG-CoA synthesis in ketone body formation (see Fig. 17–18).

The third reaction is the committed and rate-limiting step: reduction of HMG-CoA to mevalonate, for which each of two molecules of NADPH donates two electrons. **HMG-CoA reductase,** an integral membrane protein of the smooth ER, is the major point of regulation on the pathway to cholesterol, as we shall see.

Stage (2) Conversion of Mevalonate to Two Activated Isoprenes In the next stage of cholesterol synthesis, three phosphate groups are transferred from three ATP molecules to mevalonate (Fig. 21–35). The phosphate attached to the C-3 hydroxyl group of mevalonate in the intermediate 3-phospho-5-pyrophosphomevalonate is a good

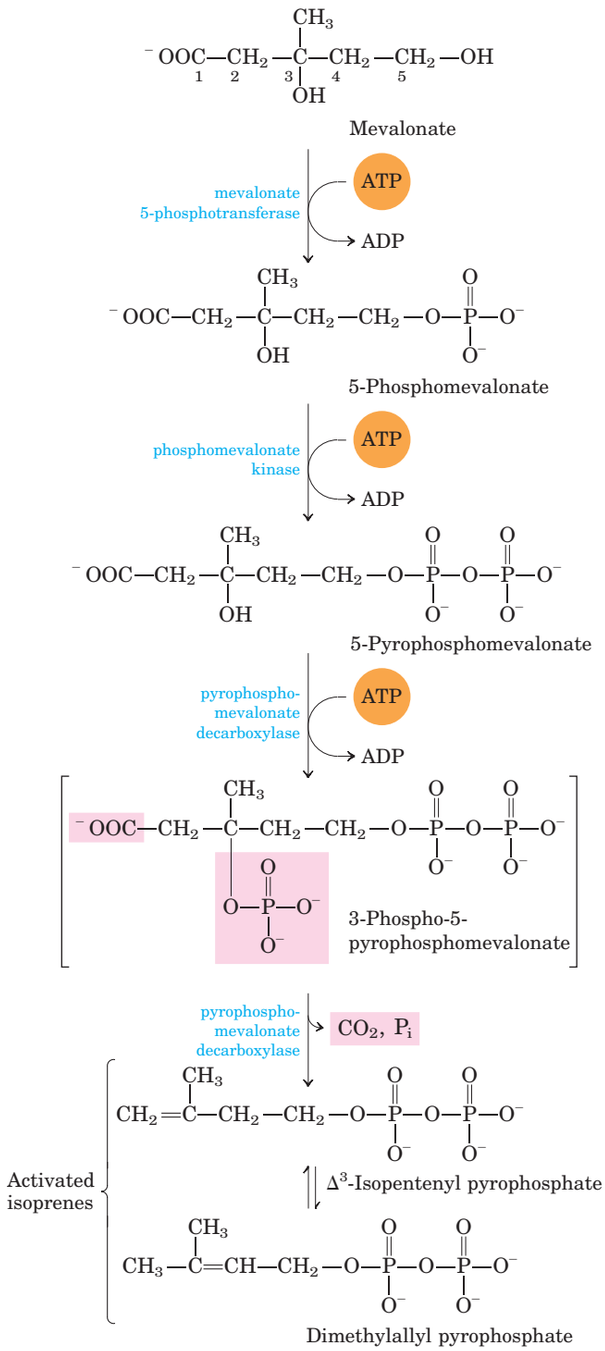


FIGURE 21–35 **Conversion of mevalonate to activated isoprene units.** Six of these activated units combine to form squalene (see Fig. 21–36). The leaving groups of 3-phospho-5-pyrophosphomevalonate are shaded pink. The bracketed intermediate is hypothetical.

leaving group; in the next step, both this phosphate and the nearby carboxyl group leave, producing a double bond in the five-carbon product, **Δ^3-isopentenyl pyrophosphate.** This is the first of the two activated isoprenes central to cholesterol formation. Isomerization of Δ^3-isopentenyl pyrophosphate yields the second activated isoprene, **dimethylallyl pyrophosphate.** Synthesis of isopentenyl pyrophosphate in the cytoplasm of plant cells follows the pathway described here. However, plant chloroplasts and many bacteria use a mevalonate-independent pathway. This alternative pathway does not occur in animals, so it is an attractive target for the development of new antibiotics.

Stage (3) Condensation of Six Activated Isoprene Units to Form Squalene Isopentenyl pyrophosphate and dimethylallyl pyrophosphate now undergo a head-to-tail condensation, in which one pyrophosphate group is displaced and a 10-carbon chain, **geranyl pyrophosphate,** is formed (Fig. 21–36). (The "head" is the end to which pyrophosphate is joined.) Geranyl pyrophosphate undergoes another head-to-tail condensation with isopentenyl pyro-

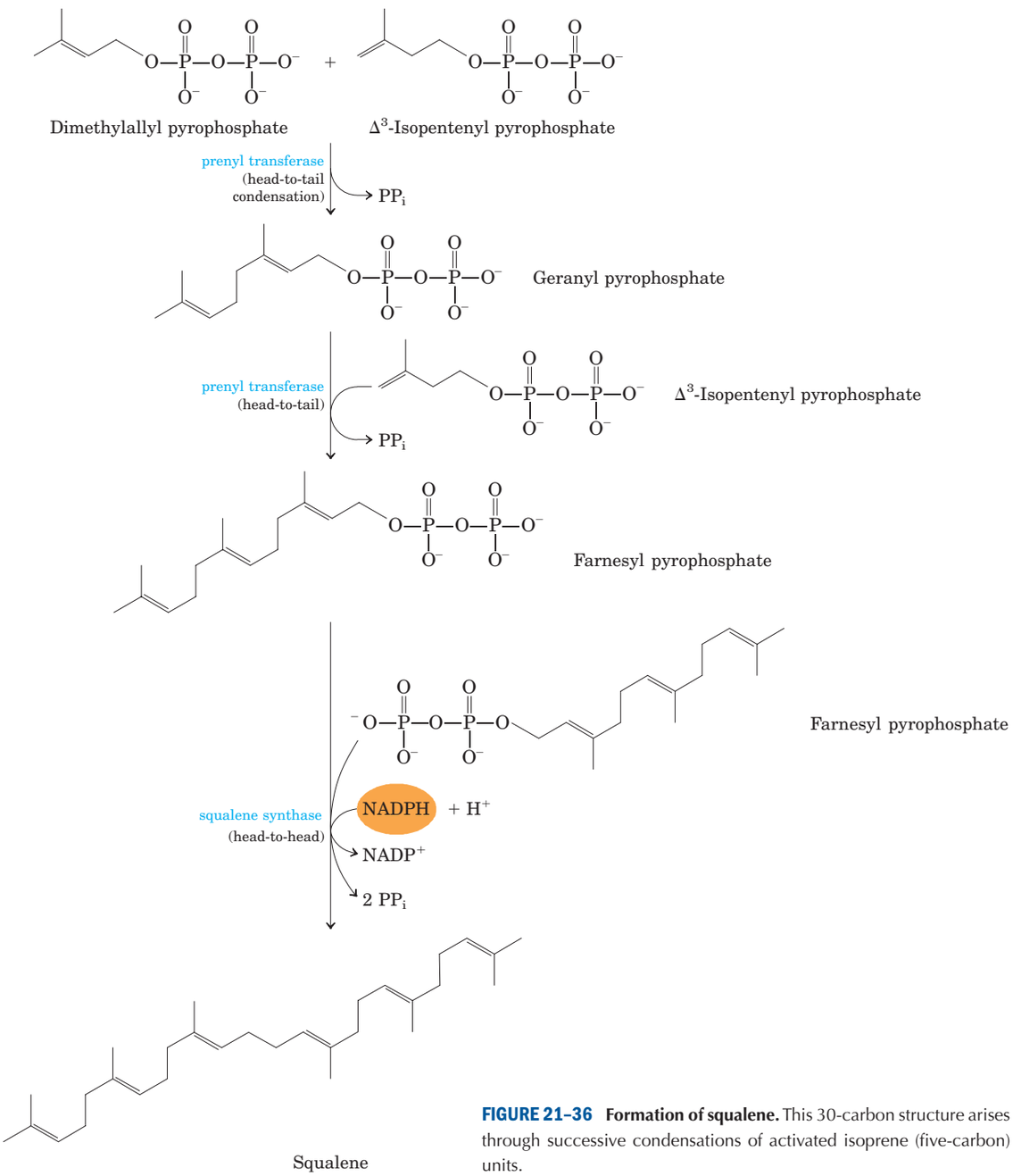


FIGURE 21–36 **Formation of squalene.** This 30-carbon structure arises through successive condensations of activated isoprene (five-carbon) units.

phosphate, yielding the 15-carbon intermediate **farnesyl pyrophosphate.** Finally, two molecules of farnesyl pyrophosphate join head to head, with the elimination of both pyrophosphate groups, to form **squalene.**

The common names of these intermediates derive from the sources from which they were first isolated. Geraniol, a component of rose oil, has the aroma of geraniums, and farnesol is an aromatic compound found in the flowers of the Farnese acacia tree. Many natural scents of plant origin are synthesized from isoprene units. Squalene, first isolated from the liver of sharks (genus *Squalus*), has 30 carbons, 24 in the main chain and 6 in the form of methyl group branches.

