22

> Time passes rapidly when you are having fun. The thrill of seeing people get well who might otherwise have died of disease . . . cannot be described in words. The Nobel Prize was only the icing on the cake.
>
> —*Gertrude Elion, quoted in an article in* Science, *2002*

Biosynthesis of Amino Acids, Nucleotides, and Related Molecules

Nitrogen ranks behind only carbon, hydrogen, and oxygen in its contribution to the mass of living systems. Most of this nitrogen is bound up in amino acids and nucleotides. In this chapter we address all aspects of the metabolism of these nitrogen-containing compounds except amino acid catabolism, which is covered in Chapter 18.

Discussing the biosynthetic pathways for amino acids and nucleotides together is a sound approach, not only because both classes of molecules contain nitrogen (which arises from common biological sources) but because the two sets of pathways are extensively intertwined, with several key intermediates in common. Certain amino acids or parts of amino acids are incorporated into the structure of purines and pyrimidines, and in one case part of a purine ring is incorporated into an amino acid (histidine). The two sets of pathways also share much common chemistry, in particular a preponderance of reactions involving the transfer of nitrogen or one-carbon groups.

The pathways described here can be intimidating to the beginning biochemistry student. Their complexity arises not so much from the chemistry itself, which in many cases is well understood, but from the sheer number of steps and variety of intermediates. These pathways are best approached by maintaining a focus on metabolic principles we have already discussed, on key intermediates and precursors, and on common classes of reactions. Even a cursory look at the chemistry can be rewarding, for some of the most unusual chemical transformations in biological systems occur in these pathways; for instance, we find prominent examples of the rare biological use of the metals molybdenum, selenium, and vanadium. The effort also offers a practical dividend, especially for students of human or veterinary medicine. Many genetic diseases of humans and animals have been traced to an absence of one or more enzymes of amino acid and nucleotide metabolism, and many pharmaceuticals in common use to combat infectious diseases are inhibitors of enzymes in these pathways—as are a number of the most important agents in cancer chemotherapy.

Regulation is crucial in the biosynthesis of the nitrogen-containing compounds. Because each amino acid and each nucleotide is required in relatively small amounts, the metabolic flow through most of these pathways is not nearly as great as the biosynthetic flow leading to carbohydrate or fat in animal tissues. Because the different amino acids and nucleotides must be made in the correct ratios and at the right time for protein and nucleic acid synthesis, their biosynthetic pathways must be accurately regulated and coordinated with each other. And because amino acids and nucleotides are charged molecules, their levels must be regulated to maintain electrochemical balance in the cell. As discussed in earlier chapters, pathways can be controlled by changes in either the activity or the amounts of specific enzymes. The pathways we encounter in this chapter provide some of the best-understood examples of the regulation of enzyme activity. Control of the *amounts* of different enzymes in a cell (that is, of their synthesis and degradation) is a topic covered in Chapter 28.

22.1 Overview of Nitrogen Metabolism

The biosynthetic pathways leading to amino acids and nucleotides share a requirement for nitrogen. Because soluble, biologically useful nitrogen compounds are generally scarce in natural environments, most organisms maintain strict economy in their use of ammonia, amino acids, and nucleotides. Indeed, as we shall see, free amino acids, purines, and pyrimidines formed during metabolic turnover of proteins and nucleic acids are often salvaged and reused. We first examine the pathways by which nitrogen from the environment is introduced into biological systems.

The Nitrogen Cycle Maintains a Pool of Biologically Available Nitrogen

The most important source of nitrogen is air, which is four-fifths molecular nitrogen (N_2). However, relatively few species can convert atmospheric nitrogen into forms useful to living organisms. In the biosphere, the metabolic processes of different species function interdependently to salvage and reuse biologically available nitrogen in a vast **nitrogen cycle** **(Fig. 22–1)**. The first step in the cycle is **fixation** (reduction) of atmospheric nitrogen by nitrogen-fixing bacteria to yield ammonia (NH_3 or NH_4^+). Although ammonia can be used by most living organisms, soil bacteria that derive their energy by oxidizing ammonia to nitrite (NO_2^-) and ultimately nitrate (NO_3^-) are so abundant and active that nearly all ammonia reaching the soil is oxidized to nitrate. This process is known as **nitrification**. Plants and many bacteria can take up and readily reduce nitrate and nitrite through the action of nitrate and nitrite reductases. The ammonia so formed is incorporated into amino acids by plants. Animals then use plants as a source of amino acids, both nonessential and essential, to build their proteins. When organisms die, microbial degradation of their proteins returns ammonia to the soil, where nitrifying bacteria again convert it to nitrite and nitrate. A balance is maintained between fixed nitrogen and atmospheric nitrogen by bacteria that convert nitrate to N_2 under anaerobic conditions, a process called **denitrification** (Fig. 22–1). These soil bacteria use NO_3^- rather than O_2 as the ultimate electron acceptor in a series of reactions that (like oxidative phosphorylation) generates a transmembrane proton gradient, which is used to synthesize ATP.

The nitrogen cycle is short-circuited by a recently discovered group of bacteria that promote anaerobic ammonia oxidation, or **anammox** (Fig. 22–1), a process that converts ammonia and nitrite to N_2. As much as 50% to 70% of the NH_3-to-N_2 conversion in the biosphere may occur through this pathway, undetected until the 1980s. The obligate anaerobes that promote anammox are fascinating in their own right and are providing some useful solutions to waste-treatment problems (Box 22–1).

Now let's examine the process of nitrogen fixation, the first step in the nitrogen cycle.

Nitrogen Is Fixed by Enzymes of the Nitrogenase Complex

Only certain bacteria and archaea can fix atmospheric nitrogen. These include the cyanobacteria of soils and fresh and salt waters, methanogenic archaea (strict anaerobes that obtain energy and carbon by converting H_2 and CO_2 to methane), other kinds of free-living soil bacteria such as *Azotobacter* species, and the nitrogen-fixing bacteria that live as **symbionts** in the root nodules of leguminous plants. The first important product of nitrogen fixation is ammonia, which can be used by all organisms either directly or after its conversion to other soluble compounds such as nitrites, nitrates, or amino acids.

The reduction of nitrogen to ammonia is an exergonic reaction:

$$N_2 + 3H_2 \longrightarrow 2NH_3 \qquad \Delta G'^\circ = -33.5 \text{ kJ/mol}$$

The N≡N triple bond, however, is very stable, with a bond energy of 930 kJ/mol. Nitrogen fixation therefore has an extremely high activation energy, and atmospheric nitrogen is almost chemically inert under normal conditions. Ammonia is produced industrially by the Haber process (named for its inventor, Fritz Haber),

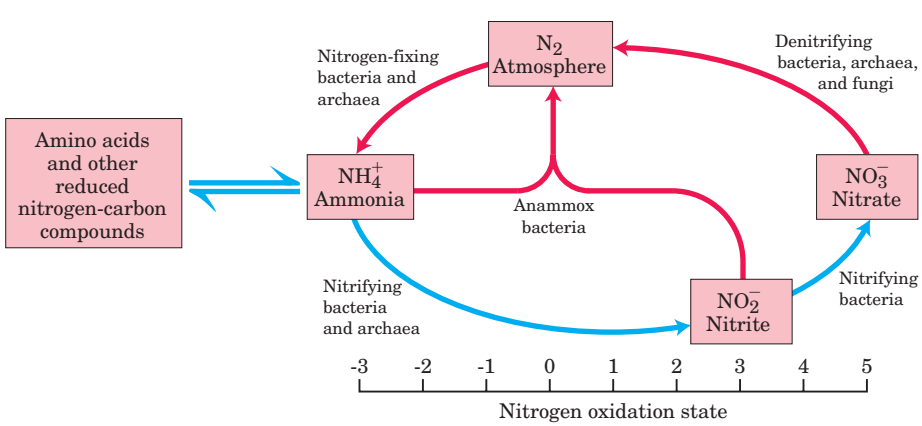


FIGURE 22–1 The nitrogen cycle. The total amount of nitrogen fixed annually in the biosphere exceeds 10^{11} kg. Reactions with red arrows occur largely or entirely in anaerobic environments. The redox states of the various nitrogen species are depicted at the bottom of the figure.

BOX 22–1 Unusual Lifestyles of the Obscure but Abundant

Air-breathers that we are, we can easily overlook the bacteria and archaea that thrive in anaerobic environments. Although rarely featured in introductory biochemistry textbooks, these organisms constitute much of the biomass of this planet, and their contributions to the balance of carbon and nitrogen in the biosphere are essential to all forms of life.

As detailed in earlier chapters, the energy used to maintain living systems relies on the generation of proton gradients across membranes. Electrons derived from a reduced substrate are made available to electron carriers in membranes and pass through a series of electron transfers to a final electron acceptor. As a byproduct of this process, protons are released on one side of the membrane, generating the transmembrane proton gradient. The proton gradient is used to synthesize ATP or to drive other energy-requiring processes. For all eukaryotes, the reduced substrate is generally a carbohydrate (glucose or pyruvate) and the electron acceptor is oxygen.

Many bacteria and archaea are much more versatile. In anaerobic environments such as marine and freshwater sediments, the variety of life strategies is extraordinary. Almost any available redox pair can be an energy source for some specialized organism or group of organisms. For example, a large number of lithotrophic bacteria (a lithotroph is a chemotroph that uses inorganic energy sources; see Fig. 1–5) have a hydrogenase that uses molecular hydrogen to reduce NAD^+:

$$H_2 + NAD^+ \xrightarrow{\text{hydrogenase}} NADH + H^+$$

The NADH is a source of electrons for a variety of membrane-bound electron acceptors, generating the proton gradient needed for ATP synthesis. Other lithotrophs oxidize sulfur compounds (H_2S, elemental sulfur, or thiosulfate) or ferrous iron. A widespread group of archaea called methanogens, all strict anaerobes, extract energy from the reduction of CO_2 to methane. And this is just a small sampling of what anaerobic organisms do for a living. Their metabolic pathways are replete with interesting reactions and highly specialized cofactors unknown in our own world of obligate aerobic metabolism. Study of these organisms can yield practical dividends. It can also provide clues about the origins of life on an early earth, in an atmosphere that lacked molecular oxygen.

The nitrogen cycle depends on a wide range of specialized bacteria. There are two groups of nitrifying bacteria: those that oxidize ammonia to nitrites and those that oxidize the resulting nitrites to nitrates (see Fig. 22–1). Nitrate is second only to O_2 as a biological electron acceptor, and a great many bacteria and archaea can catalyze the denitrification of nitrates to nitrogen, which the nitrogen-fixing bacteria then convert back into ammonia. Ammonia is a major pollutant in sewage and in farm animal waste, and is a byproduct of fertilizer manufacture and oil refining. Waste-treatment plants have generally made use of communities of nitrifying and denitrifying bacteria to convert ammonia waste to atmospheric nitrogen. The process is expensive, requiring inputs of organic carbon and oxygen.

In the 1960s and 1970s, a few articles appeared in the research literature suggesting that ammonia could be oxidized to nitrogen anaerobically, using nitrite as an electron acceptor; this process was called anammox. The reports received little notice until bacteria promoting anammox were discovered in a waste-treatment system in Delft, the Netherlands, in the mid-1980s. A team of Dutch microbiologists led by Gijs Kuenen and Mike Jetten began to study these bacteria, which were soon identified as belonging to an unusual bacterial phylum, Planctomycetes. Some surprises were to follow.

The biochemistry underlying the anammox process was slowly unraveled (Fig. 1). Hydrazine (N_2H_4), a highly reactive molecule used as a rocket fuel, was an unexpected intermediate. As a small molecule, hydrazine is both highly toxic and difficult to contain. It readily diffuses across typical phospholipid membranes. The

(*continued on next page*)

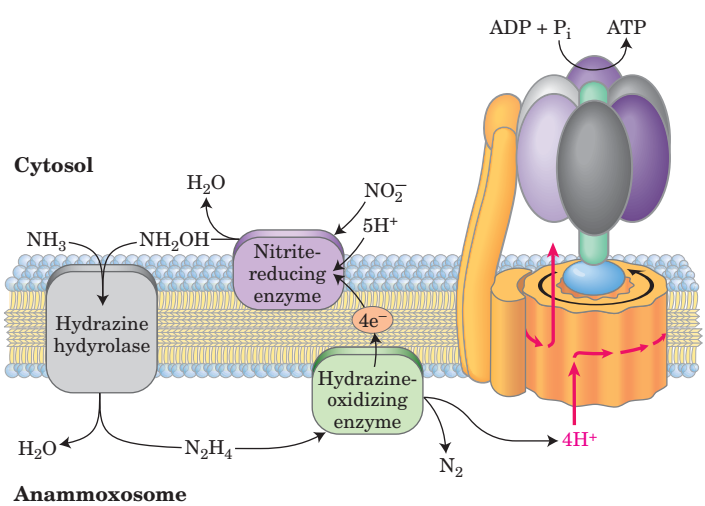


FIGURE 1 The anammox reactions. Ammonia and ammonium hydroxide are converted to hydrazine and H_2O by hydrazine hydrolase, and the hydrazine is oxidized by hydrazine-oxidizing enzyme, generating N_2 and protons. The protons generate a proton gradient for ATP synthesis. On the anammoxosome exterior, protons are used by the nitrite-reducing enzyme, producing ammonium oxide and completing the cycle. All of the anammox enzymes are embedded in the anammoxosome membrane.

BOX 22–1 Unusual Lifestyles of the Obscure but Abundant (*continued from previous page*)

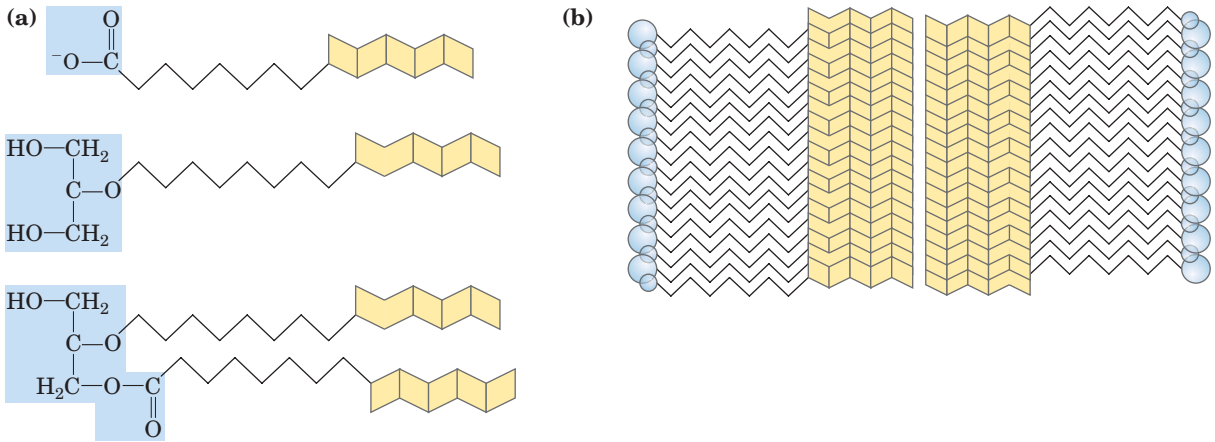


FIGURE 2 **(a)** Ladderane lipids of the anammoxosome membrane. The mechanism for synthesis of the unstable fused cyclobutane ring structures is unknown. **(b)** Ladderanes can stack to form a very dense, impermeable, hydrophobic membrane structure, allowing sequestration of the hydrazine produced in the anammox reactions.

anammox bacteria solve this problem by sequestering hydrazine in a specialized organelle, dubbed the **anammoxosome**. The membrane of this organelle is composed of lipids known as **ladderanes** (Fig. 2), never before encountered in biology. The fused cyclobutane rings of ladderanes stack tightly to form a very dense barrier, greatly slowing the release of hydrazine. Cyclobutane rings are strained and difficult to synthesize; the bacterial mechanisms for synthesizing these lipids are not yet known.

The anammoxosome was a surprising finding. Bacterial cells generally do not have compartments, and the lack of a membrane-enclosed nucleus is often cited as the primary distinction between eukaryotes and bacteria. One type of organelle in a bacterium was interesting enough, but planctomycetes also have a nucleus: their chromosomal DNA is contained within a membrane (Fig. 3). Discovery of this subcellular organization has prompted further research to trace the origin of the planctomycetes and the evolution of eukaryotic nuclei. Planctomycetes are an ancient bacterial line with multiple genera, three of which are known to carry out the anammox reactions. Further study of this group may ultimately bring us closer to a key goal of evolutionary biology: a description of the organism affectionately referred to as LUCA—the Last Universal Common Ancestor of all life on our planet.

For now, the anammox bacteria offer a major advance in waste treatment, reducing the cost of ammonia removal by as much as 90% (the conventional denitrification steps are eliminated completely, and the aeration costs associated with nitrification are lower) and reducing the release of polluting byproducts. Clearly, a greater familiarity with the bacterial underpinnings of the biosphere can pay big dividends as we deal with the environmental challenges of the twenty-first century.