Stage (4) Conversion of Squalene to the Four-Ring Steroid Nucleus When the squalene molecule is represented as in Figure 21–37, the relationship of its linear structure to the cyclic structure of the sterols becomes apparent. All

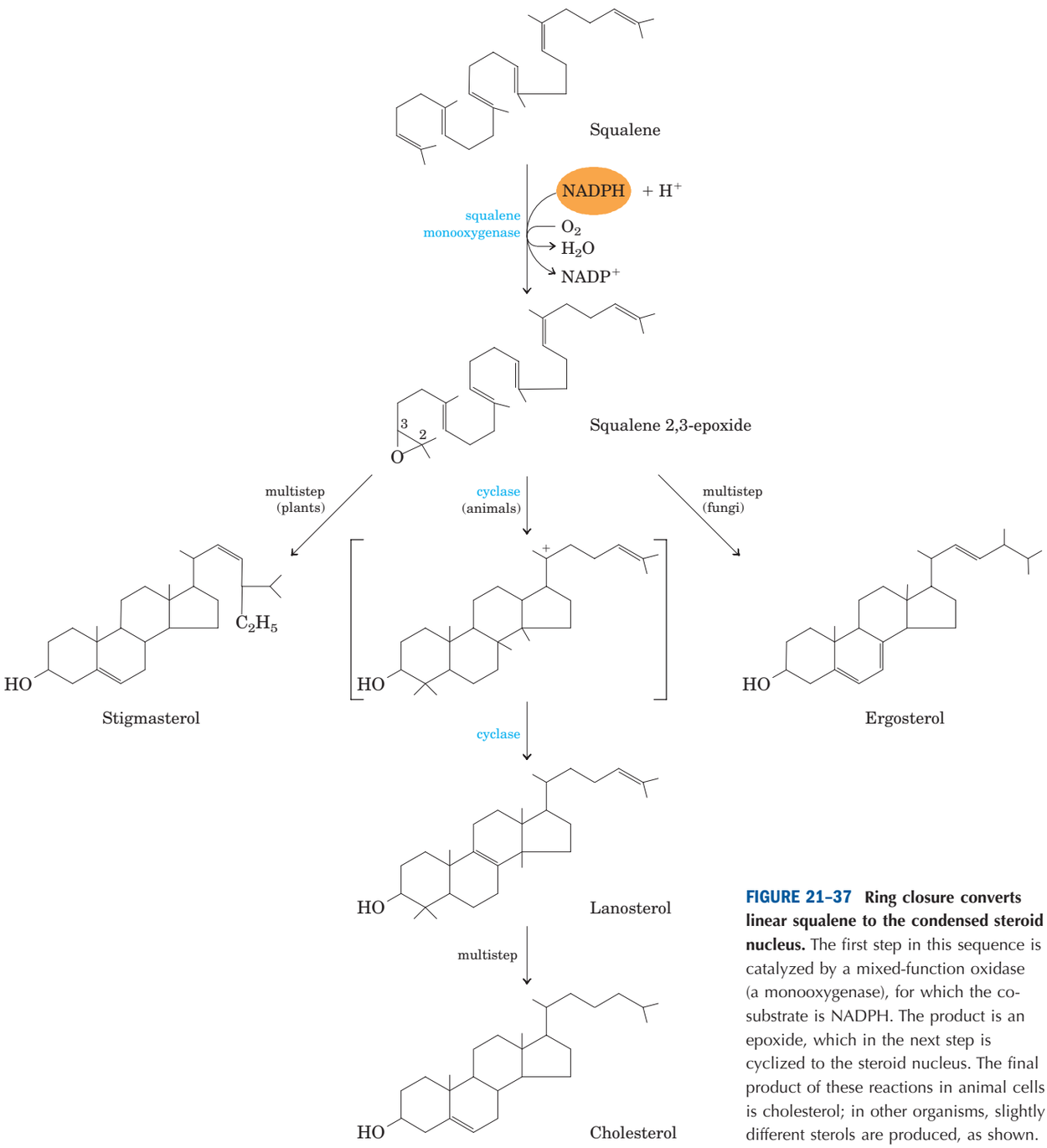


FIGURE 21–37 Ring closure converts linear squalene to the condensed steroid nucleus. The first step in this sequence is catalyzed by a mixed-function oxidase (a monooxygenase), for which the co-substrate is NADPH. The product is an epoxide, which in the next step is cyclized to the steroid nucleus. The final product of these reactions in animal cells is cholesterol; in other organisms, slightly different sterols are produced, as shown.

sterols have the four fused rings that form the steroid nucleus, and all are alcohols, with a hydroxyl group at C-3—thus the name "sterol." The action of **squalene monooxygenase** adds one oxygen atom from O_2 to the end of the squalene chain, forming an epoxide. This enzyme is another mixed-function oxidase (Box 21–1); NADPH reduces the other oxygen atom of O_2 to H_2O. The double bonds of the product, **squalene 2,3-epoxide,** are positioned so that a remarkable concerted reaction can convert the linear squalene epoxide to a cyclic structure. In animal cells, this cyclization results in the formation of **lanosterol,** which contains the four rings characteristic of the steroid nucleus. Lanosterol is finally converted to cholesterol in a series of about 20 reactions that include the migration of some methyl groups and the removal of others. Elucidation of this extraordinary biosynthetic pathway, one of the most complex known, was accomplished by Konrad Bloch, Feodor Lynen, John Cornforth, and George Popják in the late 1950s.

Cholesterol is the sterol characteristic of animal cells; plants, fungi, and protists make other, closely related sterols instead. They use the same synthetic pathway as far as squalene 2,3-epoxide, at which point the pathways diverge slightly, yielding other sterols, such as stigmasterol in many plants and ergosterol in fungi (Fig. 21–37).

Konrad Bloch,
1912–2000

Feodor Lynen,
1911–1979

John Cornforth

George Popják

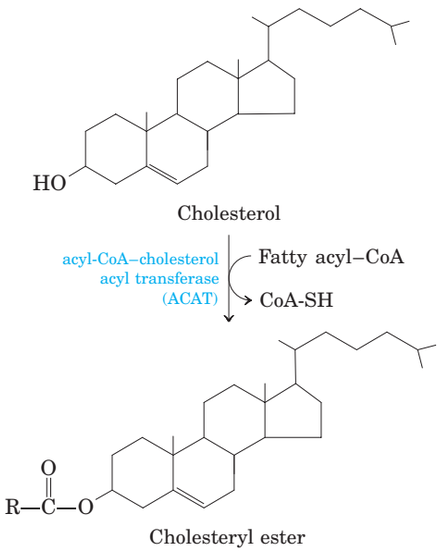


FIGURE 21–38 Synthesis of cholesteryl esters. Esterification converts cholesterol to an even more hydrophobic form for storage and transport.

Cholesterol Has Several Fates

Much of the cholesterol synthesis in vertebrates takes place in the liver. A small fraction of the cholesterol made there is incorporated into the membranes of hepatocytes, but most of it is exported in one of three forms: biliary cholesterol, bile acids, or cholesteryl esters. **Bile acids** and their salts are relatively hydrophilic cholesterol derivatives that are synthesized in the liver and aid in lipid digestion (see Fig. 17–1). **Cholesteryl esters** are formed in the liver through the action of **acyl-CoA–cholesterol acyl transferase (ACAT).** This enzyme catalyzes the transfer of a fatty acid from coenzyme A to the hydroxyl group of cholesterol (Fig. 21–38), converting the cholesterol to a more hydrophobic form. Cholesteryl esters are transported in secreted lipoprotein particles to other tissues that use cholesterol, or they are stored in the liver.

All growing animal tissues need cholesterol for membrane synthesis, and some organs (adrenal gland and gonads, for example) use cholesterol as a precursor for steroid hormone production (discussed below). Cholesterol is also a precursor of vitamin D (see Fig. 10–20a).

Cholesterol and Other Lipids Are Carried on Plasma Lipoproteins

Cholesterol and cholesteryl esters, like triacylglycerols and phospholipids, are essentially insoluble in water, yet must be moved from the tissue of origin to the tissues in which they will be stored or consumed. They are carried in the blood plasma as **plasma lipoproteins,**

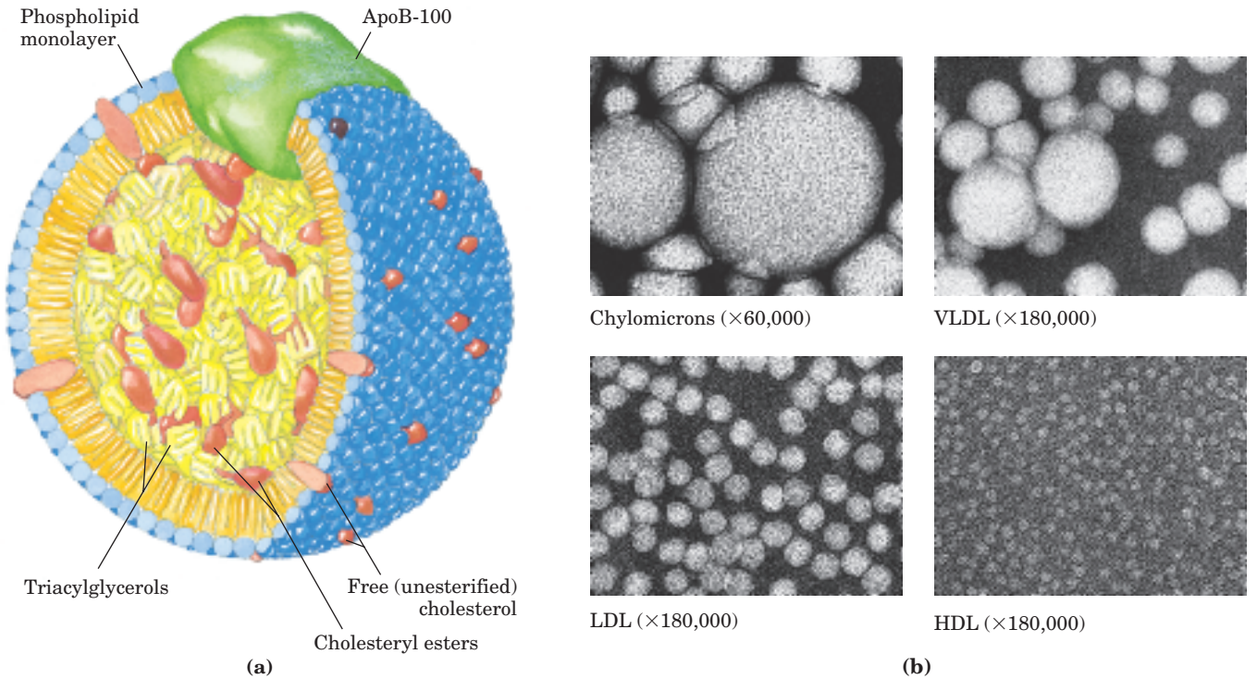


FIGURE 21–39 Lipoproteins. (a) Structure of a low-density lipoprotein (LDL). Apolipoprotein B-100 (apoB-100) is one of the largest single polypeptide chains known, with 4,636 amino acid residues (M_r 513,000). **(b)** Four classes of lipoproteins, visualized in the electron microscope after negative staining. Clockwise from top left: chylomicrons, 50 to 200 nm in diameter; VLDL, 28 to 70 nm; HDL, 8 to 11 nm; and LDL, 20 to 25 nm. For properties of lipoproteins, see Table 21–2.

macromolecular complexes of specific carrier proteins, **apolipoproteins,** with various combinations of phospholipids, cholesterol, cholesteryl esters, and triacylglycerols.

Apolipoproteins ("apo" designates the protein in its lipid-free form) combine with lipids to form several classes of lipoprotein particles, spherical complexes with hydrophobic lipids in the core and hydrophilic amino acid side chains at the surface (Fig. 21–39a). Different combinations of lipids and proteins produce particles of different densities, ranging from chylomicrons to high-density lipoproteins. These particles can be separated by ultracentrifugation (Table 21–2) and visualized by electron microscopy (Fig. 21–39b).

Each class of lipoprotein has a specific function, determined by its point of synthesis, lipid composition, and apolipoprotein content. At least nine different apolipoproteins are found in the lipoproteins of human plasma (Table 21–3), distinguishable by their size, their reactions with specific antibodies, and their characteristic distribution in the lipoprotein classes. These protein components act as signals, targeting lipoproteins to specific tissues or activating enzymes that act on the lipoproteins.

Chylomicrons, discussed in Chapter 17 in connection with the movement of dietary triacylglycerols from the intestine to other tissues, are the largest of the lipoproteins and the least dense, containing a high

TABLE 21–2 Major Classes of Human Plasma Lipoproteins: Some Properties

		Composition (wt %)				
Lipoprotein	*Density (g/mL)*	*Protein*	*Phospholipids*	*Free cholesterol*	*Cholesteryl esters*	*Triacylglycerols*
Chylomicrons	<1.006	2	9	1	3	85
VLDL	0.95–1.006	10	18	7	12	50
LDL	1.006–1.063	23	20	8	37	10
HDL	1.063–1.210	55	24	2	15	4

Source: Modified from Kritchevsky, D. (1986) Atherosclerosis and nutrition. *Nutr. Int.* **2,** 290–297.

proportion of triacylglycerols (see Fig. 17–2). Chylomicrons are synthesized in the ER of epithelial cells that line the small intestine, then move through the lymphatic system and enter the bloodstream via the left subclavian vein. The apolipoproteins of chylomicrons include apoB-48 (unique to this class of lipoproteins), apoE, and apoC-II (Table 21–3). ApoC-II activates lipoprotein lipase in the capillaries of adipose, heart, skeletal muscle, and lactating mammary tissues, allowing the release of free fatty acids to these tissues. Chylomicrons thus carry dietary fatty acids to tissues where they will be consumed or stored as fuel (Fig. 21–40). The remnants of chylomicrons (depleted of most of their triacylglycerols but still containing cholesterol, apoE, and apoB-48) move through the bloodstream to the liver. Receptors in the liver bind to the apoE in the chylomicron remnants and mediate their uptake by endocytosis. In the liver, the remnants release their cholesterol and are degraded in lysosomes.

When the diet contains more fatty acids than are needed immediately as fuel, they are converted to triacylglycerols in the liver and packaged with specific apolipoproteins into **very-low-density lipoprotein (VLDL).** Excess carbohydrate in the diet can also be converted to triacylglycerols in the liver and exported as VLDLs (Fig. 21–40a). In addition to triacylglycerols, VLDLs contain some cholesterol and cholesteryl esters, as well as apoB-100, apoC-I, apoC-II, apoC-III, and apoE (Table 21–3). These lipoproteins are transported in the blood from the liver to muscle and adipose tissue, where activation of lipoprotein lipase by apoC-II causes the release of free fatty acids from the VLDL triacylglycerols. Adipocytes take up these fatty acids, reconvert them to triacylglycerols, and store the products in intracellular lipid droplets; myocytes, in contrast, primarily oxidize the fatty acids to supply energy. Most VLDL remnants are removed from the circulation by hepatocytes. The uptake, like that for chylomicrons, is