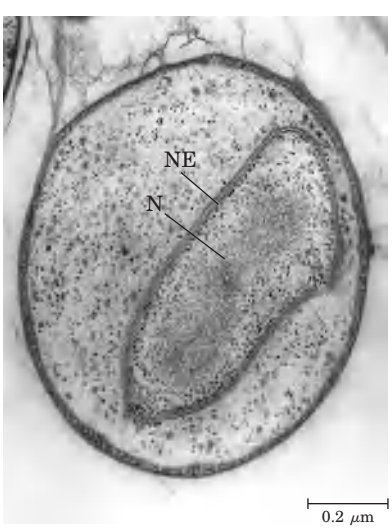


FIGURE 3 Transmission electron micrograph of a cross section through *Gemmata obscuriglobus*, showing the DNA in a nucleus (N) with enclosing nuclear envelope (NE). Bacteria of the *Gemmata* genus (phylum Planctomycetes) do not promote the anammox reactions.

which requires temperatures of 400 to 500 °C and nitrogen and hydrogen at pressures of tens of thousands of kilopascals (several hundred atmospheres) to provide the necessary activation energy. Biological nitrogen fixation, however, must occur at biological temperatures and at 0.8 atm of nitrogen, and the high activation barrier is overcome by other means. This is accomplished, at least in part, by the binding and hydrolysis of ATP. The overall reaction can be written

$$N_2 + 10H^+ + 8e^- + 16ATP \longrightarrow 2NH_4^+ + 16ADP + 16P_i + H_2$$

Biological nitrogen fixation is carried out by a highly conserved complex of proteins called the **nitrogenase complex** (Fig. 22–2), the crucial components

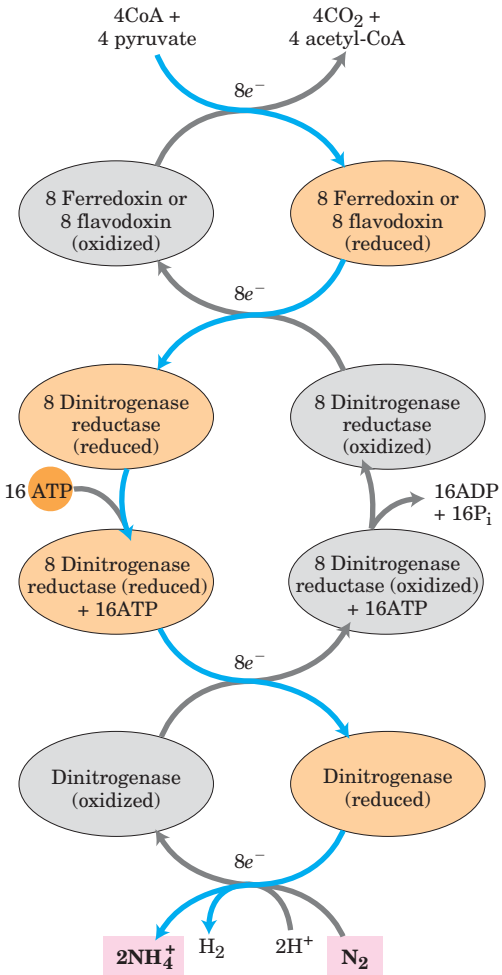


FIGURE 22–2 Nitrogen fixation by the nitrogenase complex. Electrons are transferred from pyruvate to dinitrogenase via ferredoxin (or flavodoxin) and dinitrogenase reductase. Dinitrogenase reductase reduces dinitrogenase one electron at a time, with at least six electrons required to fix one molecule of N_2. An additional two electrons are used to reduce 2 H^+ to H_2 in a process that obligatorily accompanies nitrogen fixation in anaerobes, making a total of eight electrons required per N_2 molecule. The subunit structures and metal cofactors of the dinitrogenase reductase and dinitrogenase proteins are described in the text and in Figure 22–3.

of which are **dinitrogenase reductase** and **dinitrogenase** **(Fig. 22–3)**. Dinitrogenase reductase (M_r 60,000) is a dimer of two identical subunits. It contains a single 4Fe-4S redox center (see Fig. 19–5), bound between the subunits, and can be oxidized and reduced by one electron. It also has two binding sites for ATP/ADP (one site on each subunit). Dinitrogenase (M_r 240,000), a tetramer with two copies of two different subunits, contains both iron and molybdenum; its redox centers have a total of 2 Mo, 32 Fe, and 30 S per tetramer. About half of the iron and sulfur is present as two bridged pairs of 4Fe-4S centers called P clusters; the remainder is present as part of a novel iron-molybdenum cofactor. A form of nitrogenase that contains vanadium rather than molybdenum has been discovered, and some bacterial species can produce both types of nitrogenase system. The vanadium-containing enzyme may be the primary nitrogen-fixing system under some environmental conditions, but it is

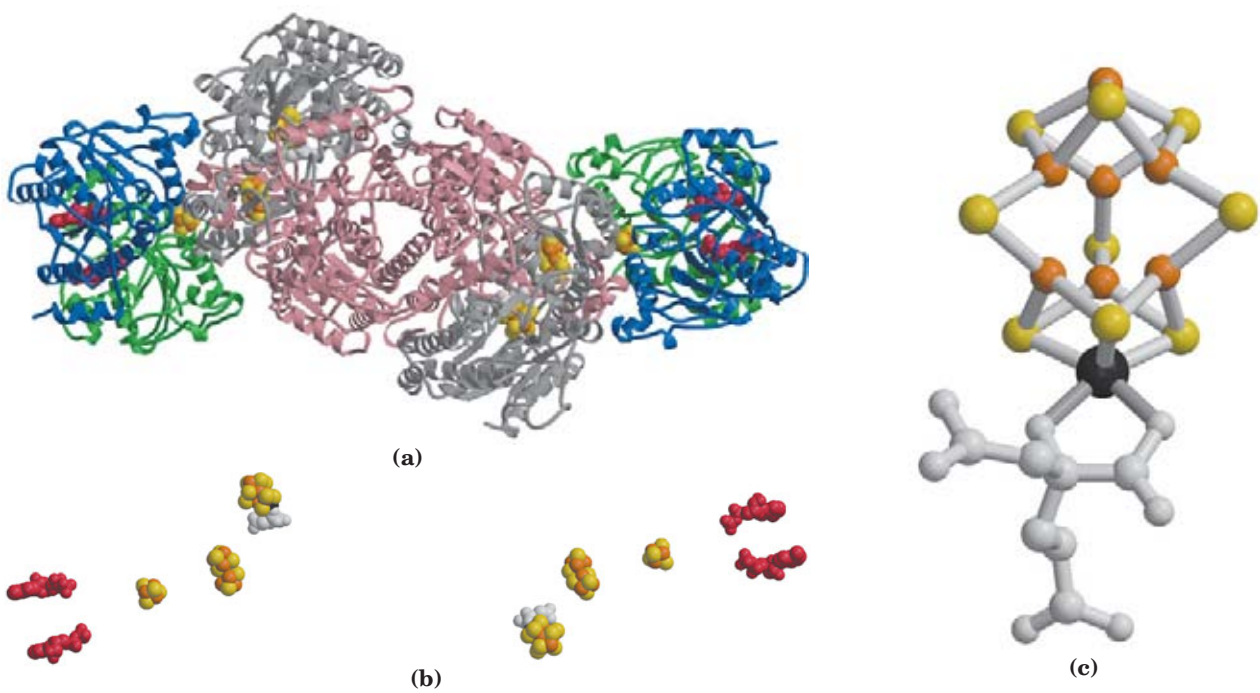


FIGURE 22–3 Enzymes and cofactors of the nitrogenase complex. (PDB ID 1N2C) **(a)** In this ribbon diagram, the dinitrogenase subunits are shown in gray and pink, the dinitrogenase reductase subunits in blue and green. The bound ADP is red. Note the 4Fe-4S complex (Fe atoms orange, S atoms yellow) and the iron-molybdenum cofactor (Mo black, homocitrate light gray). The P clusters (bridged pairs of 4Fe-4S complexes) are also shown. **(b)** The dinitrogenase complex cofactors without the protein (colors as in **(a)**). **(c)** The iron-molybdenum cofactor contains 1 Mo (black), 7 Fe (orange), 9 S (yellow), and one molecule of homocitrate (gray).

not yet as well characterized as the molybdenum-dependent enzyme.

Nitrogen fixation is carried out by a highly reduced form of dinitrogenase and requires eight electrons: six for the reduction of N_2 and two to produce one molecule of H_2 as an obligate part of the reaction mechanism. Dinitrogenase is reduced by the transfer of electrons from dinitrogenase reductase (Fig. 22–2). The dinitrogenase tetramer has two binding sites for the reductase. The required eight electrons are transferred from reductase to dinitrogenase one at a time: a reduced reductase molecule binds to the dinitrogenase and transfers a single electron, then the oxidized reductase dissociates from dinitrogenase, in a repeating cycle. Each turn of the cycle requires the hydrolysis of two ATP molecules by the dimeric reductase. The immediate source of electrons to reduce dinitrogenase reductase varies, with reduced **ferredoxin** (p. 753; see also Fig. 19–5), reduced flavodoxin, and perhaps other sources playing a role. In at least one species, the ultimate source of electrons to reduce ferredoxin is pyruvate (Fig. 22–2).

The role of ATP in this process is somewhat unusual. As you will recall, ATP can contribute not only chemical energy, through the hydrolysis of one or more of its phosphoanhydride bonds, but also binding energy (pp. 189, 297), through noncovalent interactions that lower the activation energy. In the reaction carried out by dinitrogenase reductase, both ATP binding and ATP hydrolysis bring about protein conformational changes that help overcome the high activation energy of nitrogen fixation. The binding of two ATP molecules to the reductase shifts the reduction potential ($E'°$) of this protein from −300 to −420 mV, an enhancement of its reducing power that is required to transfer electrons to dinitrogenase. The ATP molecules are then hydrolyzed just before the actual transfer of one electron to dinitrogenase.

Another important characteristic of the nitrogenase complex is its extreme lability in the presence of oxygen. The reductase is inactivated in air, with a half-life of 30 seconds; dinitrogenase has a half-life of 10 minutes in air. Free-living bacteria that fix nitrogen cope with this problem in a variety of ways. Some live only anaerobically or repress nitrogenase synthesis when oxygen is present. Some aerobic species, such as *Azotobacter vinelandii*, partially uncouple electron transfer from ATP synthesis so that oxygen is burned off as rapidly as it enters the cell (see Box 19–1). When fixing nitrogen, cultures of these bacteria actually increase in temperature as a result of their efforts to rid themselves of oxygen.

The symbiotic relationship between leguminous plants and the nitrogen-fixing bacteria in their root nodules **(Fig. 22–4)** takes care of both the energy requirements and the oxygen lability of the nitrogenase complex. The energy required for nitrogen fixation was probably the evolutionary driving force for this plant-bacteria association. The bacteria in root nodules have access to a large reservoir of energy in the form of abundant carbohydrate and citric acid cycle intermediates made available by the plant. This may allow the bacteria to fix hundreds of times more nitrogen than do their free-living cousins under conditions generally encountered in soils. To solve the oxygen-toxicity problem, the bacteria in root nodules are bathed in a solution of the oxygen-binding heme protein **leghemoglobin**, produced by the plant (although the heme may be contributed by the bacteria). Leghemoglobin binds all available oxygen so that it cannot interfere with nitrogen fixation, and efficiently delivers the oxygen to the bacterial electron-transfer system. The benefit to the plant, of course, is a ready supply of reduced nitrogen. The efficiency of the symbiosis between plants and bacteria is evident in the enrichment of soil

FIGURE 22–4 Nitrogen-fixing nodules. (a) Root nodules of bird's-foot trefoil, a legume. The flower of this common plant is shown in the inset. **(b)** Artificially colorized electron micrograph of a thin section through a pea root nodule. Symbiotic nitrogen-fixing bacteria, or bacteroids (red), live inside the nodule cell, surrounded by the peribacteroid membrane (blue). Bacteroids produce the nitrogenase complex that converts atmospheric nitrogen (N_2) to ammonium (NH_4^+); without the bacteroids, the plant is unable to utilize N_2. The infected root cell provides some factors essential for nitrogen fixation, including leghemoglobin; this heme protein has a very high binding affinity for oxygen, which strongly inhibits nitrogenase. (The cell nucleus is shown in yellow/green. Not visible in this micrograph are other organelles of the infected root cell that are normally found in plant cells.)

nitrogen brought about by leguminous plants. This enrichment is the basis of crop rotation methods, in which plantings of nonleguminous plants (such as maize) that extract fixed nitrogen from the soil are alternated every few years with plantings of legumes such as alfalfa, peas, or clover.

Nitrogen fixation is the subject of intense study, because of its immense practical importance. Industrial production of ammonia for use in fertilizers requires a large and expensive input of energy, and this has spurred a drive to develop recombinant or transgenic organisms that can fix nitrogen. Recombinant DNA techniques (Chapter 9) are being used to transfer the DNA that encodes the enzymes of nitrogen fixation into non-nitrogen-fixing bacteria and plants. Success in these efforts will depend on overcoming the problem of oxygen toxicity in any cell that produces nitrogenase.

Ammonia Is Incorporated into Biomolecules through Glutamate and Glutamine

Reduced nitrogen in the form of NH_4^+ is assimilated into amino acids and then into other nitrogen-containing biomolecules. Two amino acids, **glutamate** and **glutamine**, provide the critical entry point. Recall that these same two amino acids play central roles in the catabolism of ammonia and amino groups in amino acid oxidation (Chapter 18). Glutamate is the source of amino groups for most other amino acids, through transamination reactions (the reverse of the reaction shown in Fig. 18–4). The amide nitrogen of glutamine is a source of amino groups in a wide range of biosynthetic processes. In most types of cells, and in extracellular fluids in higher organisms, one or both of these amino acids are present at higher concentrations—sometimes an order of magnitude or more higher—than other amino acids. An *Escherichia coli* cell requires so much glutamate that this amino acid is one of the primary solutes in the cytosol. Its concentration is regulated not only in response to the cell's nitrogen requirements but also to maintain an osmotic balance between the cytosol and the external medium.

The biosynthetic pathways to glutamate and glutamine are simple, and all or some of the steps occur in most organisms. The most important pathway for the assimilation of NH_4^+ into glutamate requires two reactions. First, **glutamine synthetase** catalyzes the reaction of glutamate and NH_4^+ to yield glutamine. This reaction takes place in two steps, with enzyme-bound γ-glutamyl phosphate as an intermediate (see Fig. 18–8):

(1) Glutamate + ATP $\longrightarrow$ γ-glutamyl phosphate + ADP

(2) γ-Glutamyl phosphate + NH_4^+ $\longrightarrow$ glutamine + P_i + H^+

Sum: Glutamate + NH_4^+ + ATP $\longrightarrow$ glutamine + ADP + P_i + H^+ (22–1)

Glutamine synthetase is found in all organisms. In addition to its importance for NH_4^+ assimilation in bacteria, it has a central role in amino acid metabolism in mammals, converting free NH_4^+, which is toxic, to glutamine for transport in the blood (Chapter 18).

In bacteria and plants, glutamate is produced from glutamine in a reaction catalyzed by **glutamate synthase**. α-Ketoglutarate, an intermediate of the citric acid cycle, undergoes reductive amination with glutamine as nitrogen donor:

α-Ketoglutarate + glutamine + NADPH + H^+ $\longrightarrow$ 2 glutamate + $NADP^+$ (22–2)

The net reaction of glutamine synthetase and glutamate synthase (Eqns 22–1 and 22–2) is

α-Ketoglutarate + NH_4^+ + NADPH + ATP $\longrightarrow$ L-glutamate + $NADP^+$ + ADP + P_i

Glutamate synthase is not present in animals, which instead maintain high levels of glutamate by processes such as the transamination of α-ketoglutarate during amino acid catabolism.

Glutamate can also be formed in yet another, albeit minor, pathway: the reaction of α-ketoglutarate and NH_4^+ to form glutamate in one step. This is catalyzed by L-glutamate dehydrogenase, an enzyme present in all organisms. Reducing power is furnished by NADPH:

α-Ketoglutarate + NH_4^+ + NADPH $\longrightarrow$ L-glutamate + $NADP^+$ + H_2O

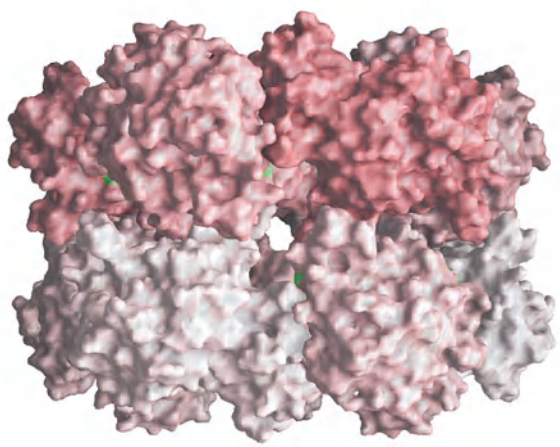
We encountered this reaction in the catabolism of amino acids (see Fig. 18–7). In eukaryotic cells, L-glutamate dehydrogenase is located in the mitochondrial matrix. The reaction equilibrium favors the reactants, and the K_m for NH_4^+ (~1 mM) is so high that the reaction probably makes only a modest contribution to NH_4^+ assimilation into amino acids and other metabolites. (Recall that the glutamate dehydrogenase reaction, in reverse (see Fig. 18–10), is one source of NH_4^+ destined for the urea cycle.) Concentrations of NH_4^+ high enough for the glutamate dehydrogenase reaction to make a significant contribution to glutamate levels generally occur only when NH_3 is added to the soil or when organisms are grown in a laboratory in the presence of high NH_3 concentrations. In general, soil bacteria and plants rely on the two-enzyme pathway outlined above (Eqns 22–1, 22–2).