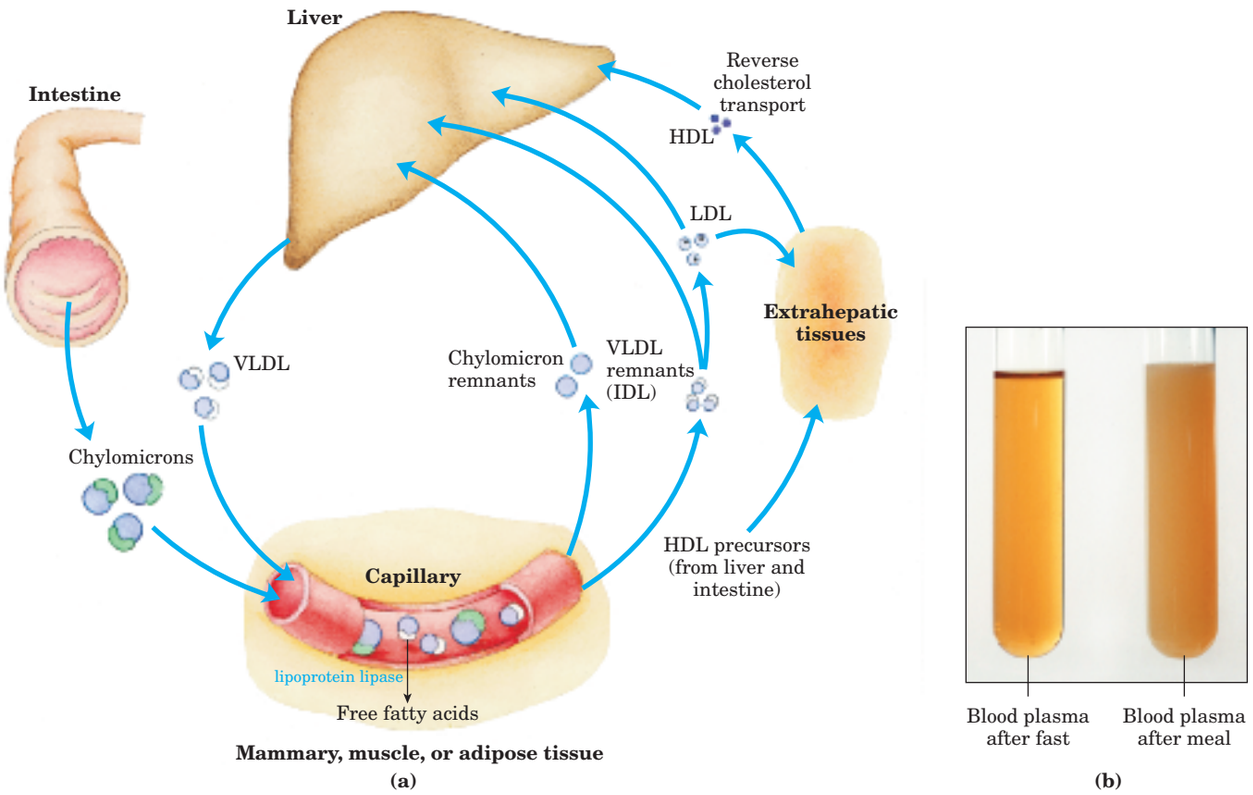


FIGURE 21–40 Lipoproteins and lipid transport. (a) Lipids are transported in the bloodstream as lipoproteins, which exist as several variants that have different functions, different protein and lipid compositions (see Tables 21–2, 21–3), and thus different densities. Dietary lipids are packaged into chylomicrons; much of their triacylglycerol content is released by lipoprotein lipase to adipose and muscle tissues during transport through capillaries. Chylomicron remnants (containing largely protein and cholesterol) are taken up by the liver. Endogenous lipids and cholesterol from the liver are delivered to adipose and muscle tissue by VLDL. Extraction of lipid from VLDL (along with loss of some apolipoproteins) gradually converts some of it to LDL, which delivers cholesterol to extrahepatic tissues or returns to the liver. The liver takes up LDL, VLDL remnants, and chylomicron remnants by receptor-mediated endocytosis. Excess cholesterol in extrahepatic tissues is transported back to the liver as HDL. In the liver, some cholesterol is converted to bile salts.

(b) Blood plasma samples collected after a fast (left) and after a high-fat meal (right). Chylomicrons produced after a fatty meal give the plasma a milky appearance.

TABLE 21–3 Apolipoproteins of the Human Plasma Lipoproteins

Apolipoprotein	*Molecular weight*	*Lipoprotein association*	*Function (if known)*
ApoA-I	28,331	HDL	Activates LCAT; interacts with ABC transporter
ApoA-II	17,380	HDL	
ApoA-IV	44,000	Chylomicrons, HDL	
ApoB-48	240,000	Chylomicrons	
ApoB-100	513,000	VLDL, LDL	Binds to LDL receptor
ApoC-I	7,000	VLDL, HDL	
ApoC-II	8,837	Chylomicrons, VLDL, HDL	Activates lipoprotein lipase
ApoC-III	8,751	Chylomicrons, VLDL, HDL	Inhibits lipoprotein lipase
ApoD	32,500	HDL	
ApoE	34,145	Chylomicrons, VLDL, HDL	Triggers clearance of VLDL and chylomicron remnants

Source: Modified from Vance, D.E. & Vance, J.E. (eds) (1985) *Biochemistry of Lipids and Membranes.* The Benjamin/Cummings Publishing Company, Menlo Park, CA.

receptor-mediated and depends on the presence of apoE in the VLDL remnants (Box 21–3 describes a link between apoE and Alzheimer's disease).

The loss of triacylglycerol converts some VLDL to VLDL remnants (also called intermediate density lipoprotein, IDL); further removal of triacylglycerol from VLDL produces **low-density lipoprotein (LDL)** (Table 21–2). Very rich in cholesterol and cholesteryl esters and containing apoB-100 as their major apolipoprotein, LDLs carry cholesterol to extrahepatic tissues that have specific plasma membrane receptors that recognize apoB-100. These receptors mediate the uptake of cholesterol and cholesteryl esters in a process described below.

The fourth major lipoprotein type, **high-density lipoprotein (HDL),** originates in the liver and small intestine as small, protein-rich particles that contain relatively little cholesterol and no cholesteryl esters (Fig. 21–40). HDLs contain apoA-I, apoC-I, apoC-II, and other apolipoproteins (Table 21–3), as well as the enzyme **lecithin-cholesterol acyl transferase (LCAT),** which catalyzes the formation of cholesteryl esters from lecithin (phosphatidylcholine) and cholesterol (Fig. 21–41). LCAT on the surface of nascent (newly forming) HDL particles converts the cholesterol and phosphatidylcholine of chylomicron and VLDL remnants to cholesteryl esters, which begin to form a core, transforming the disk-shaped nascent HDL to a mature, spherical HDL particle. This cholesterol-rich lipoprotein then returns to the liver, where the cholesterol is unloaded; some of this cholesterol is converted to bile salts.

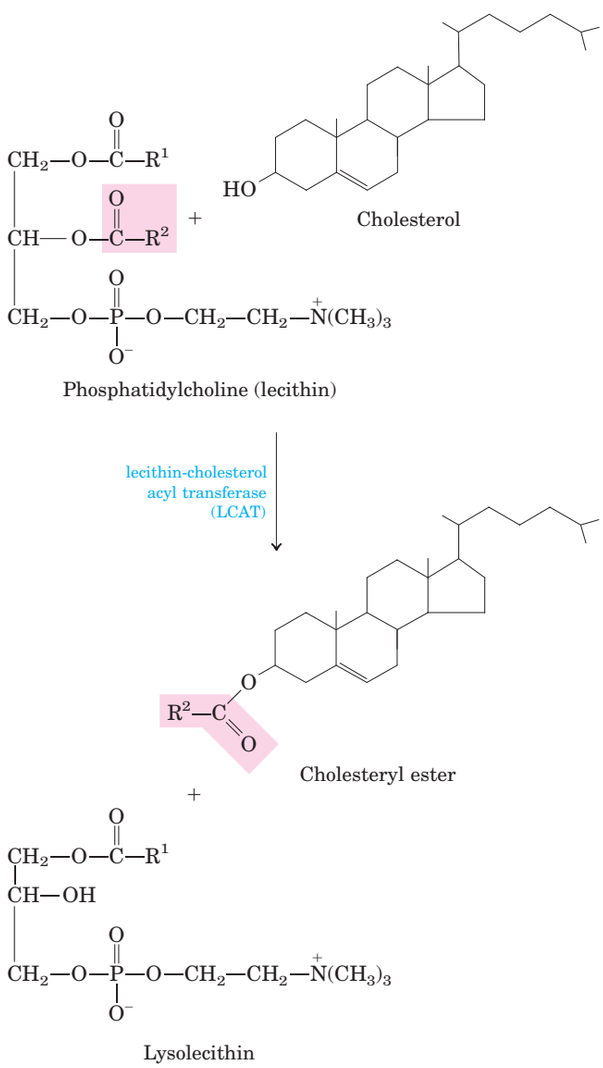


FIGURE 21–41 Reaction catalyzed by lecithin-cholesterol acyl transferase (LCAT). This enzyme is present on the surface of HDL and is stimulated by the HDL component apoA-I. Cholesteryl esters accumulate within nascent HDLs, converting them to mature HDLs.

BOX 21–3 BIOCHEMISTRY IN MEDICINE

ApoE Alleles Predict Incidence of Alzheimer's Disease

In the human population there are three common variants, or alleles, of the gene encoding apolipoprotein E. The most common, accounting for about 78% of human apoE alleles, is *APOE3;* alleles *APOE4* and *APOE2* account for 15% and 7%, respectively. The *APOE4* allele is particularly common in humans with Alzheimer's disease, and the link is highly predictive. Individuals who inherit *APOE4* have an increased risk of late-onset Alzheimer's disease. Those who are homozygous for *APOE4* have a 16-fold increased risk of developing the disease; for those who do, the mean age of onset is just under 70 years. For people who inherit two copies of *APOE3,* by contrast, the mean age of onset of Alzheimer's disease exceeds 90 years.

The molecular basis for the association between apoE4 and Alzheimer's disease is not yet known. Speculation has focused on a possible role for apoE in stabilizing the cytoskeletal structure of neurons. The apoE2 and apoE3 proteins bind to a number of proteins associated with neuronal microtubules, whereas apoE4 does not. This may accelerate the death of neurons. Whatever the mechanism proves to be, these observations promise to expand our understanding of the biological functions of apolipoproteins.

HDL may be taken up in the liver by receptor-mediated endocytosis, but at least some of the cholesterol in HDL is delivered to other tissues by a novel mechanism. HDL can bind to plasma membrane receptor proteins called SR-BI in hepatic and steroidogenic tissues such as the adrenal gland. These receptors mediate not endocytosis but a partial and selective transfer of cholesterol and other lipids in HDL into the cell. Depleted HDL then dissociates to recirculate in the bloodstream and extract more lipids from chylomicron and VLDL remnants. Depleted HDL can also pick up cholesterol stored in extrahepatic tissues and carry it to the liver, in **reverse cholesterol transport** pathways (Fig. 21–40). In one reverse transport path, interaction of nascent HDL with SR-BI receptors in cholesterol-rich cells triggers passive movement of cholesterol from the cell surface into HDL, which then carries it back to the liver. In a second pathway, apoA-I in depleted HDL interacts with an active transporter, the ABC1 protein, in a cholesterol-rich cell. The apoA-I (and presumably the HDL) is taken up by endocytosis, then resecreted with a load of cholesterol, which it transports to the liver.

Michael Brown and Joseph Goldstein

The ABC1 protein is a member of a large family of multidrug transporters, sometimes called ABC transporters because they all have *A*TP-*b*inding *c*assettes; they also have two transmembrane domains with six transmembrane helices (Chapter 11). These proteins actively transport a variety of ions, amino acids, vitamins, steroid hormones, and bile salts across plasma membranes. The CFTR protein that is defective in cystic fibrosis (see Box 11–3) is another member of this ABC family of multidrug transporters.

Cholesteryl Esters Enter Cells by Receptor-Mediated Endocytosis

Each LDL particle in the bloodstream contains apoB-100, which is recognized by specific surface receptor proteins, **LDL receptors,** on cells that need to take up cholesterol. The binding of LDL to an LDL receptor initiates endocytosis, which conveys the LDL and its receptor into the cell within an endosome (Fig. 21–42). The endosome eventually fuses with a lysosome, which contains enzymes that hydrolyze the cholesteryl esters, releasing cholesterol and fatty acid into the cytosol. The apoB-100 of LDL is also degraded to amino acids that are released to the cytosol, but the LDL receptor escapes degradation and is returned to the cell surface, to function again in LDL uptake. ApoB-100 is also present in VLDL, but its receptor-binding domain is not available for binding to the LDL receptor; conversion of VLDL to LDL exposes the receptor-binding domain of apoB-100. This pathway for the transport of cholesterol in blood and its **receptor-mediated endocytosis** by target tissues was elucidated by Michael Brown and Joseph Goldstein.

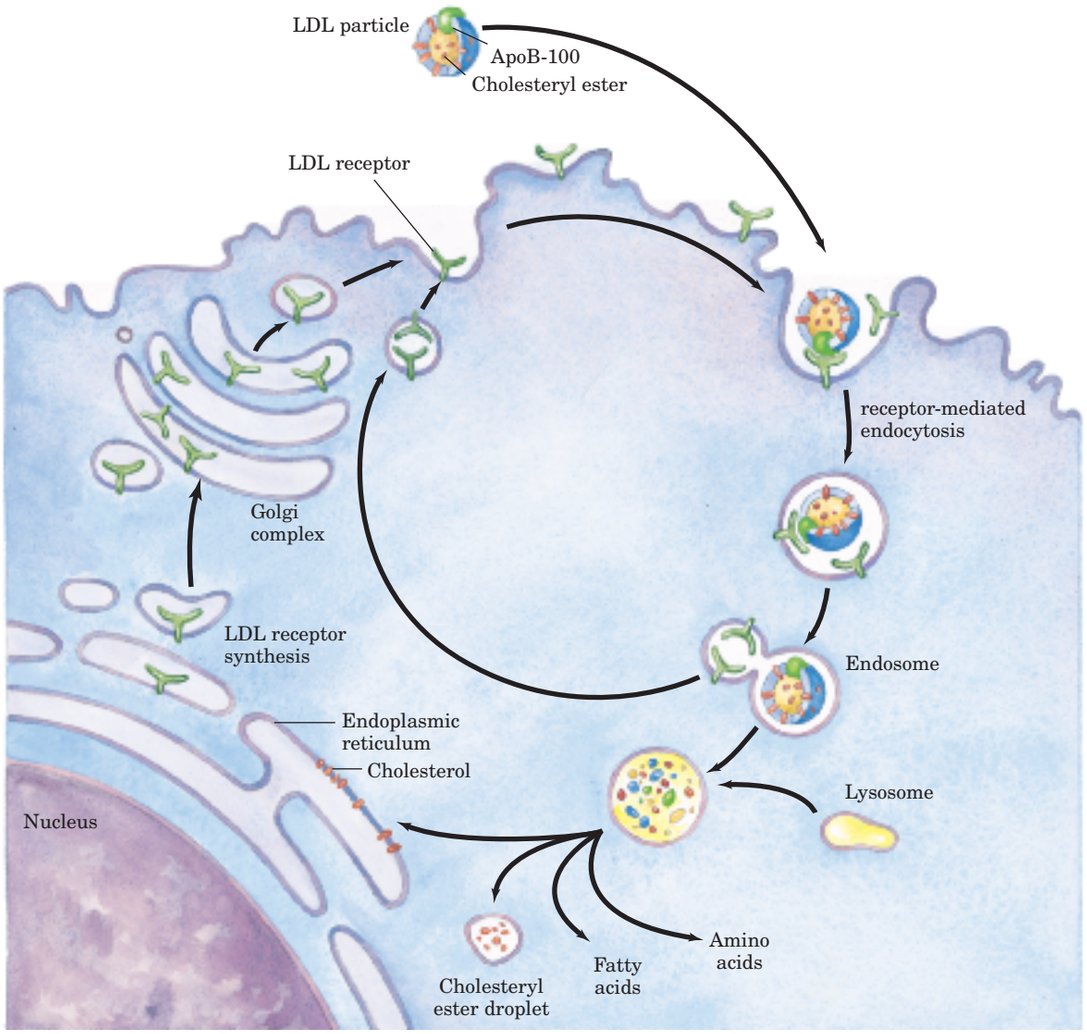


FIGURE 21–42 Uptake of cholesterol by receptor-mediated endocytosis.

Cholesterol that enters cells by this path may be incorporated into membranes or reesterified by ACAT (Fig. 21–38) for storage within cytosolic lipid droplets. Accumulation of excess intracellular cholesterol is prevented by reducing the rate of cholesterol synthesis when sufficient cholesterol is available from LDL in the blood.

The LDL receptor also binds to apoE and plays a significant role in the hepatic uptake of chylomicrons and VLDL remnants. However, if LDL receptors are unavailable (as, for example, in a mouse strain that lacks the gene for the LDL receptor), VLDL remnants and chylomicrons are still taken up by the liver even though LDL is not. This indicates the presence of a back-up system for receptor-mediated endocytosis of VLDL remnants and chylomicrons. One back-up receptor is *l*ipoprotein *r*eceptor-related *p*rotein (LRP), which binds to apoE as well as to a number of other ligands.

Cholesterol Biosynthesis Is Regulated at Several Levels

Cholesterol synthesis is a complex and energy-expensive process, so it is clearly advantageous to an organism to regulate the biosynthesis of cholesterol to complement dietary intake. In mammals, cholesterol production is regulated by intracellular cholesterol concentration and by the hormones glucagon and insulin. The rate-limiting step in the pathway to cholesterol (and a major site of regulation) is the conversion of HMG-CoA to mevalonate (Fig. 21–34), the reaction catalyzed by HMG-CoA reductase.