Glutamine Synthetase Is a Primary Regulatory Point in Nitrogen Metabolism

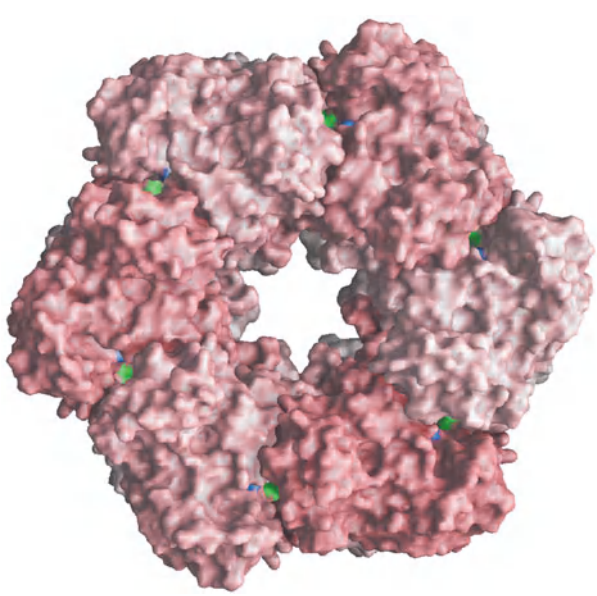
The activity of glutamine synthetase is regulated in virtually all organisms—not surprising, given its central metabolic role as an entry point for reduced nitrogen. In enteric bacteria such as *E. coli*, the regulation is unusually complex. The enzyme has 12 identical subunits of

M_r 50,000 **(Fig. 22–5)** and is regulated both allosterically and by covalent modification. Alanine, glycine, and at least six end products of glutamine metabolism are allosteric inhibitors of the enzyme **(Fig. 22–6)**. Each inhibitor alone produces only partial inhibition, but the effects of multiple inhibitors are more than additive, and all eight together virtually shut down the enzyme. This control mechanism provides a constant adjustment of glutamine levels to match immediate metabolic requirements.

Superimposed on the allosteric regulation is inhibition by adenylylation of (addition of AMP to) Tyr^{397}, located near the enzyme's active site **(Fig. 22–7)**. This covalent modification increases sensitivity to the allosteric inhibitors, and activity decreases as more



(a)



(b)

FIGURE 22–5 Subunit structure of glutamine synthetase as determined by x-ray diffraction. (PDB ID 2GLS) **(a)** Side view. The 12 subunits are identical; they are differently colored to illustrate packing and placement. **(b)** Top view, showing active sites (green).

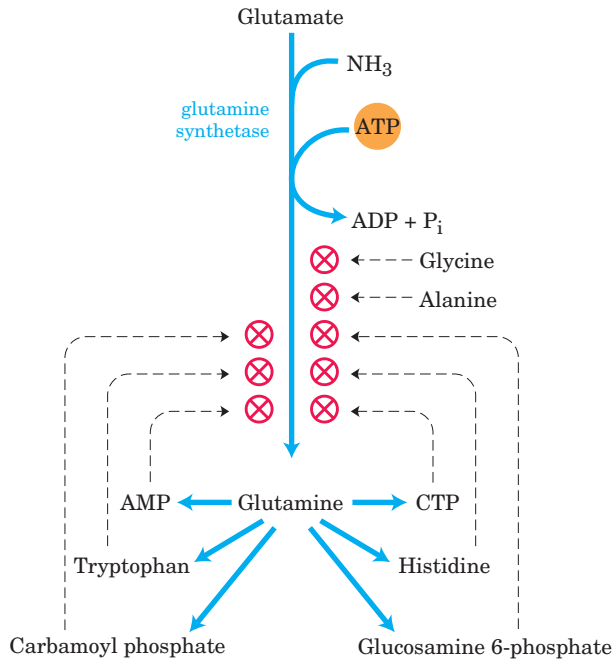


FIGURE 22–6 Allosteric regulation of glutamine synthetase. The enzyme undergoes cumulative regulation by six end products of glutamine metabolism. Alanine and glycine probably serve as indicators of the general status of amino acid metabolism in the cell.

subunits are adenylylated. Both adenylylation and deadenylylation are promoted by **adenylyltransferase** (AT in Fig. 22–7), part of a complex enzymatic cascade that responds to levels of glutamine, α-ketoglutarate, ATP, and P_i. The activity of adenylyltransferase is modulated by binding to a regulatory protein called P_{II}, and the activity of P_{II}, in turn, is regulated by covalent modification (uridylylation), again at a Tyr residue. The adenylyltransferase complex with uridylylated P_{II} (P_{II}-UMP) stimulates deadenylylation, whereas the same complex with deuridylylated P_{II} stimulates adenylylation of glutamine synthetase. Both uridylylation and deuridylylation of P_{II} are brought about by a single enzyme, **uridylyltransferase**. Uridylylation is inhibited by binding of glutamine and P_i to uridylyltransferase and is stimulated by binding of α-ketoglutarate and ATP to P_{II}.

The regulation does not stop there. The uridylylated P_{II} also mediates the activation of transcription of the gene encoding glutamine synthetase, thus increasing the cellular concentration of the enzyme; the deuridylylated P_{II} brings about a decrease in transcription of the same gene. This mechanism involves an interaction of P_{II} with additional proteins involved in gene regulation, of a type described in Chapter 28. The net result of this elaborate system of controls is a decrease in glutamine synthetase activity when glutamine levels are high, and an increase in activity when glutamine levels are low and α-ketoglutarate and ATP (substrates for the synthetase reaction) are available. The multiple layers of regulation

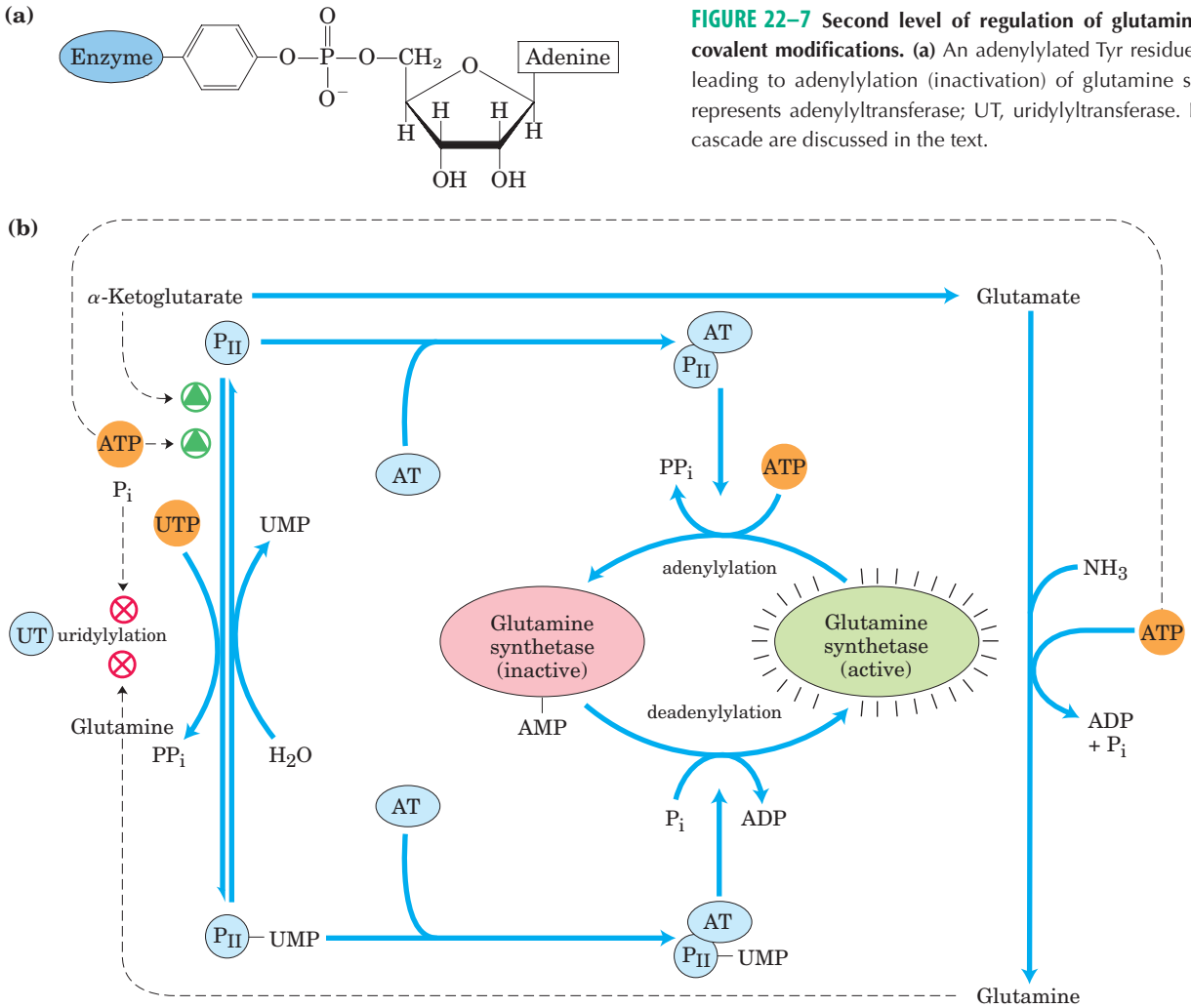


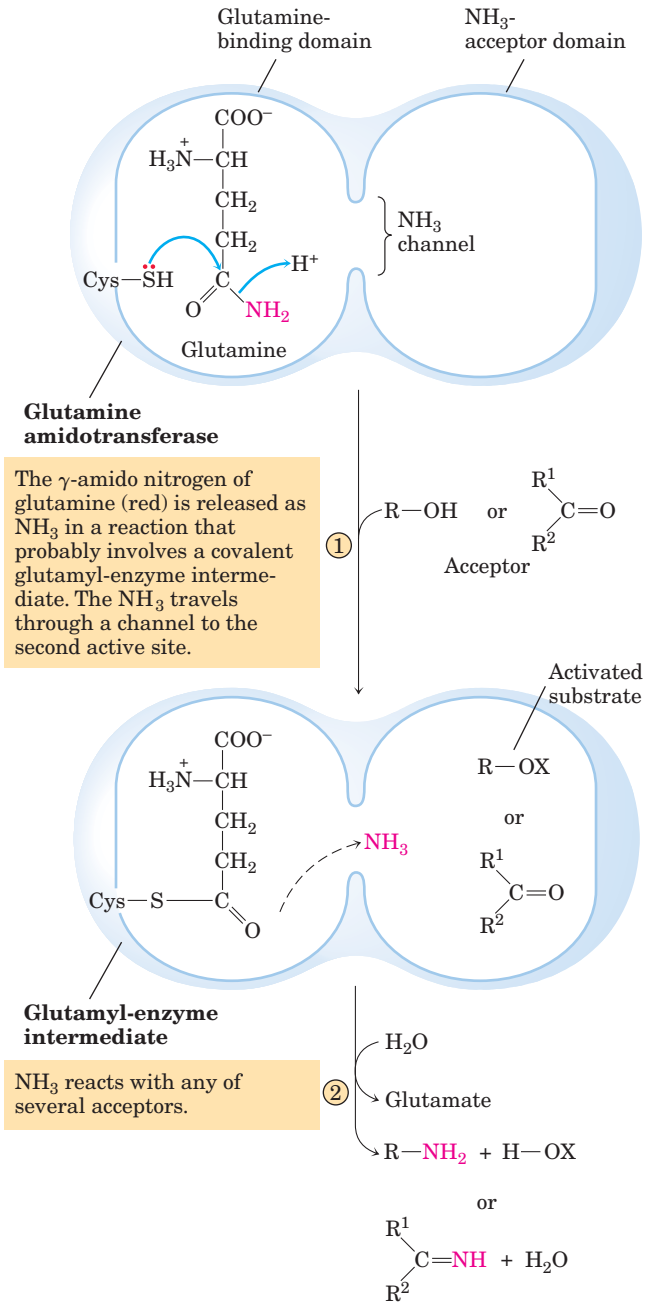
FIGURE 22–7 Second level of regulation of glutamine synthetase: covalent modifications. (a) An adenylylated Tyr residue. **(b)** Cascade leading to adenylylation (inactivation) of glutamine synthetase. AT represents adenylyltransferase; UT, uridylyltransferase. Details of this cascade are discussed in the text.

permit a sensitive response in which glutamine synthesis is tailored to cellular needs.

Several Classes of Reactions Play Special Roles in the Biosynthesis of Amino Acids and Nucleotides

The pathways described in this chapter include a variety of interesting chemical rearrangements. Several of these recur and deserve special note before we progress to the pathways themselves. These are (1) transamination reactions and other rearrangements promoted by enzymes containing pyridoxal phosphate; (2) transfer of one-carbon groups, with either tetrahydrofolate (usually at the —CHO and —CH_2OH oxidation levels) or *S*-adenosylmethionine (at the —CH_3 oxidation level) as cofactor; and (3) transfer of amino groups derived from the amide nitrogen of glutamine. Pyridoxal phosphate (PLP), tetrahydrofolate (H_4 folate), and *S*-adenosylmethionine (adoMet) are described in some detail in Chapter 18 (see Figs 18–6, 18–17, and 18–18). Here we focus on amino group transfer involving the amide nitrogen of glutamine.

More than a dozen known biosynthetic reactions use glutamine as the major physiological source of amino groups, and most of these occur in the pathways outlined in this chapter. As a class, the enzymes catalyzing these reactions are called **glutamine amidotransferases**. All have two structural domains: one binding glutamine, the other binding the second substrate, which serves as amino group acceptor **(Fig. 22–8)**. A conserved Cys residue in the glutamine-binding domain is believed to act as a nucleophile, cleaving the amide bond of glutamine and forming a covalent glutamyl-enzyme intermediate. The NH_3 produced in this reaction is not released, but instead is transferred through an "ammonia channel" to a second active site, where it reacts with the second substrate to form the aminated product. The covalent intermediate is hydrolyzed to the free enzyme and glutamate. If the second substrate must be activated, the usual method is the use of ATP to generate an acyl phosphate intermediate (R—OX in Fig. 22–8, with X as a phosphoryl group). The enzyme glutaminase acts in a similar fashion but uses H_2O as the second substrate, yielding NH_4^+ and glutamate (see Fig. 18–8).



MECHANISM FIGURE 22–8 Proposed mechanism for glutamine amidotransferases. Each enzyme has two domains. The glutamine-binding domain contains structural elements conserved among many of these enzymes, including a Cys residue required for activity. The NH_3-acceptor (second-substrate) domain varies. Two types of amino acceptors are shown. X represents an activating group, typically a phosphoryl group derived from ATP, that facilitates displacement of a hydroxyl group from R—OH by NH_3.

SUMMARY 22.1 Overview of Nitrogen Metabolism

- The molecular nitrogen that makes up 80% of the earth's atmosphere is unavailable to most living organisms until it is reduced. This fixation of atmospheric N_2 takes place in certain free-living bacteria and in symbiotic bacteria in the root nodules of leguminous plants.
- The nitrogen cycle entails formation of ammonia by bacterial fixation of N_2, nitrification of ammonia to nitrate by soil organisms, conversion of nitrate to ammonia by higher plants, synthesis of amino acids from ammonia by all organisms, and conversion of nitrate to N_2 by denitrifying soil bacteria. The anammox bacteria anaerobically oxidize ammonia to nitrogen, using nitrite as an electron acceptor.
- Fixation of N_2 as NH_3 is carried out by the nitrogenase complex, in a reaction that requires ATP. The nitrogenase complex is highly labile in the presence of O_2.
- In living systems, reduced nitrogen is incorporated first into amino acids and then into a variety of other biomolecules, including nucleotides. The key entry point is the amino acid glutamate. Glutamate and glutamine are the nitrogen donors in a wide range of biosynthetic reactions. Glutamine synthetase, which catalyzes the formation of glutamine from glutamate, is a main regulatory enzyme of nitrogen metabolism.
- The amino acid and nucleotide biosynthetic pathways make repeated use of the biological cofactors pyridoxal phosphate, tetrahydrofolate, and *S*-adenosylmethionine. Pyridoxal phosphate is required for transamination reactions involving glutamate and for other amino acid transformations. One-carbon transfers require *S*-adenosylmethionine and tetrahydrofolate. Glutamine amidotransferases catalyze reactions that incorporate nitrogen derived from glutamine.

22.2 Biosynthesis of Amino Acids

All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway **(Fig. 22–9)**. Nitrogen enters these pathways by way of glutamate and glutamine. Some pathways are simple, others are not. Ten of the amino acids are just one or several steps removed from the common metabolite from which they are derived. The biosynthetic pathways for others, such as the aromatic amino acids, are more complex.

Organisms vary greatly in their ability to synthesize the 20 common amino acids. Whereas most bacteria and plants can synthesize all 20, mammals can synthesize only about half of them—generally those with simple pathways. These are the **nonessential amino acids**, not needed in the diet (see Table 18–1). The remainder, the **essential amino acids**, must be obtained from food. Unless otherwise indicated, the pathways for the 20 common amino acids presented below are those operative in bacteria.

A useful way to organize these biosynthetic pathways is to group them into six families corresponding

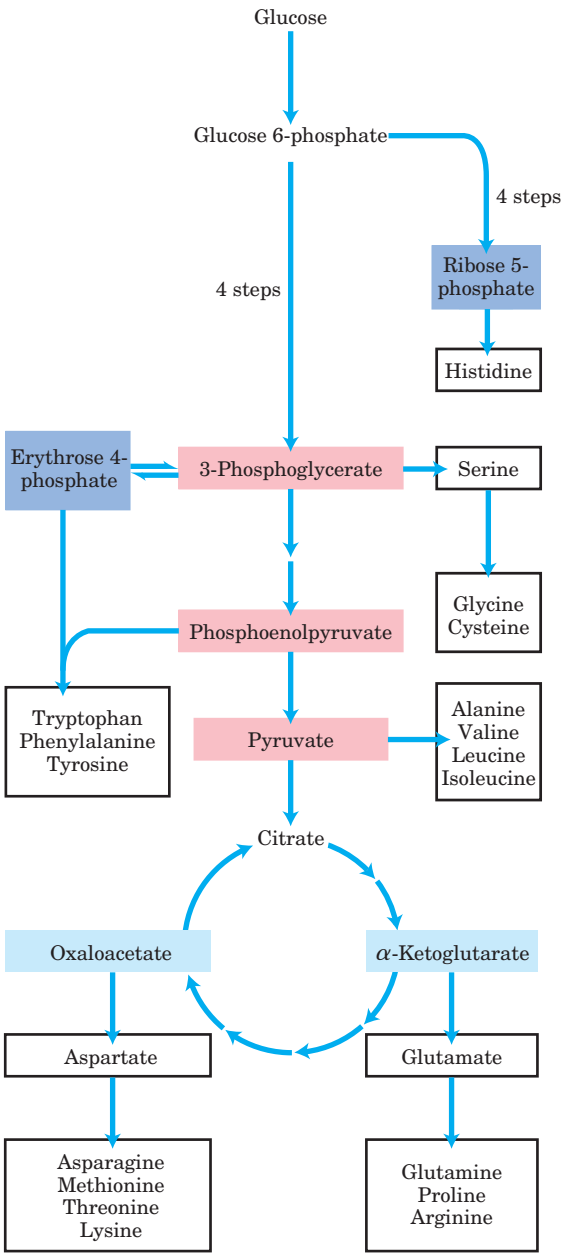


FIGURE 22–9 Overview of amino acid biosynthesis. The carbon skeleton precursors derive from three sources: glycolysis (pink), the citric acid cycle (blue), and the pentose phosphate pathway (purple).

to their metabolic precursors (Table 22–1), and we use this approach to structure the detailed descriptions that follow. In addition to these six precursors, there is a notable intermediate in several pathways of amino acid and nucleotide synthesis: **5-phosphoribosyl-1-pyrophosphate (PRPP)**:

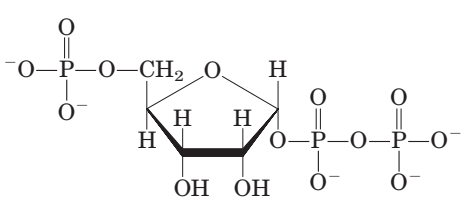


TABLE 22–1 Amino Acid Biosynthetic Families, Grouped by Metabolic Precursor

α-Ketoglutarate	**Pyruvate**
Glutamate	Alanine
Glutamine	Valine*
Proline	Leucine*
Arginine	Isoleucine*
3-Phosphoglycerate	**Phosphoenolpyruvate and erythrose 4-phosphate**
Serine	Tryptophan*
Glycine	Phenylalanine*
Cysteine	Tyrosine†
Oxaloacetate	**Ribose 5-phosphate**
Aspartate	Histidine*
Asparagine	
Methionine*	
Threonine*	
Lysine*	

*Essential amino acids.

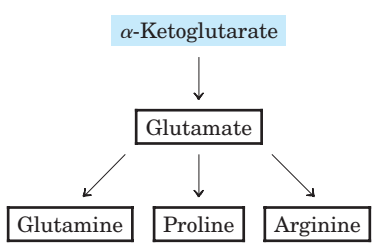
†Derived from phenylalanine in mammals.

PRPP is synthesized from ribose 5-phosphate derived from the pentose phosphate pathway (see Fig. 14–21), in a reaction catalyzed by **ribose phosphate pyrophosphokinase**:

$$\text{Ribose 5-phosphate} + \text{ATP} \longrightarrow \text{5-phosphoribosyl-1-pyrophosphate} + \text{AMP}$$

This enzyme is allosterically regulated by many of the biomolecules for which PRPP is a precursor.

α-Ketoglutarate Gives Rise to Glutamate, Glutamine, Proline, and Arginine



We have already described the biosynthesis of **glutamate** and **glutamine**. **Proline** is a cyclized derivative of glutamate **(Fig. 22–10)**. In the first step of proline synthesis, ATP reacts with the γ-carboxyl group of glutamate to form an acyl phosphate, which is reduced by NADPH or NADH to glutamate γ-semialdehyde. This intermediate undergoes rapid spontaneous cyclization and is then reduced further to yield proline.

Arginine is synthesized from glutamate via ornithine and the urea cycle in animals (Chapter 18). In principle, ornithine could also be synthesized from glutamate γ-semialdehyde by transamination, but the spontaneous cyclization of the semialdehyde in the proline pathway precludes a sufficient supply of this

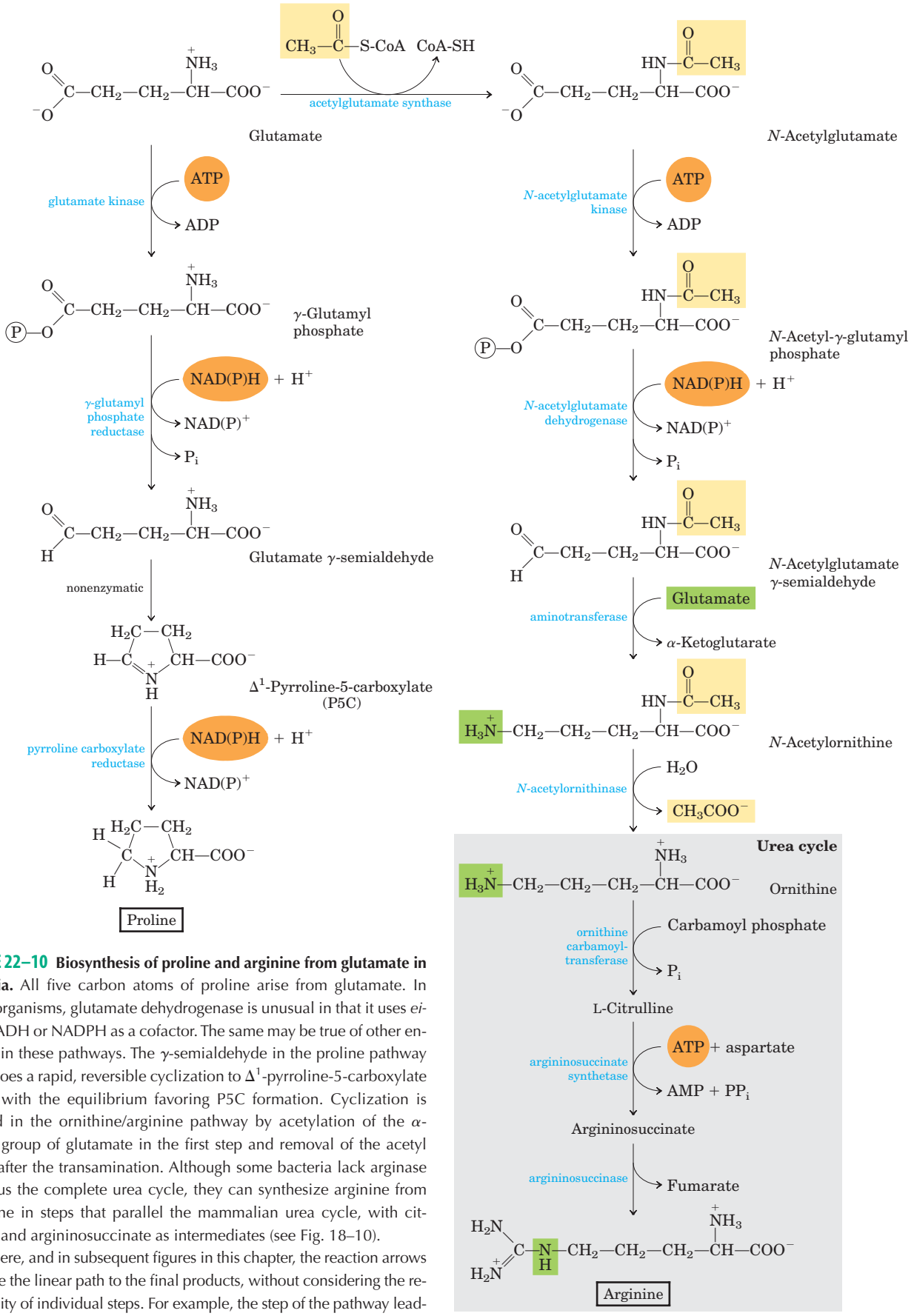


FIGURE 22–10 Biosynthesis of proline and arginine from glutamate in bacteria. All five carbon atoms of proline arise from glutamate. In many organisms, glutamate dehydrogenase is unusual in that it uses *either* NADH or NADPH as a cofactor. The same may be true of other enzymes in these pathways. The γ-semialdehyde in the proline pathway undergoes a rapid, reversible cyclization to Δ^1-pyrroline-5-carboxylate (P5C), with the equilibrium favoring P5C formation. Cyclization is averted in the ornithine/arginine pathway by acetylation of the α-amino group of glutamate in the first step and removal of the acetyl group after the transamination. Although some bacteria lack arginase and thus the complete urea cycle, they can synthesize arginine from ornithine in steps that parallel the mammalian urea cycle, with citrulline and argininosuccinate as intermediates (see Fig. 18–10).

Here, and in subsequent figures in this chapter, the reaction arrows indicate the linear path to the final products, without considering the reversibility of individual steps. For example, the step of the pathway leading to arginine that is catalyzed by *N*-acetylglutamate dehydrogenase is chemically similar to the glyceraldehyde 3-phosphate dehydrogenase reaction in glycolysis (see Fig. 14–7), and is readily reversible.

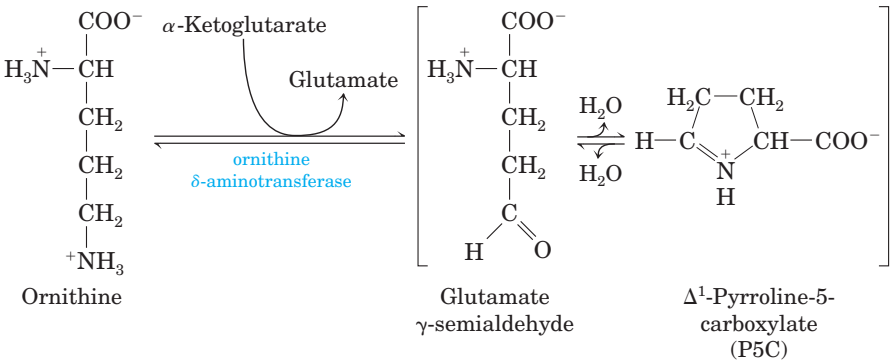
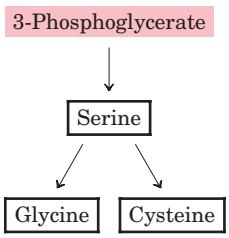


FIGURE 22–11 Ornithine δ-aminotransferase reaction: a step in the mammalian pathway to proline. This enzyme is found in the mitochondrial matrix of most tissues. Although the equilibrium favors P5C formation, the reverse reaction is the only mammalian pathway for synthesis of ornithine (and thus arginine) when arginine levels are insufficient for protein synthesis.

intermediate for ornithine synthesis. Bacteria have a de novo biosynthetic pathway for ornithine (and thus arginine) that parallels some steps of the proline pathway but includes two additional steps that avoid the problem of the spontaneous cyclization of glutamate γ-semialdehyde (Fig. 22–10). In the first step, the α-amino group of glutamate is blocked by an acetylation requiring acetyl-CoA; then, after the transamination step, the acetyl group is removed to yield ornithine.

The pathways to proline and arginine are somewhat different in mammals. Proline can be synthesized by the pathway shown in Figure 22–10, but it is also formed from arginine obtained from dietary or tissue protein. Arginase, a urea cycle enzyme, converts arginine to ornithine and urea (see Figs 18–10, 18–26). The ornithine is converted to glutamate γ-semialdehyde by the enzyme **ornithine δ-aminotransferase (Fig. 22–11)**. The semialdehyde cyclizes to Δ^1-pyrroline-5-carboxylate, which is then converted to proline (Fig. 22–10). The pathway for arginine synthesis shown in Figure 22–10 is absent in mammals. When arginine from dietary intake or protein turnover is insufficient for protein synthesis, the ornithine δ-aminotransferase reaction operates in the direction of ornithine formation. Ornithine is then converted to citrulline and arginine in the urea cycle.

Serine, Glycine, and Cysteine Are Derived from 3-Phosphoglycerate



The major pathway for the formation of **serine** is the same in all organisms **(Fig. 22–12)**. In the first step, the hydroxyl group of 3-phosphoglycerate is oxidized by a dehydrogenase (using NAD^+) to yield 3-phosphohydroxypyruvate. Transamination from glutamate yields 3-phosphoserine, which is hydrolyzed to free serine by phosphoserine phosphatase.

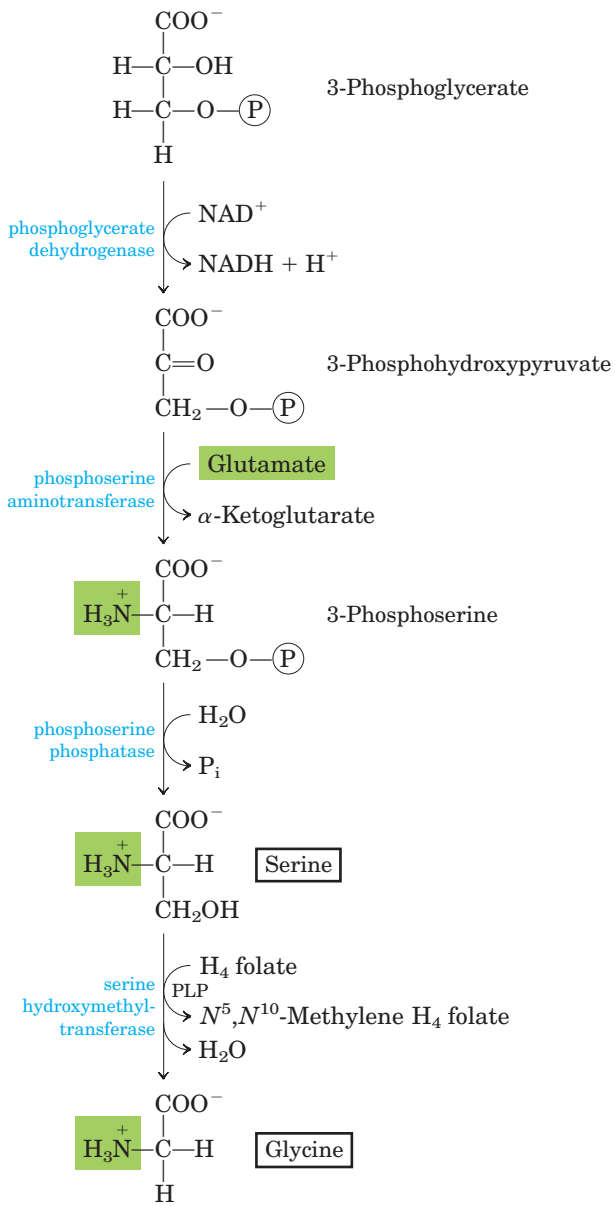


FIGURE 22–12 Biosynthesis of serine from 3-phosphoglycerate and of glycine from serine in all organisms. Glycine is also made from CO_2 and NH_4^+ by the action of glycine synthase, with N^5,N^{10}-methylenetetrahydrofolate as methyl group donor (see text).

Serine (three carbons) is the precursor of **glycine** (two carbons) through removal of a carbon atom by **serine hydroxymethyltransferase** (Fig. 22–12). Tetrahydrofolate accepts the β carbon (C-3) of serine, which forms a methylene bridge between N-5 and N-10 to yield N^5,N^{10}-methylenetetrahydrofolate (see Fig. 18–17). The overall reaction, which is reversible, also requires pyridoxal phosphate. In the liver of vertebrates, glycine can be made by another route: the reverse of the reaction shown in Figure 18–20c, catalyzed by **glycine synthase** (also called **glycine cleavage enzyme**):

$$CO_2 + NH_4^+ + N^5,N^{10}\text{-methylenetetrahydrofolate} + NADH + H^+ \longrightarrow \text{glycine} + \text{tetrahydrofolate} + NAD^+$$

Plants and bacteria produce the reduced sulfur required for the synthesis of **cysteine** (and methionine, described later) from environmental sulfates; the pathway is shown on the right side of **Figure 22–13**. Sulfate is activated in two steps to produce 3′-phosphoadenosine 5′-phosphosulfate (PAPS), which undergoes an eight-electron reduction to sulfide. The sulfide is then used in the formation of cysteine from serine in a two-step pathway. Mammals synthesize cysteine from two amino acids: methionine furnishes the sulfur atom, and serine furnishes the carbon skeleton. Methionine is first converted to *S*-adenosylmethionine (see Fig. 18–18), which can lose its methyl group to any of a number of acceptors to form *S*-adenosylhomocysteine (adoHcy). This demethylated product is hydrolyzed to free homocysteine, which undergoes a reaction with serine, catalyzed by **cystathionine β-synthase**, to yield cystathionine **(Fig. 22–14)**. Finally, **cystathionine γ-lyase**, a PLP-requiring enzyme, catalyzes removal of ammonia and cleavage of cystathionine to yield free cysteine.