Regulation in response to cholesterol levels is mediated by an elegant system of transcriptional regulation of the gene encoding HMG-CoA reductase. This gene, along with more than 20 other genes encoding enzymes that mediate the uptake and synthesis of cholesterol and

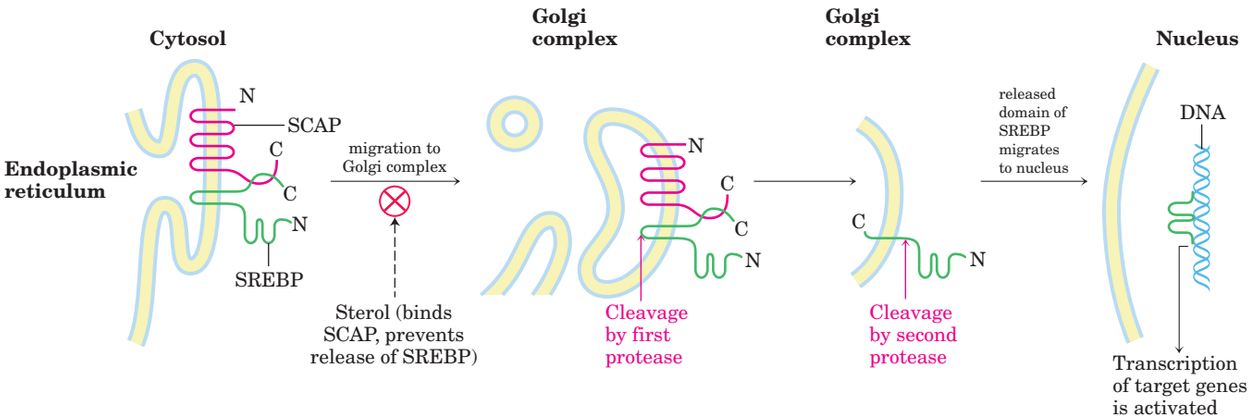


FIGURE 21–43 SREBP activation. Sterol regulatory element-binding proteins (SREBPs, shown in green) are embedded in the ER when first synthesized, in a complex with the protein SREBP cleavage-activating protein (SCAP, red). (N and C represent the amino and carboxyl termini of the proteins.) When bound to SCAP, SREBPs are inactive. When sterol levels decline, the complex migrates to the Golgi complex, and SREBP is cleaved by two different proteases in succession. The liberated amino-terminal domain of SREBP migrates to the nucleus, where it activates transcription of sterol-regulated genes.

unsaturated fatty acids, is controlled by a small family of proteins called sterol regulatory element-binding proteins (SREBPs). When newly synthesized, these proteins are embedded in the ER. Only the soluble amino-terminal domain of an SREBP functions as a transcriptional activator, using mechanisms discussed in Chapter 28. However, this domain has no access to the nucleus and cannot participate in gene activation while it remains part of the SREBP molecule. To activate transcription of the HMG-CoA reductase gene and other genes, the transcriptionally active domain is separated from the rest of the SREBP by proteolytic cleavage. When cholesterol levels are high, SREBPs are inactive, secured to the ER in a complex with another protein called SREBP cleavage-activating protein (SCAP) (Fig. 21–43). It is SCAP that binds cholesterol and a number of other sterols, thus acting as a sterol sensor. When sterol levels are high, the SCAP-SREBP complex probably interacts with another protein that retains the entire complex in the ER. When the level of sterols in the cell declines, a conformational change in SCAP causes release of the SCAP-SREBP complex from the ER-retention activity, and the complex migrates within vesicles to the Golgi complex. In the Golgi complex, SREBP is cleaved twice by two different proteases, the second cleavage releasing the amino-terminal domain into the cytosol. This domain travels to the nucleus and activates transcription of its target genes. The amino-terminal domain of SREBP has a short half-life and is rapidly degraded by proteasomes (see Fig. 27–42). When sterol levels increase sufficiently, the proteolytic release of SREBP amino-terminal domains is again blocked, and proteasome degradation of the existing active domains results in a rapid shut-down of the gene targets.

Several other mechanisms also regulate cholesterol synthesis (Fig. 21–44). Hormonal control is mediated by covalent modification of HMG-CoA reductase itself. The enzyme exists in phosphorylated (inactive) and dephosphorylated (active) forms. Glucagon stimulates phosphorylation (inactivation), and insulin promotes dephosphorylation, activating the enzyme and favoring cholesterol synthesis. High intracellular concentrations of cholesterol activate ACAT, which increases esterification of cholesterol for storage. Finally, a high cellular cholesterol level diminishes transcription of the gene that encodes the LDL receptor, reducing production of the receptor and thus the uptake of cholesterol from the blood.

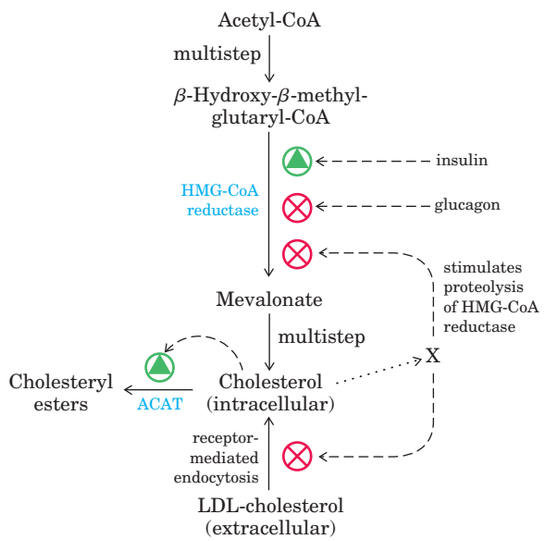
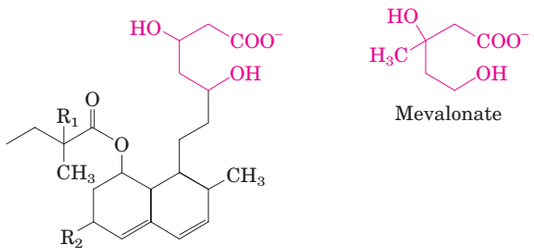


FIGURE 21–44 Regulation of cholesterol formation balances synthesis with dietary uptake. Glucagon promotes phosphorylation (inactivation) of HMG-CoA reductase; insulin promotes dephosphorylation (activation). X represents unidentified metabolites of cholesterol that stimulate proteolysis of HMG-CoA reductase.

Unregulated cholesterol production can lead to serious human disease. When the sum of cholesterol synthesized and cholesterol obtained in the diet exceeds the amount required for the synthesis of membranes, bile salts, and steroids, pathological accumulations of cholesterol in blood vessels (atherosclerotic plaques) can develop, resulting in obstruction of blood vessels **(atherosclerosis).** Heart failure due to occluded coronary arteries is a leading cause of death in industrialized societies. Atherosclerosis is linked to high levels of cholesterol in the blood, and particularly to high levels of LDL-bound cholesterol; there is a *negative* correlation between HDL levels and arterial disease.

In familial hypercholesterolemia, a human genetic disorder, blood levels of cholesterol are extremely high and severe atherosclerosis develops in childhood. These individuals have a defective LDL receptor and lack receptor-mediated uptake of cholesterol carried by LDL. Consequently, cholesterol is not cleared from the blood; it accumulates and contributes to the formation of atherosclerotic plaques. Endogenous cholesterol synthesis continues despite the excessive cholesterol in the blood, because extracellular cholesterol cannot enter the cell to regulate intracellular synthesis (Fig. 21–44). Two products derived from fungi, **lovastatin** and **compactin,** are used to treat patients with familial hypercholesterolemia. Both these compounds, and several synthetic analogs, resemble mevalonate (Fig. 21–45) and are competitive inhibitors of HMG-CoA reductase, thus inhibiting cholesterol synthesis. Lovastatin treatment lowers serum cholesterol by as much as 30% in individuals having one defective copy of the gene for the LDL receptor. When combined with an edible resin that binds bile acids and prevents their reabsorption from the intestine, the drug is even more effective.

In familial HDL deficiency, HDL levels are very low; they are almost undetectable in Tangier disease. Both genetic disorders are the result of mutations in the ABC1 protein. Cholesterol-depleted HDL cannot take up cholesterol from cells that lack ABC1 protein, and cholesterol-poor HDL is rapidly removed from the blood and destroyed. Both familial HDL deficiency and Tangier disease are very rare (worldwide, fewer than 100 families with Tangier disease are known), but the existence of these diseases establishes a role for ABC1 protein in the regulation of plasma HDL levels. Because low plasma HDL levels correlate with a high incidence of coronary artery disease, the ABC1 protein may prove a useful target for drugs to control HDL levels. ■

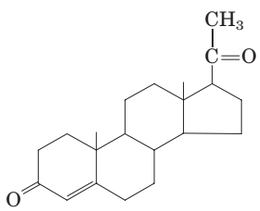


$R_1 = H$	$R_2 = H$	Compactin
$R_1 = CH_3$	$R_2 = CH_3$	Simvastatin (Zocor)
$R_1 = H$	$R_2 = OH$	Pravastatin (Pravachol)
$R_1 = H$	$R_2 = CH_3$	Lovastatin (Mevacor)

FIGURE 21–45 **Inhibitors of HMG-CoA reductase.** A comparison of the structures of mevalonate and four pharmaceutical compounds that inhibit HMG-CoA reductase.

Steroid Hormones Are Formed by Side-Chain Cleavage and Oxidation of Cholesterol

Humans derive all their steroid hormones from cholesterol (Fig. 21–46). Two classes of steroid hormones are synthesized in the cortex of the adrenal gland: **mineralocorticoids,** which control the reabsorption of inorganic ions (Na^+, Cl^-, and HCO_3^-) by the kidney, and **glucocorticoids,** which help regulate gluconeogenesis and reduce the inflammatory response. Sex hormones are produced in male and female gonads and the placenta. They include **progesterone,** which regulates the female reproductive cycle, and **androgens** (such as testosterone) and **estrogens** (such as estradiol), which influence the development of



Progesterone

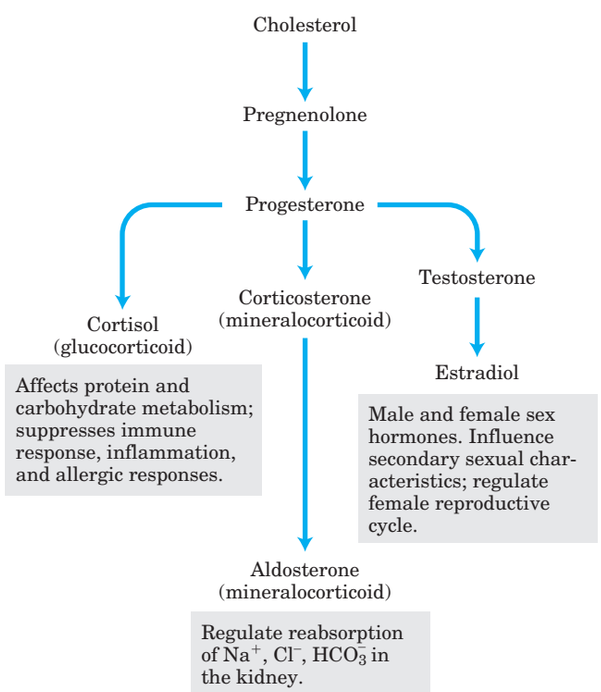


FIGURE 21–46 **Some steroid hormones derived from cholesterol.** The structures of some of these compounds are shown in Figure 10–19.

secondary sexual characteristics in males and females, respectively. Steroid hormones are effective at very low concentrations and are therefore synthesized in relatively small quantities. In comparison with the bile salts, their production consumes relatively little cholesterol.

Synthesis of steroid hormones requires removal of some or all of the carbons in the "side chain" on C-17 of the D ring of cholesterol. Side-chain removal takes place in the mitochondria of steroidogenic tissues. Removal involves the hydroxylation of two adjacent carbons in the side chain (C-20 and C-22) followed by cleavage of the bond between them (Fig. 21–47). Formation of the various hormones also involves the introduction of oxygen atoms. All the hydroxylation and oxygenation reactions in steroid biosynthesis are catalyzed by mixed-function oxidases (Box 21–1) that use NADPH, O_2, and mitochondrial cytochrome P-450.

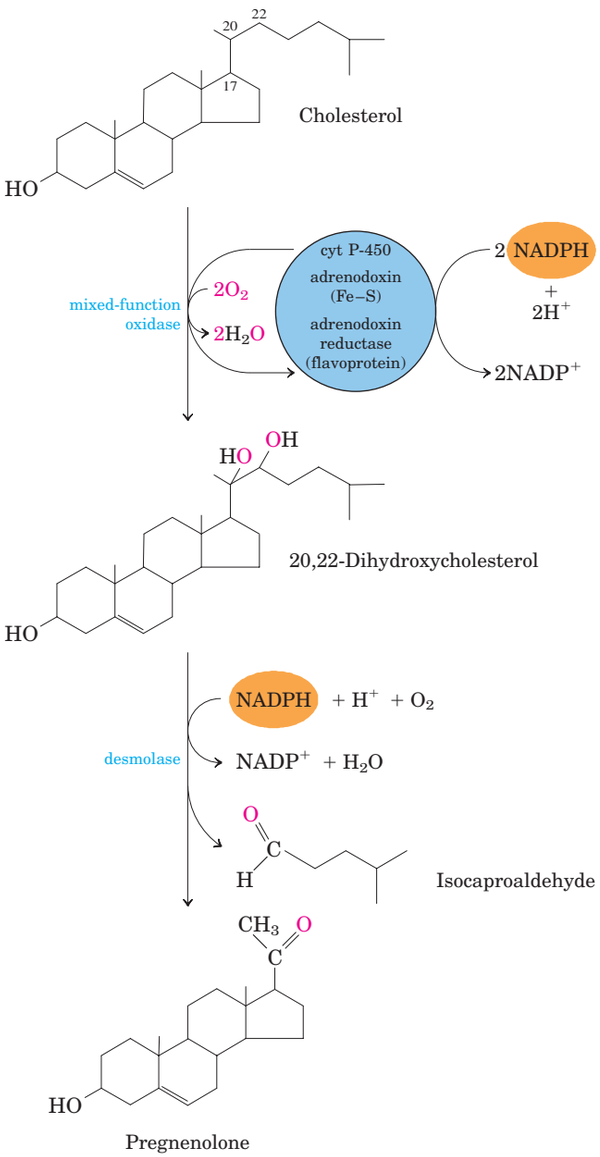


FIGURE 21–47 Side-chain cleavage in the synthesis of steroid hormones. Cytochrome P-450 acts as electron carrier in this mixed-function oxidase system that oxidizes adjacent carbons. The process also requires the electron-transferring proteins adrenodoxin and adrenodoxin reductase. This system for cleaving side chains is found in mitochondria of the adrenal cortex, where active steroid production occurs. Pregnenolone is the precursor of all other steroid hormones (see Fig. 21–46).

Intermediates in Cholesterol Biosynthesis Have Many Alternative Fates

In addition to its role as an intermediate in cholesterol biosynthesis, isopentenyl pyrophosphate is the activated precursor of a huge array of biomolecules with diverse biological roles (Fig. 21–48). They include vitamins A, E, and K; plant pigments such as carotene and the phytol chain of chlorophyll; natural rubber; many essential oils (such as the fragrant principles of lemon oil, eucalyptus, and musk); insect juvenile hormone, which controls metamorphosis; dolichols, which serve as lipid-soluble carriers in complex polysaccharide synthesis; and ubiquinone and plastoquinone, electron carriers in mitochondria and chloroplasts. Collectively, these molecules are called isoprenoids. More than

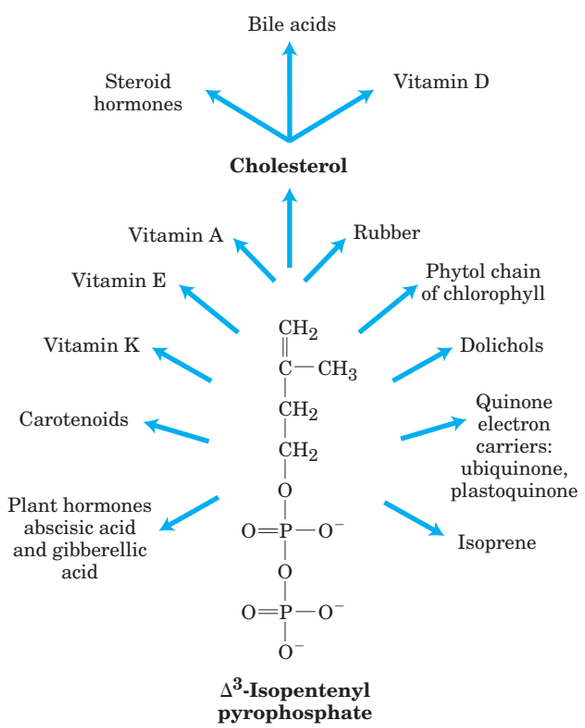


FIGURE 21–48 Overview of isoprenoid biosynthesis. The structures of most of the end products shown here are given in Chapter 10.

20,000 different isoprenoid molecules have been discovered in nature, and hundreds of new ones are reported each year.

Prenylation (covalent attachment of an isoprenoid; see Fig. 27–30) is a common mechanism by which proteins are anchored to the inner surface of cellular membranes in mammals (see Fig. 11–14). In some of these proteins the attached lipid is the 15-carbon farnesyl group; others have the 20-carbon geranylgeranyl group. Different enzymes attach the two types of lipids. It is possible that prenylation reactions target proteins to different membranes, depending on which lipid is attached. Protein prenylation is another important role for the isoprene derivatives of the pathway to cholesterol.