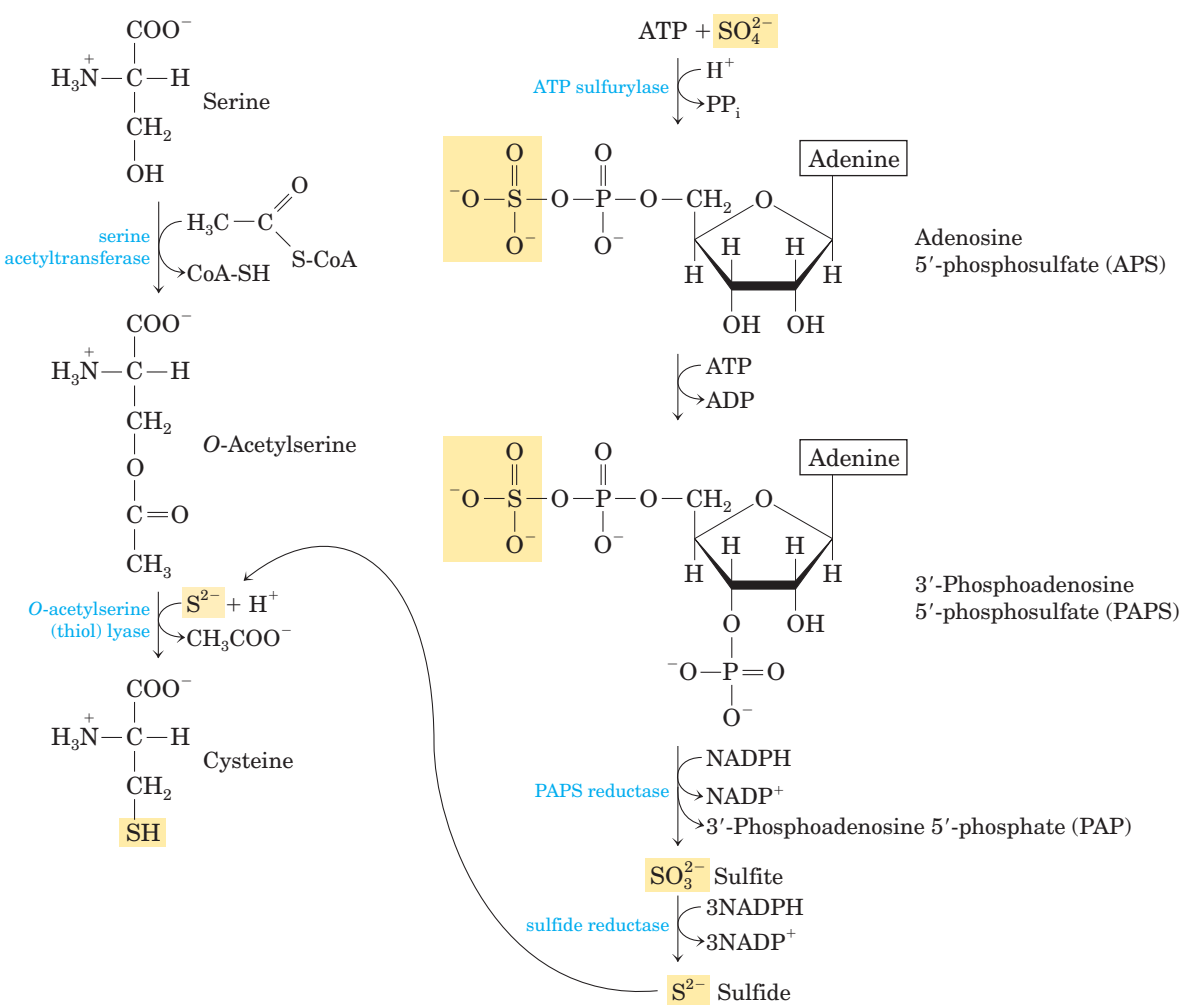


FIGURE 22–13 Biosynthesis of cysteine from serine in bacteria and plants. The origin of reduced sulfur is shown in the pathway on the right.

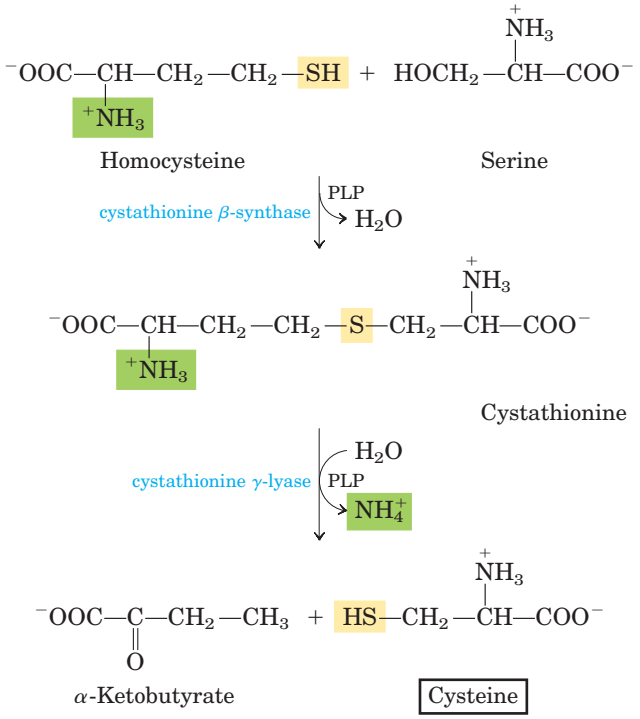
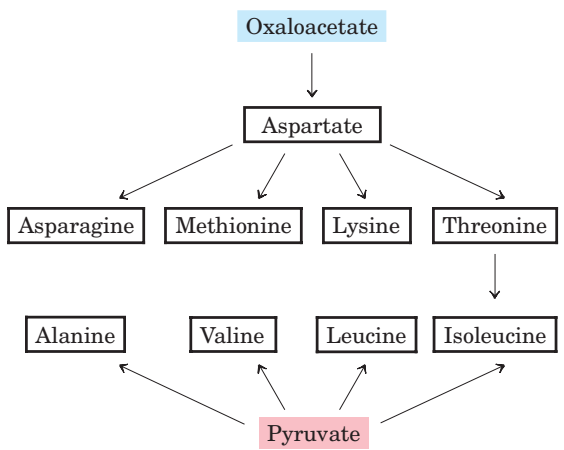


FIGURE 22–14 Biosynthesis of cysteine from homocysteine and serine in mammals. The homocysteine is formed from methionine, as described in the text.

Three Nonessential and Six Essential Amino Acids Are Synthesized from Oxaloacetate and Pyruvate



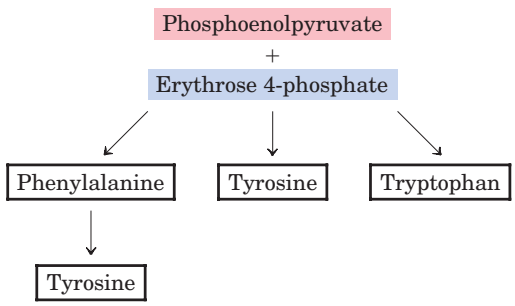
Alanine and **aspartate** are synthesized from pyruvate and oxaloacetate, respectively, by transamination from glutamate. **Asparagine** is synthesized by amidation of aspartate, with glutamine donating the NH_4^+. These are nonessential amino acids, and their simple biosynthetic pathways occur in all organisms.

For reasons incompletely understood, the malignant lymphocytes present in childhood acute lymphoblastic leukemia (ALL) require serum asparagine for growth. The chemotherapy for ALL is administered together with an L-asparaginase derived from bacteria, with the enzyme functioning to reduce serum asparagine. The combined treatment results in a greater than 95% remission rate in cases of childhood ALL (L-asparaginase treatment alone produces remission in 40% to 60% of cases). However, the asparaginase treatment has some deleterious side effects, and about 10% of patients who achieve remission eventually suffer relapse, with tumors resistant to drug therapy. Researchers are now developing inhibitors of human asparagine synthetase to augment these therapies for childhood ALL. ■

Methionine, threonine, lysine, isoleucine, valine, and leucine are essential amino acids. Their biosynthetic pathways are complex and interconnected **(Fig. 22–15)**. In some cases, the pathways in bacteria, fungi, and plants differ significantly. Figure 22–15 shows the bacterial pathways.

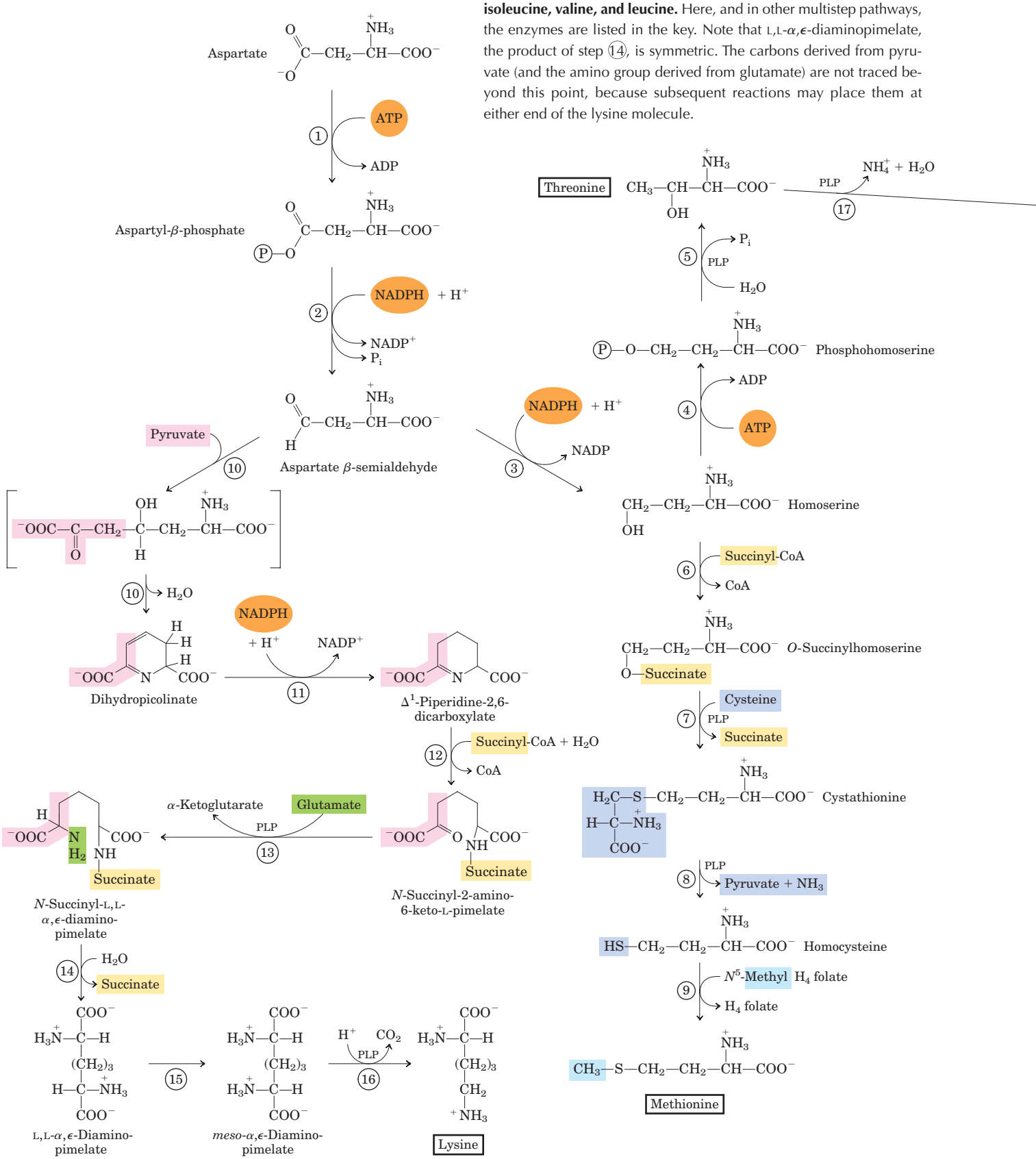
Aspartate gives rise to **methionine**, **threonine**, and **lysine**. Branch points occur at aspartate β-semialdehyde, an intermediate in all three pathways, and at homoserine, a precursor of threonine and methionine. Threonine, in turn, is one of the precursors of isoleucine. The **valine** and **isoleucine** pathways share four enzymes (Fig. 22–15, steps (18) to (21)). Pyruvate gives rise to valine and isoleucine in pathways that begin with condensation of two carbons of pyruvate (in the form of hydroxyethyl thiamine pyrophosphate; see Fig. 14–14) with another molecule of pyruvate (the valine path) or with α-ketobutyrate (the isoleucine path). The α-ketobutyrate is derived from threonine in a reaction that requires pyridoxal phosphate (Fig. 22–15, step (17)). An intermediate in the valine pathway, α-ketoisovalerate, is the starting point for a four-step branch pathway leading to **leucine** (steps (22) to (25)).

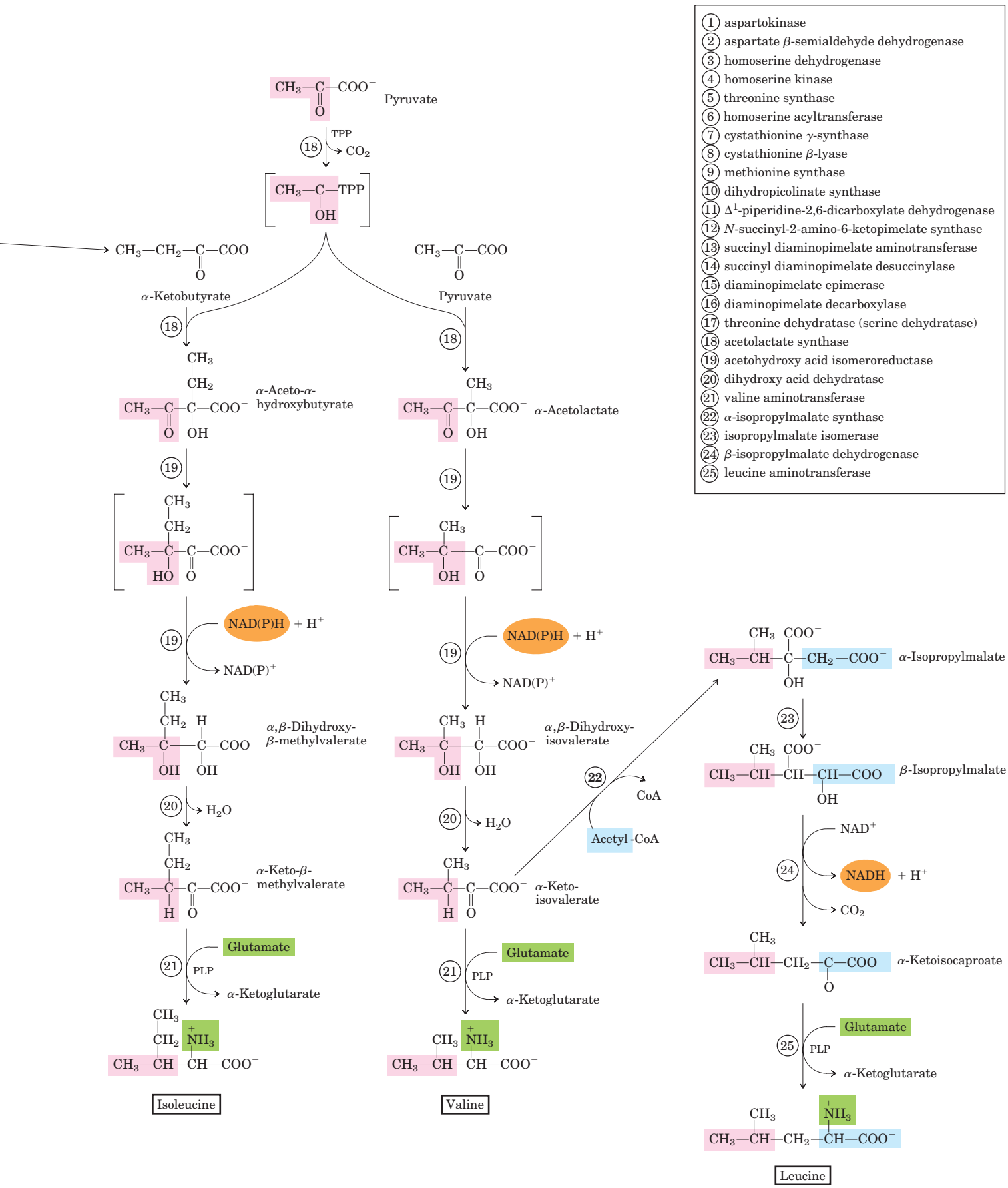
Chorismate Is a Key Intermediate in the Synthesis of Tryptophan, Phenylalanine, and Tyrosine



Aromatic rings are not readily available in the environment, even though the benzene ring is very stable. The branched pathway to tryptophan, phenylalanine, and tyrosine, occurring in bacteria, fungi, and plants, is the main biological route of aromatic ring formation. It proceeds through ring closure of an aliphatic precursor followed by stepwise addition of double bonds. The first

FIGURE 22–15 Biosynthesis of six essential amino acids from oxaloacetate and pyruvate in bacteria: methionine, threonine, lysine, isoleucine, valine, and leucine. Here, and in other multistep pathways, the enzymes are listed in the key. Note that L,L-α,ε-diaminopimelate, the product of step (14), is symmetric. The carbons derived from pyruvate (and the amino group derived from glutamate) are not traced beyond this point, because subsequent reactions may place them at either end of the lysine molecule.




1 aspartokinase
2 aspartate β-semialdehyde dehydrogenase
3 homoserine dehydrogenase
4 homoserine kinase
5 threonine synthase
6 homoserine acyltransferase
7 cystathionine γ-synthase
8 cystathionine β-lyase
9 methionine synthase
10 dihydropicolinate synthase
11 Δ1-piperidine-2,6-dicarboxylate dehydrogenase
12 N-succinyl-2-amino-6-ketopimelate synthase
13 succinyl diaminopimelate aminotransferase
14 succinyl diaminopimelate desuccinylase
15 diaminopimelate epimerase
16 diaminopimelate decarboxylase
17 threonine dehydratase (serine dehydratase)
18 acetolactate synthase
19 acetohydroxy acid isomeroreductase
20 dihydroxy acid dehydratase
21 valine aminotransferase
22 α-isopropylmalate synthase
23 isopropylmalate isomerase
24 β-isopropylmalate dehydrogenase
25 leucine aminotransferase
Pyruvate
TPP
CO2
α-Ketobutyrate
Pyruvate
α-Aceto-α-hydroxybutyrate
α-Acetolactate
NAD(P)H + H+
NAD(P)+
α,β-Dihydroxy-β-methylvalerate
α,β-Dihydroxy-isovalerate
H2O
α-Keto-β-methylvalerate
α-Keto-isovalerate
CoA
Acetyl-CoA
Glutamate
PLP
α-Ketoglutarate
Isoleucine
Valine
α-Isopropylmalate
β-Isopropylmalate
NAD+
NADH + H+
CO2
α-Ketoisocaproate
Leucine

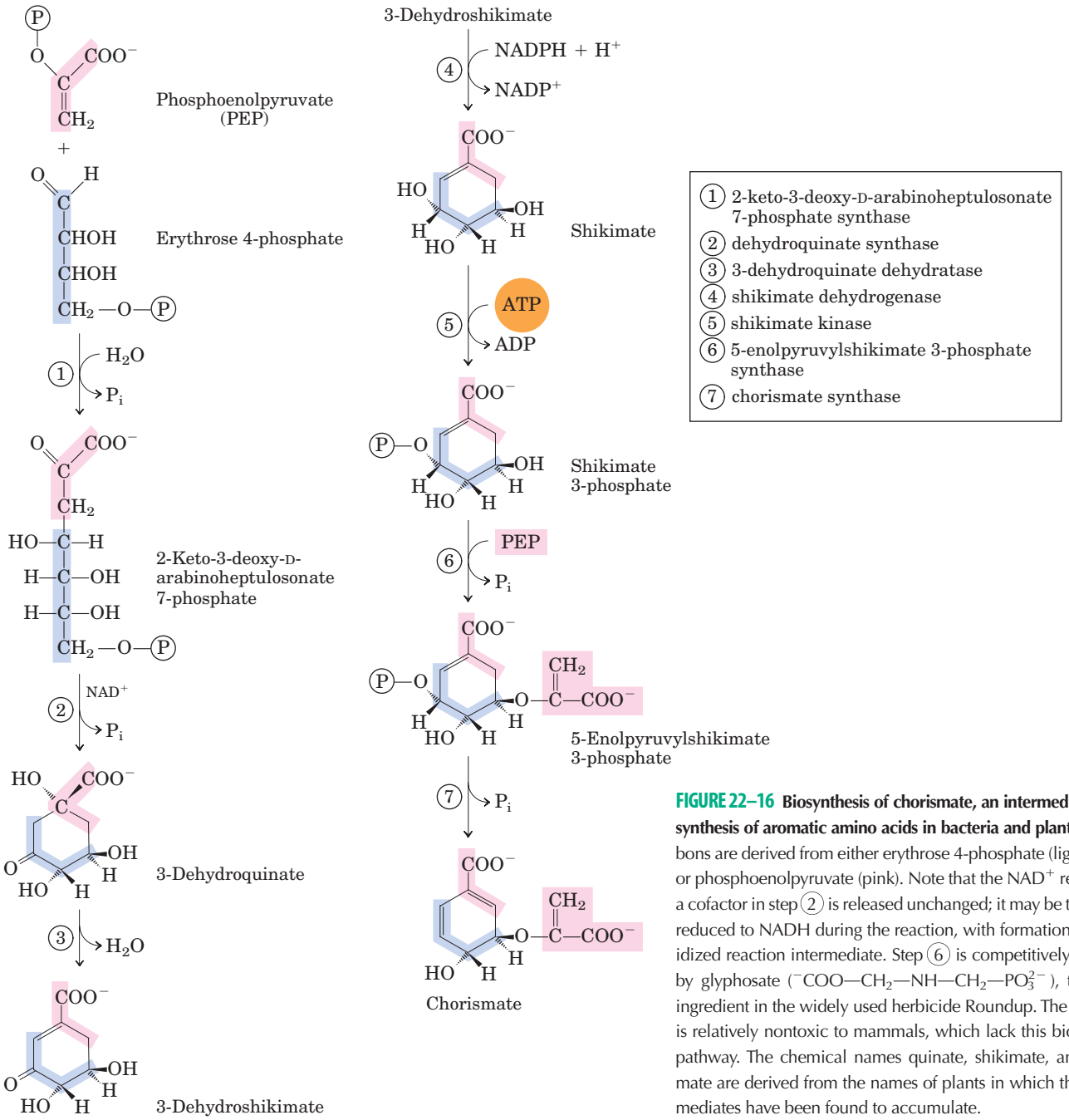


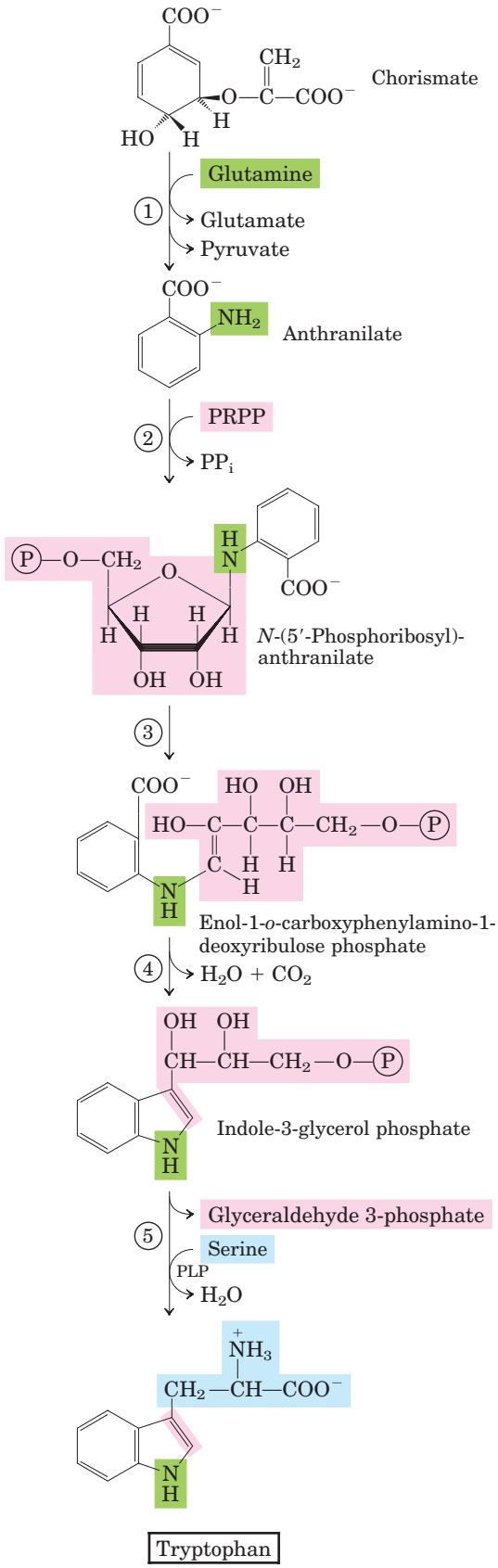
FIGURE 22–16 Biosynthesis of chorismate, an intermediate in the synthesis of aromatic amino acids in bacteria and plants. All carbons are derived from either erythrose 4-phosphate (light purple) or phosphoenolpyruvate (pink). Note that the NAD^+ required as a cofactor in step (2) is released unchanged; it may be transiently reduced to NADH during the reaction, with formation of an oxidized reaction intermediate. Step (6) is competitively inhibited by glyphosate ($^-OOC—CH_2—NH—CH_2—PO_3^{2-}$), the active ingredient in the widely used herbicide Roundup. The herbicide is relatively nontoxic to mammals, which lack this biosynthetic pathway. The chemical names quinate, shikimate, and chorismate are derived from the names of plants in which these intermediates have been found to accumulate.

four steps produce shikimate, a seven-carbon molecule derived from erythrose 4-phosphate and phosphoenolpyruvate **(Fig. 22–16)**. Shikimate is converted to chorismate in three steps that include the addition of three more carbons from another molecule of phosphoenolpyruvate. Chorismate is the first branch point of the pathway, with one branch leading to tryptophan, the other to phenylalanine and tyrosine.

In the **tryptophan** branch **(Fig. 22–17)**, chorismate is converted to anthranilate in a reaction in which glutamine donates the nitrogen that will become part of the indole ring. Anthranilate then condenses with PRPP. The indole ring of tryptophan is derived from the ring carbons and amino group of anthranilate plus two carbons derived from PRPP. The final reaction in the sequence is catalyzed by **tryptophan synthase**. This enzyme has an $\alpha_2\beta_2$ subunit structure and can be dissociated into two α subunits and a β_2 unit that catalyze different parts of the overall reaction:

$$\text{Indole-3-glycerol phosphate} \xrightarrow[\alpha\text{ subunit}]{} \text{indole} + \text{glyceraldehyde 3-phosphate}$$

$$\text{Indole} + \text{serine} \xrightarrow[\beta_2\text{ subunit}]{} \text{tryptophan} + H_2O$$



1. anthranilate synthase
2. anthranilate phosphoribosyltransferase
3. *N*-(5'-phosphoribosyl)-anthranilate isomerase
4. indole-3-glycerol phosphate synthase
5. tryptophan synthase

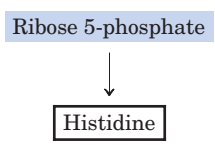
FIGURE 22–17 Biosynthesis of tryptophan from chorismate in bacteria and plants. In *E. coli*, enzymes catalyzing steps 1 and 2 are subunits of a single complex.

The second part of the reaction requires pyridoxal phosphate **(Fig. 22–18)**. Indole formed in the first part is not released by the enzyme, but instead moves through a channel from the α-subunit active site to one of the β-subunit active sites, where it condenses with a Schiff base intermediate derived from serine and PLP. Intermediate channeling of this type may be a feature of the entire pathway from chorismate to tryptophan. Enzyme active sites catalyzing different steps (sometimes not sequential steps) of the pathway to tryptophan are found on single polypeptides in some species of fungi and bacteria, but are separate proteins in other species. In addition, the activity of some of these enzymes requires a noncovalent association with other enzymes of the pathway. These observations suggest that all the pathway enzymes are components of a large, multienzyme complex in both bacteria and eukaryotes. Such complexes are generally not preserved intact when the enzymes are isolated using traditional biochemical methods, but evidence for the existence of multienzyme complexes is accumulating for this and other metabolic pathways (p. 619).

In plants and bacteria, **phenylalanine** and **tyrosine** are synthesized from chorismate in pathways much less complex than the tryptophan pathway. The common intermediate is prephenate **(Fig. 22–19)**. The final step in both cases is transamination with glutamate.

Animals can produce tyrosine directly from phenylalanine through hydroxylation at C-4 of the phenyl group by **phenylalanine hydroxylase**; this enzyme also participates in the degradation of phenylalanine (see Figs 18–23, 18–24). Tyrosine is considered a conditionally essential amino acid, or as nonessential insofar as it can be synthesized from the essential amino acid phenylalanine.

Histidine Biosynthesis Uses Precursors of Purine Biosynthesis



The pathway to **histidine** in all plants and bacteria differs in several respects from other amino acid biosynthetic pathways. Histidine is derived from three precursors **(Fig. 22–20)**: PRPP contributes five carbons,

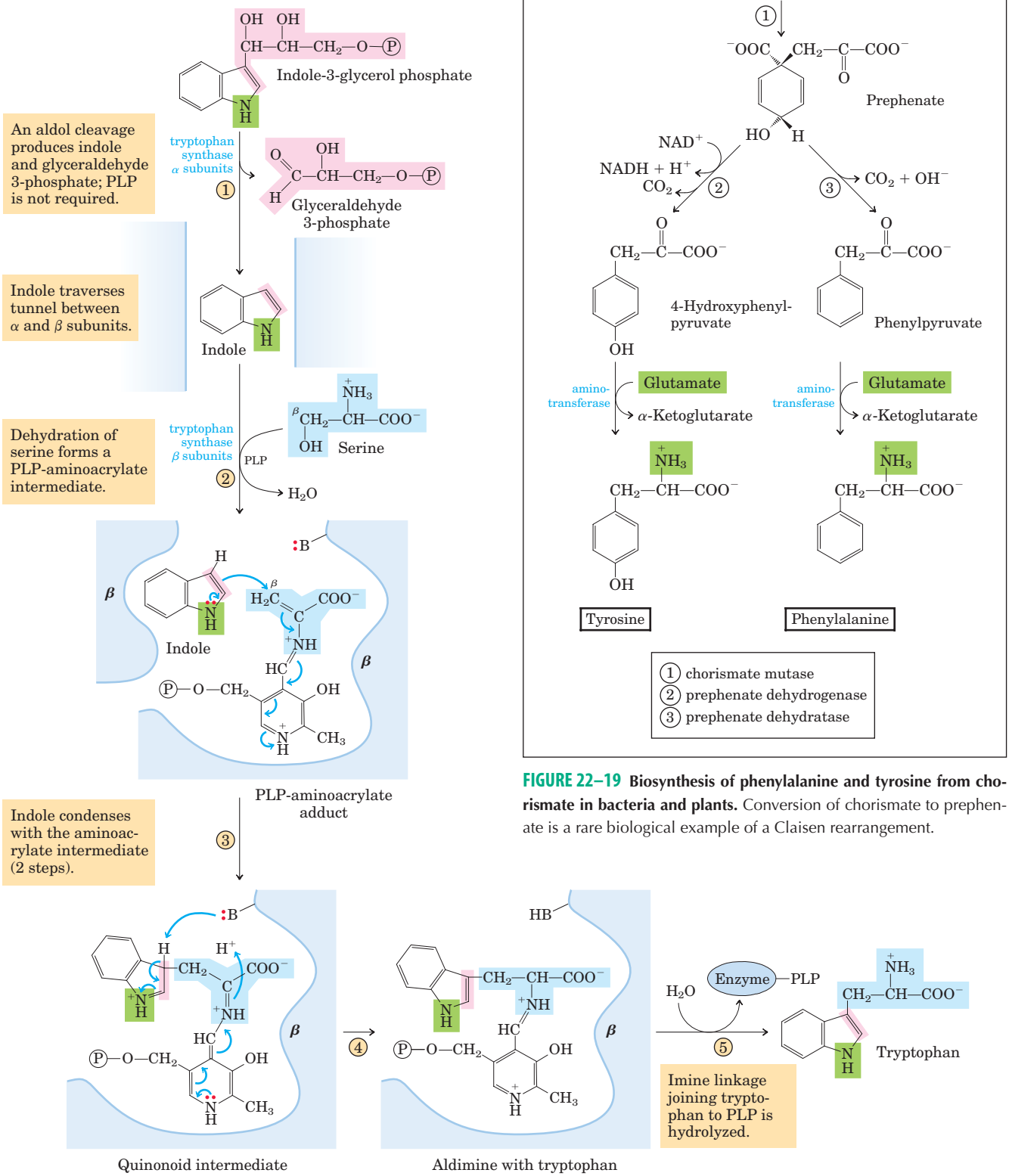


FIGURE 22–19 Biosynthesis of phenylalanine and tyrosine from chorismate in bacteria and plants. Conversion of chorismate to prephenate is a rare biological example of a Claisen rearrangement.