SUMMARY 21.4 Biosynthesis of Cholesterol, Steroids, and Isoprenoids

- Cholesterol is formed from acetyl-CoA in a complex series of reactions, through the intermediates β-hydroxy-β-methylglutaryl-CoA, mevalonate, and two activated isoprenes, dimethylallyl pyrophosphate and isopentenyl pyrophosphate. Condensation of isoprene units produces the noncyclic squalene, which is cyclized to yield the steroid ring system and side chain.
- Cholesterol synthesis is under hormonal control and is also inhibited by elevated concentrations of intracellular cholesterol, which acts through covalent modification and transcriptional regulation mechanisms.
- Cholesterol and cholesteryl esters are carried in the blood as plasma lipoproteins. VLDL carries cholesterol, cholesteryl esters, and triacylglycerols from the liver to other tissues, where the triacylglycerols are degraded by lipoprotein lipase, converting VLDL to LDL. The LDL, rich in cholesterol and its esters, is taken up by receptor-mediated endocytosis, in which the apolipoprotein B-100 of LDL is recognized by receptors in the plasma membrane. HDL removes cholesterol from the blood, carrying it to the liver. Dietary conditions or genetic defects in cholesterol metabolism may lead to atherosclerosis and heart disease.
- The steroid hormones (glucocorticoids, mineralocorticoids, and sex hormones) are produced from cholesterol by alteration of the side chain and introduction of oxygen atoms into the steroid ring system. In addition to cholesterol, a wide variety of isoprenoid compounds are derived from mevalonate through condensations of isopentenyl pyrophosphate and dimethylallyl pyrophosphate.
- Prenylation of certain proteins targets them for association with cellular membranes and is essential for their biological activity.

Key Terms

Terms in bold are defined in the glossary.

acetyl-CoA carboxylase 787
fatty acid synthase 789
acyl carrier protein (ACP) 790
fatty acyl-CoA desaturase 798
mixed-function oxidases 799
mixed-function oxygenases 799
cytochrome P-450 799
essential fatty acids 800
prostaglandins 800
cyclooxygenase (COX) 800
prostaglandin H_2 synthase 800
thromboxane synthase 800
thromboxanes 800
leukotrienes 800
glycerol 3-phosphate dehydrogenase 804
triacylglycerol cycle 806
glyceroneogenesis 806
thiazolidinediones 807
phosphatidylserine 811
phosphatidylglycerol 811
phosphatidylethanolamine 811
cardiolipin 811
phosphatidylcholine 812
plasmalogen 813
platelet-activating factor 813
cerebroside 813
sphingomyelin 813
gangliosides 813
isoprene 816
mevalonate 817
β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) 817
thiolase 817
HMG-CoA synthase 817
HMG-CoA reductase 817
bile acids 820
cholesteryl esters 820
apolipoproteins 821
chylomicron 821
very-low-density lipoprotein (VLDL) 822
low-density lipoprotein (LDL) 823
high-density lipoprotein (HDL) 823
reverse cholesterol transport 824
LDL receptors 824
receptor-mediated endocytosis 824
atherosclerosis 827
lovastatin 827
mineralocorticoids 827
glucocorticoids 827
progesterone 827
androgens 827
estrogens 827

Further Reading

The general references in Chapters 10 and 17 are also useful.

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Problems

1. Pathway of Carbon in Fatty Acid Synthesis Using your knowledge of fatty acid biosynthesis, provide an explanation for the following experimental observations:

(a) Addition of uniformly labeled [^{14}C]acetyl-CoA to a soluble liver fraction yields palmitate uniformly labeled with ^{14}C.

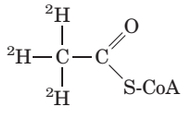
(b) However, addition of a *trace* of uniformly labeled [^{14}C]acetyl-CoA in the presence of an excess of unlabeled malonyl-CoA to a soluble liver fraction yields palmitate labeled with ^{14}C only in C-15 and C-16.

2. Synthesis of Fatty Acids from Glucose After a person has ingested large amounts of sucrose, the glucose and fructose that exceed caloric requirements are transformed to fatty acids for triacylglycerol synthesis. This fatty acid synthesis consumes acetyl-CoA, ATP, and NADPH. How are these substances produced from glucose?

3. Net Equation of Fatty Acid Synthesis Write the net equation for the biosynthesis of palmitate in rat liver, starting from mitochondrial acetyl-CoA and cytosolic NADPH, ATP, and CO_2.

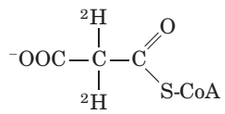
4. Pathway of Hydrogen in Fatty Acid Synthesis Consider a preparation that contains all the enzymes and cofactors necessary for fatty acid biosynthesis from added acetyl-CoA and malonyl-CoA.

(a) If [2-^{2}H]acetyl-CoA (labeled with deuterium, the heavy isotope of hydrogen)



and an excess of unlabeled malonyl-CoA are added as substrates, how many deuterium atoms are incorporated into every molecule of palmitate? What are their locations? Explain.

(b) If unlabeled acetyl-CoA and [2-^{2}H]malonyl-CoA



are added as substrates, how many deuterium atoms are incorporated into every molecule of palmitate? What are their locations? Explain.

5. Energetics of β-Ketoacyl-ACP Synthase In the condensation reaction catalyzed by β-ketoacyl-ACP synthase (see Fig. 21–5), a four-carbon unit is synthesized by the combination of a two-carbon unit and a three-carbon unit, with the release of CO_2. What is the thermodynamic advantage of this process over one that simply combines two two-carbon units?

6. Modulation of Acetyl-CoA Carboxylase Acetyl-CoA carboxylase is the principal regulation point in the biosynthesis of fatty acids. Some of the properties of the enzyme are described below.

(a) Addition of citrate or isocitrate raises the V_{max} of the enzyme as much as 10-fold.

(b) The enzyme exists in two interconvertible forms that differ markedly in their activities:

$$\text{Protomer (inactive)} \rightleftharpoons \text{filamentous polymer (active)}$$

Citrate and isocitrate bind preferentially to the filamentous form, and palmitoyl-CoA binds preferentially to the protomer.

Explain how these properties are consistent with the regulatory role of acetyl-CoA carboxylase in the biosynthesis of fatty acids.

7. Shuttling of Acetyl Groups across the Mitochondrial Inner Membrane The acetyl group of acetyl-CoA, produced by the oxidative decarboxylation of pyruvate in the

mitochondrion, is transferred to the cytosol by the acetyl group shuttle outlined in Figure 21-10.

(a) Write the overall equation for the transfer of one acetyl group from the mitochondrion to the cytosol.

(b) What is the cost of this process in ATPs per acetyl group?

(c) In Chapter 17 we encountered an acyl group shuttle in the transfer of fatty acyl–CoA from the cytosol to the mitochondrion in preparation for β oxidation (see Fig. 17–6). One result of that shuttle was separation of the mitochondrial and cytosolic pools of CoA. Does the acetyl group shuttle also accomplish this? Explain.

8. Oxygen Requirement for Desaturases The biosynthesis of palmitoleate (see Fig. 21–12), a common unsaturated fatty acid with a cis double bond in the Δ^9 position, uses palmitate as a precursor. Can this be carried out under strictly anaerobic conditions? Explain.

9. Energy Cost of Triacylglycerol Synthesis Use a net equation for the biosynthesis of tripalmitoylglycerol (tripalmitin) from glycerol and palmitate to show how many ATPs are required per molecule of tripalmitin formed.

10. Turnover of Triacylglycerols in Adipose Tissue When [^{14}C]glucose is added to the balanced diet of adult rats, there is no increase in the total amount of stored triacylglycerols, but the triacylglycerols become labeled with ^{14}C. Explain.

11. Energy Cost of Phosphatidylcholine Synthesis Write the sequence of steps and the net reaction for the biosynthesis of phosphatidylcholine by the salvage pathway from oleate, palmitate, dihydroxyacetone phosphate, and choline. Starting from these precursors, what is the cost (in number of ATPs) of the synthesis of phosphatidylcholine by the salvage pathway?

12. Salvage Pathway for Synthesis of Phosphatidylcholine A young rat maintained on a diet deficient in methionine fails to thrive unless choline is included in the diet. Explain.

13. Synthesis of Isopentenyl Pyrophosphate If 2-[^{14}C]acetyl-CoA is added to a rat liver homogenate that is synthesizing cholesterol, where will the ^{14}C label appear in Δ^3-isopentenyl pyrophosphate, the activated form of an isoprene unit?

14. Activated Donors in Lipid Synthesis In the biosynthesis of complex lipids, components are assembled by transfer of the appropriate group from an activated donor. For example, the activated donor of acetyl groups is acetyl-CoA. For each of the following groups, give the form of the activated donor: (a) phosphate; (b) D-glucosyl; (c) phosphoethanolamine; (d) D-galactosyl; (e) fatty acyl; (f) methyl; (g) the two-carbon group in fatty acid biosynthesis; (h) Δ^3-isopentenyl.

15. Importance of Fats in the Diet When young rats are placed on a totally fat-free diet, they grow poorly, develop a scaly dermatitis, lose hair, and soon die—symptoms that can be prevented if linoleate or plant material is included in the diet. What makes linoleate an essential fatty acid? Why can plant material be substituted?

16. Regulation of Cholesterol Biosynthesis Cholesterol in humans can be obtained from the diet or synthesized de novo. An adult human on a low-cholesterol diet typically synthesizes 600 mg of cholesterol per day in the liver. If the amount of cholesterol in the diet is large, de novo synthesis of cholesterol is drastically reduced. How is this regulation brought about?