MECHANISM FIGURE 22–18 Tryptophan synthase reaction. This enzyme catalyzes a multistep reaction with several types of chemical rearrangements. The PLP-facilitated transformations occur at the β carbon (C-3) of the amino acid, as opposed to the α-carbon reactions described in Figure 18–6. The β carbon of serine is attached to the indole ring system. **Tryptophan Synthase Mechanism**

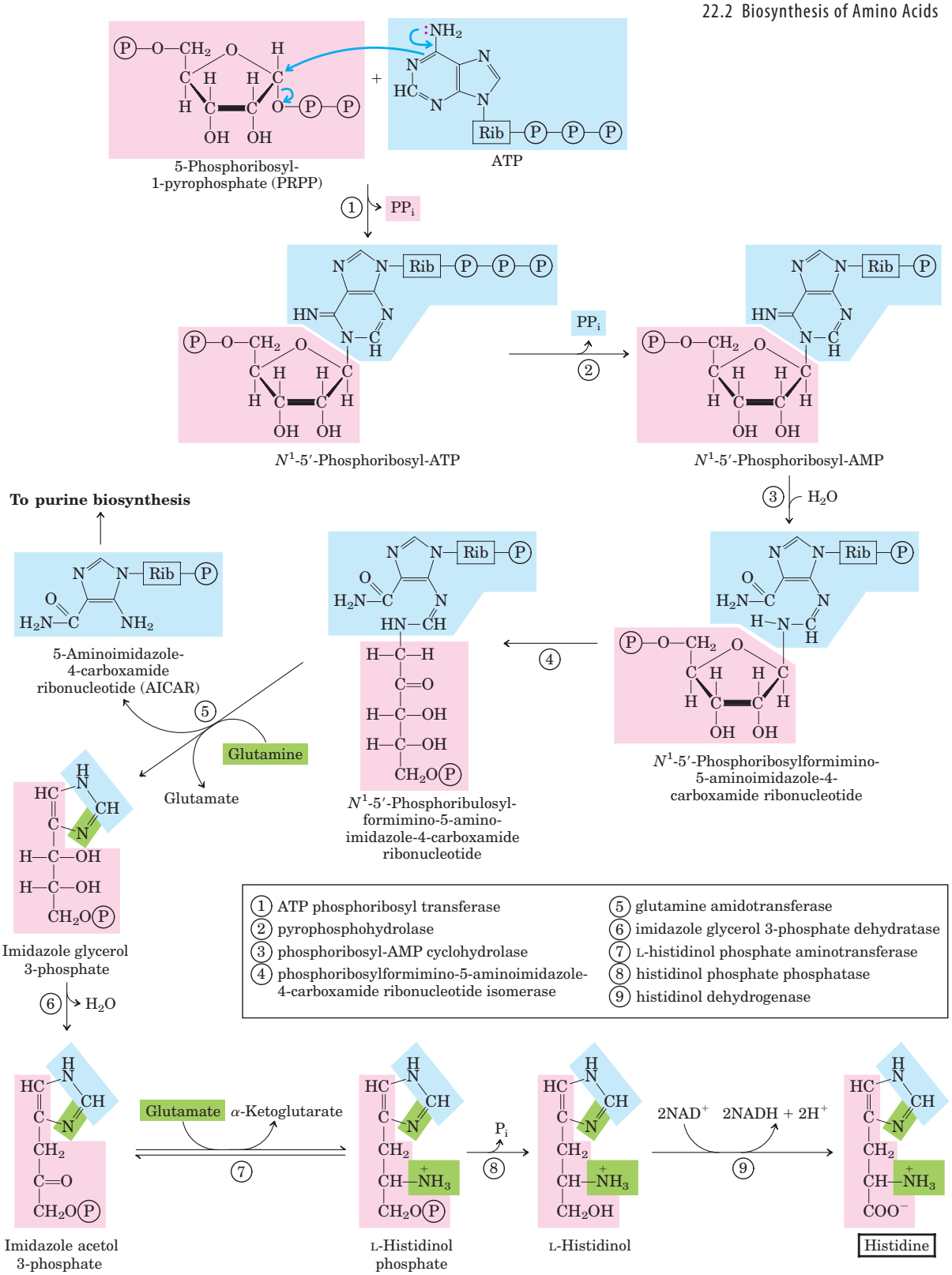


FIGURE 22–20 Biosynthesis of histidine in bacteria and plants. Atoms derived from PRPP and ATP are shaded pink and blue, respectively. Two of the histidine nitrogens are derived from glutamine and glutamate (green). Note that the derivative of ATP remaining after step ⑤ (AICAR) is an intermediate in purine biosynthesis (see Fig. 22–33, step ⑨), so ATP is rapidly regenerated.

the purine ring of ATP contributes a nitrogen and a carbon, and glutamine supplies the second ring nitrogen. The key steps are condensation of ATP and PRPP, in which N-1 of the purine ring is linked to the activated C-1 of the ribose of PRPP (step ① in Fig. 22–20); purine ring opening that ultimately leaves N-1 and C-2 of adenine linked to the ribose (step ③); and formation of the imidazole ring, a reaction in which glutamine donates a nitrogen (step ⑤). The use of ATP as a metabolite rather than a high-energy cofactor is unusual—but not wasteful, because it dovetails with the purine biosynthetic pathway. The remnant of ATP that is released after the

transfer of N-1 and C-2 is 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an intermediate of purine biosynthesis (see Fig. 22–33) that is rapidly recycled to ATP.

Amino Acid Biosynthesis Is under Allosteric Regulation

As detailed in Chapter 15, the control of flux through a metabolic pathway often reflects the activity of multiple enzymes in that pathway. In the case of amino acid synthesis, regulation takes place in part through feedback inhibition of the first reaction by the end product of the pathway. This first reaction is often catalyzed by an allosteric enzyme that plays an important role in the overall control of flux through that pathway. As an example, **Figure 22–21** shows the allosteric regulation of isoleucine synthesis from threonine (detailed in Fig. 22–15). The end product, isoleucine, is an allosteric inhibitor of the first reaction in the sequence. In bacteria, such allosteric modulation of amino acid synthesis contributes to the minute-to-minute adjustment of pathway activity to cellular needs.

Allosteric regulation of an individual enzyme can be considerably more complex. An example is the remarkable set of allosteric controls exerted on glutamine synthetase of *E. coli* (Fig. 22–6). Six products derived from glutamine serve as negative feedback modulators of the enzyme, and the overall effects of these and other modulators are more than additive. Such regulation is called **concerted inhibition**.

Additional mechanisms contribute to the regulation of the amino acid biosynthetic pathways. Because the 20 common amino acids must be made in the correct proportions for protein synthesis, cells have developed ways not only of controlling the rate of synthesis of individual amino acids but also of coordinating their formation. Such coordination is especially well developed in fast-growing bacterial cells. **Figure 22–22** shows how *E. coli* cells coordinate the synthesis of lysine, methionine, threonine, and isoleucine, all made from aspartate. Several important types of inhibition patterns are evident. The step from aspartate to aspartyl-β-phosphate is catalyzed by three isozymes, each independently controlled by different modulators. This **enzyme multiplicity** prevents one biosynthetic end product from shutting down key steps in a pathway when other products of the same pathway are required. The steps from

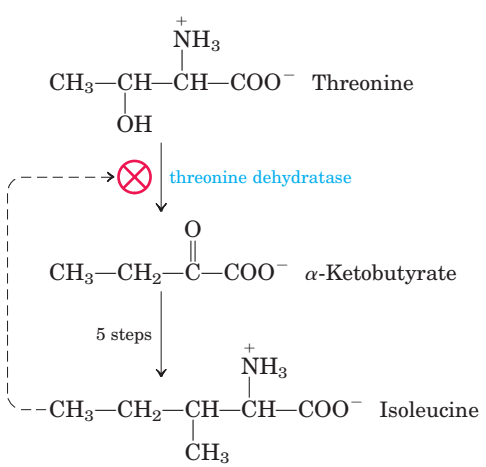


FIGURE 22–21 Allosteric regulation of isoleucine biosynthesis. The first reaction in the pathway from threonine to isoleucine is inhibited by the end product, isoleucine. This was one of the first examples of allosteric feedback inhibition to be discovered. The steps from α-ketobutyrate to isoleucine correspond to steps (18) through (21) in Figure 22–15 (five steps, because (19) is a two-step reaction).

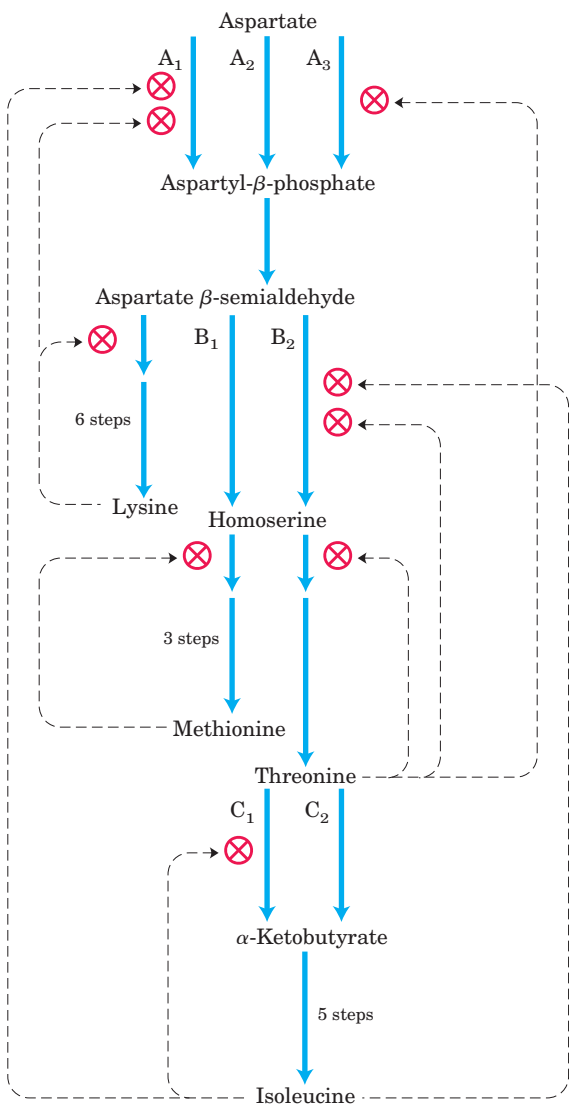


FIGURE 22–22 Interlocking regulatory mechanisms in the biosynthesis of several amino acids derived from aspartate in *E. coli*. Three enzymes (A, B, C) have either two or three isozyme forms, indicated by numerical subscripts. In each case, one isozyme (A_2, B_1, and C_2) has no allosteric regulation; these isozymes are regulated by changes in the amount synthesized (Chapter 28). Synthesis of isozymes A_2 and B_1 is repressed when methionine levels are high, and synthesis of isozyme C_2 is repressed when isoleucine levels are high. Enzyme A is aspartokinase; B, homoserine dehydrogenase; C, threonine dehydratase.

aspartate β-semialdehyde to homoserine and from threonine to α-ketobutyrate (detailed in Fig. 22–15) are also catalyzed by dual, independently controlled isozymes. One isozyme for the conversion of aspartate to aspartyl-β-phosphate is allosterically inhibited by two different modulators, lysine and isoleucine, whose action is more than additive—another example of concerted inhibition. The sequence from aspartate to isoleucine undergoes multiple, overlapping negative feedback inhibitions; for example, isoleucine inhibits the conversion of threonine to α-ketobutyrate (as described above), and threonine inhibits its own formation at three points: from homoserine, from aspartate β-semialdehyde, and from aspartate (steps ④, ③, and ① in Fig. 22–15). This overall regulatory mechanism is called **sequential feedback inhibition**.

Similar patterns are evident in the pathways leading to the aromatic amino acids. The first step of the early pathway to the common intermediate chorismate is catalyzed by the enzyme 2-keto-3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthase (step ① in Fig. 22–16). Most microorganisms and plants have three DAHP synthase isozymes. One is allosterically inhibited (feedback inhibition) by phenylalanine, another by tyrosine, and the third by tryptophan. This scheme helps the overall pathway to respond to cellular requirements for one or more of the aromatic amino acids. Additional regulation takes place after the pathway branches at chorismate. For example, the enzymes catalyzing the first two steps of the tryptophan branch are subject to allosteric inhibition by tryptophan.

SUMMARY 22.2 Biosynthesis of Amino Acids

- Plants and bacteria synthesize all 20 common amino acids. Mammals can synthesize about half; the others are required in the diet (essential amino acids).
- Among the nonessential amino acids, glutamate is formed by reductive amination of α-ketoglutarate and serves as the precursor of glutamine, proline, and arginine. Alanine and aspartate (and thus asparagine) are formed from pyruvate and oxaloacetate, respectively, by transamination. The carbon chain of serine is derived from 3-phosphoglycerate. Serine is a precursor of glycine; the β-carbon atom of serine is transferred to tetrahydrofolate. In microorganisms, cysteine is produced from serine and from sulfide produced by the reduction of environmental sulfate. Mammals produce cysteine from methionine and serine by a series of reactions requiring *S*-adenosylmethionine and cystathionine.
- Among the essential amino acids, the aromatic amino acids (phenylalanine, tyrosine, and tryptophan) form by a pathway in which chorismate occupies a key branch point. Phosphoribosyl pyrophosphate is a precursor of tryptophan and histidine. The pathway to histidine is interconnected with the purine synthetic pathway. Tyrosine can also be formed by hydroxylation of phenylalanine (and thus is considered conditionally essential). The pathways for the other essential amino acids are complex.
- The amino acid biosynthetic pathways are subject to allosteric end-product inhibition; the regulatory enzyme is usually the first in the sequence. Regulation of the various synthetic pathways is coordinated.

22.3 Molecules Derived from Amino Acids

In addition to their role as the building blocks of proteins, amino acids are precursors of many specialized biomolecules, including hormones, coenzymes, nucleotides, alkaloids, cell wall polymers, porphyrins, antibiotics, pigments, and neurotransmitters. We describe here the pathways to a number of these amino acid derivatives.

Glycine Is a Precursor of Porphyrins

The biosynthesis of **porphyrins**, for which glycine is a major precursor, is our first example because of the central importance of the porphyrin nucleus in heme proteins such as hemoglobin and the cytochromes. The porphyrins are constructed from four molecules of the monopyrrole derivative **porphobilinogen**, which itself is derived from two molecules of δ-aminolevulinate. There are two major pathways to δ-aminolevulinate. In higher eukaryotes **(Fig. 22–23a)**, glycine reacts with succinyl-CoA in the first step to yield α-amino-β-ketoadipate, which is then decarboxylated to δ-aminolevulinate. In plants, algae, and most bacteria, δ-aminolevulinate is formed from glutamate (Fig. 22–23b). The glutamate is first esterified to glutamyl-tRNA^{Glu} (see Chapter 27 on the topic of transfer RNAs); reduction by NADPH converts the glutamate to glutamate 1-semialdehyde, which is cleaved from the tRNA. An aminotransferase converts the glutamate 1-semialdehyde to δ-aminolevulinate.

In all organisms, two molecules of δ-aminolevulinate condense to form porphobilinogen and, through a series of complex enzymatic reactions, four molecules of porphobilinogen come together to form **protoporphyrin (Fig. 22–24)**. The iron atom is incorporated after the protoporphyrin has been assembled, in a step catalyzed by ferrochelatase. Porphyrin biosynthesis is regulated in higher eukaryotes by the concentration of the heme product, which serves as a feedback inhibitor of early steps in the synthetic pathway. Genetic defects in the biosynthesis of porphyrins can lead to the accumulation of pathway intermediates, causing a variety of human diseases known collectively as **porphyrias** (Box 22–2).

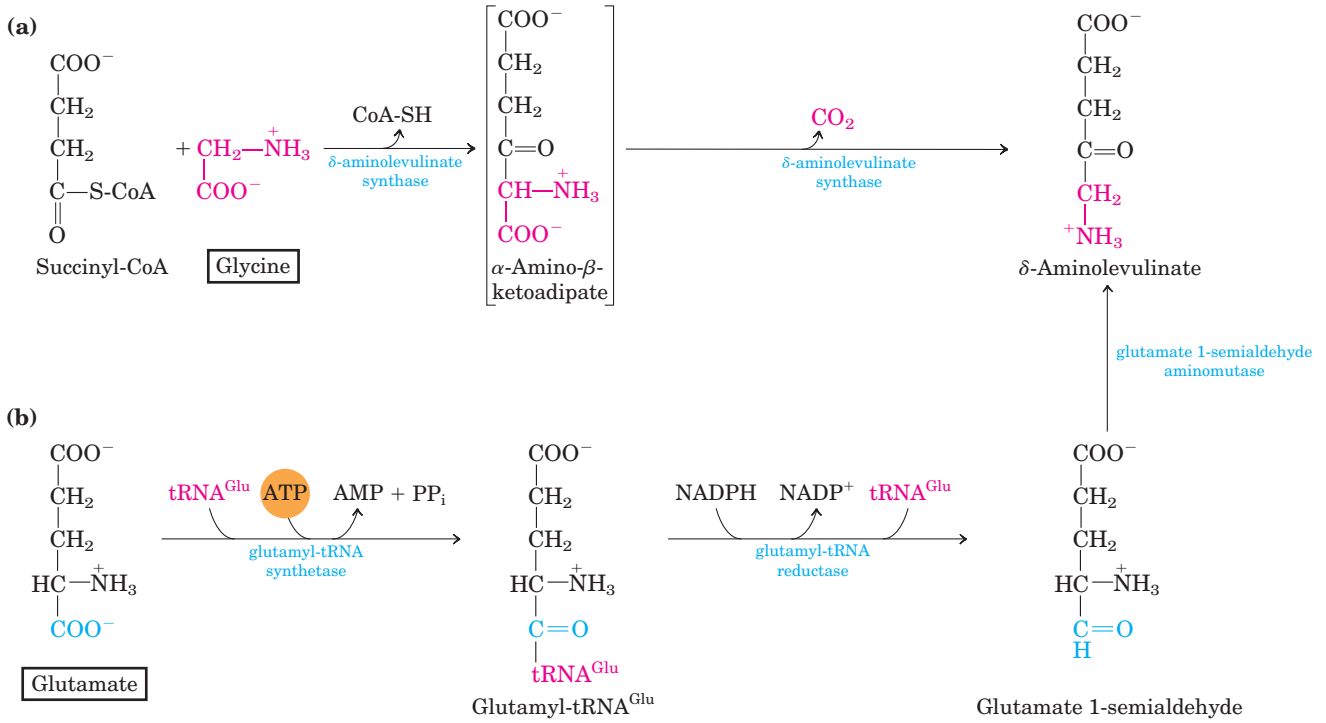


FIGURE 22–23 Biosynthesis of δ-aminolevulinate. (a) In most animals, including mammals, δ-aminolevulinate is synthesized from glycine and succinyl-CoA. The atoms furnished by glycine are shown in red. **(b)** In bacteria and plants, the precursor of δ-aminolevulinate is glutamate.

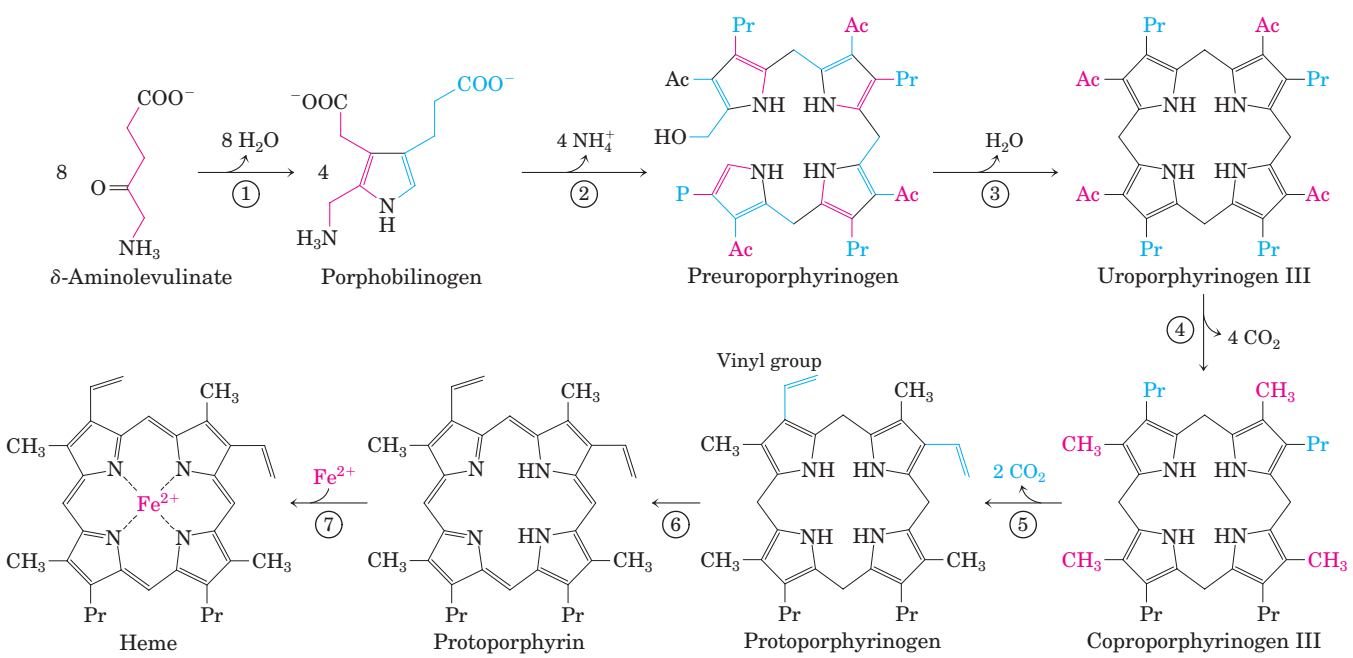


FIGURE 22–24 Biosynthesis of heme from δ-aminolevulinate. Ac represents acetyl (—CH_2COO^-); Pr, propionyl (—$CH_2CH_2COO^-$).

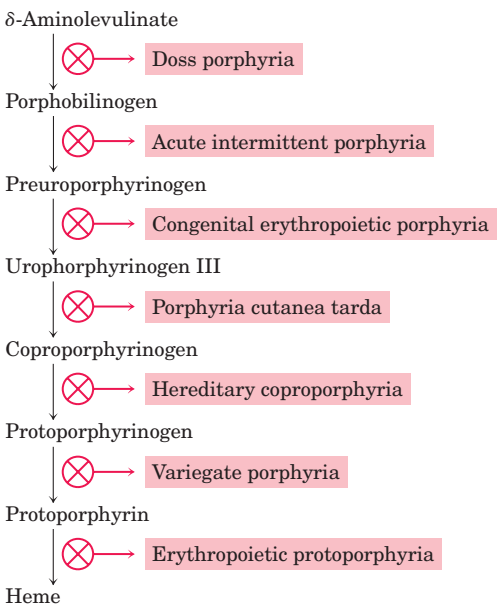
1. porphobilinogen synthase
2. uroporphyrinogen synthase
3. uroporphyrinogen III cosynthase
4. uroporphyrinogen decarboxylase
5. coproporphyrinogen oxidase
6. protoporphyrinogen oxidase
7. ferrochelatase

BOX 22–2 MEDICINE On Kings and Vampires

Porphyrias are a group of genetic diseases that result from defects in enzymes of the biosynthetic pathway from glycine to porphyrins; specific porphyrin precursors accumulate in erythrocytes, body fluids, and the liver. The most common form is acute intermittent porphyria. Most individuals inheriting this condition are heterozygotes and are usually asymptomatic, because the single copy of the normal gene provides a sufficient level of enzyme function. However, certain nutritional or environmental factors (as yet poorly understood) can cause a buildup of δ-aminolevulinate and porphobilinogen, leading to attacks of acute abdominal pain and neurological dysfunction. King George III, British monarch during the American Revolution, suffered several episodes of apparent madness that tarnished the record of this otherwise accomplished man. The symptoms of his condition suggest that George III suffered from acute intermittent porphyria.

One of the rarer porphyrias results in an accumulation of uroporphyrinogen I, an abnormal isomer of a protoporphyrin precursor. This compound stains the urine red, causes the teeth to fluoresce strongly in ultraviolet light, and makes the skin abnormally sensitive to sunlight. Many individuals with this porphyria are anemic because insufficient heme is synthesized. This genetic condition may have given rise to the vampire myths of folk legend.

The symptoms of most porphyrias are now readily controlled with dietary changes or the administration of heme or heme derivatives.



Heme Is the Source of Bile Pigments

The iron-porphyrin (heme) group of hemoglobin, released from dying erythrocytes in the spleen, is degraded to yield free Fe^{2+} and, ultimately, **bilirubin**. This pathway is arresting for its capacity to inject color into human biochemistry.

The first step in the two-step pathway, catalyzed by heme oxygenase, converts heme to biliverdin, a linear (open) tetrapyrrole derivative **(Fig. 22–25)**. The other products of the reaction are free Fe^{2+} and CO. The Fe^{2+} is quickly bound by ferritin. Carbon monoxide is a poison that binds to hemoglobin (see Box 5–1), and the production of CO by heme oxygenase ensures that, even in the absence of environmental exposure, about 1% of an individual's heme is complexed with CO.

Biliverdin is converted to bilirubin in the second step, catalyzed by biliverdin reductase. You can monitor this reaction colorimetrically in a familiar in situ experiment. When you are bruised, the black and/or purple color results from hemoglobin released from damaged erythrocytes. Over time, the color changes to the green of biliverdin, and then to the yellow of bilirubin. Bilirubin is largely insoluble, and it travels in the bloodstream as a complex with serum albumin. In the liver, bilirubin is transformed to the bile pigment bilirubin diglucuronide. This product is sufficiently water-soluble to be secreted with other components of bile into the small intestine, where microbial enzymes convert it to several products, predominantly urobilinogen. Some urobilinogen is reabsorbed into the blood and transported to the kidney, where it is converted to urobilin, the compound that gives urine its yellow color (Fig. 22–25, left branch). Urobilinogen remaining in the intestine is converted (in another microbe-dependent reaction) to stercobilin (Fig. 22–25, right branch), which imparts the red-brown color to feces.

Impaired liver function or blocked bile secretion causes bilirubin to leak from the liver into the blood, resulting in a yellowing of the skin and eyeballs, a condition called jaundice. In cases of jaundice, determination of the concentration of bilirubin in the blood may be useful in the diagnosis of underlying liver disease. Newborn infants sometimes develop jaundice because they have not yet produced enough glucuronyl bilirubin transferase to process their bilirubin. A traditional treatment to reduce excess bilirubin, exposure to a fluorescent lamp, causes a photochemical conversion of bilirubin to compounds that are more soluble and easily excreted.

These pathways of heme breakdown play significant roles in protecting cells from oxidative damage and in regulating certain cellular functions. The CO produced by heme oxygenase is toxic at high concentrations, but

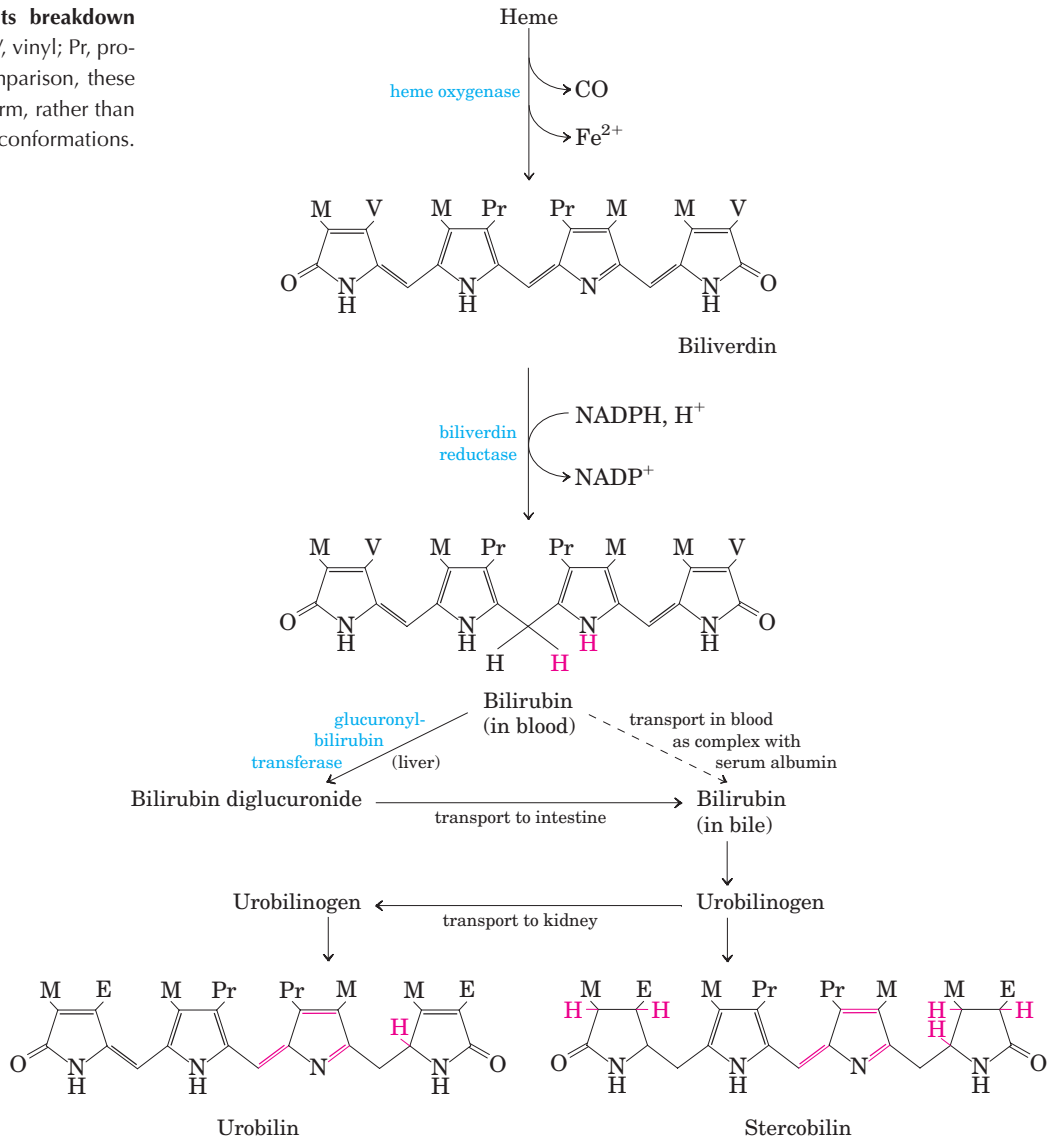


FIGURE 22–25 Bilirubin and its breakdown products. M represents methyl; V, vinyl; Pr, propionyl; E, ethyl. For ease of comparison, these structures are shown in linear form, rather than in their correct stereochemical conformations.

at the very low concentrations generated during heme degradation it seems to have some regulatory and/or signaling functions. It acts as a vasodilator, much the same as (but less potent than) nitric oxide (discussed below). Low levels of CO also have some regulatory effects on neurotransmission. Bilirubin is the most abundant antioxidant in mammalian tissues and is responsible for most of the antioxidant activity in serum. Its protective effects seem to be especially important in the developing brain of newborn infants. The cell toxicity associated with jaundice may be due to bilirubin levels in excess of the serum albumin needed to solubilize it.

Given these varied roles of heme degradation products, the degradative pathway is subject to regulation, mainly at the first step. Humans have at least three isozymes of heme oxygenase (HO). HO-1 is highly regulated; the expression of its gene is induced by a wide range of stress conditions (shear stress, angiogenesis (uncontrolled development of blood vessels), hypoxia, hyperoxia, heat shock, exposure to ultraviolet light, hydrogen peroxide, and many other metabolic insults). HO-2 is found mainly in brain and testes, where it is continuously expressed. The third isozyme, HO-3, is not yet well characterized. ■

Amino Acids Are Precursors of Creatine and Glutathione

Phosphocreatine, derived from **creatine**, is an important energy buffer in skeletal muscle (see Fig. 13–15). Creatine is synthesized from glycine and arginine **(Fig. 22–26)**; methionine, in the form of *S*-adenosylmethionine, acts as methyl group donor.

Glutathione (GSH), present in plants, animals, and some bacteria, often at high levels, can be thought of as a redox buffer. It is derived from glutamate, cysteine, and glycine **(Fig. 22–27)**. The γ-carboxyl group of glutamate is activated by ATP to form an acyl phosphate intermediate, which is then attacked by the

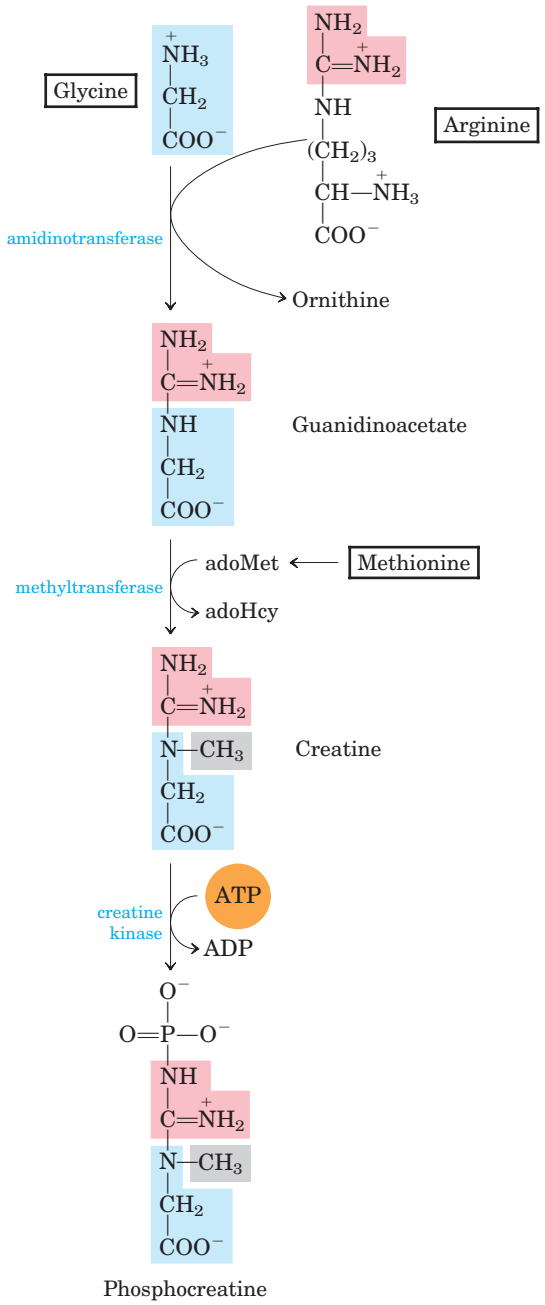


FIGURE 22–26 Biosynthesis of creatine and phosphocreatine. Creatine is made from three amino acids: glycine, arginine, and methionine. This pathway shows the versatility of amino acids as precursors of other nitrogenous biomolecules.

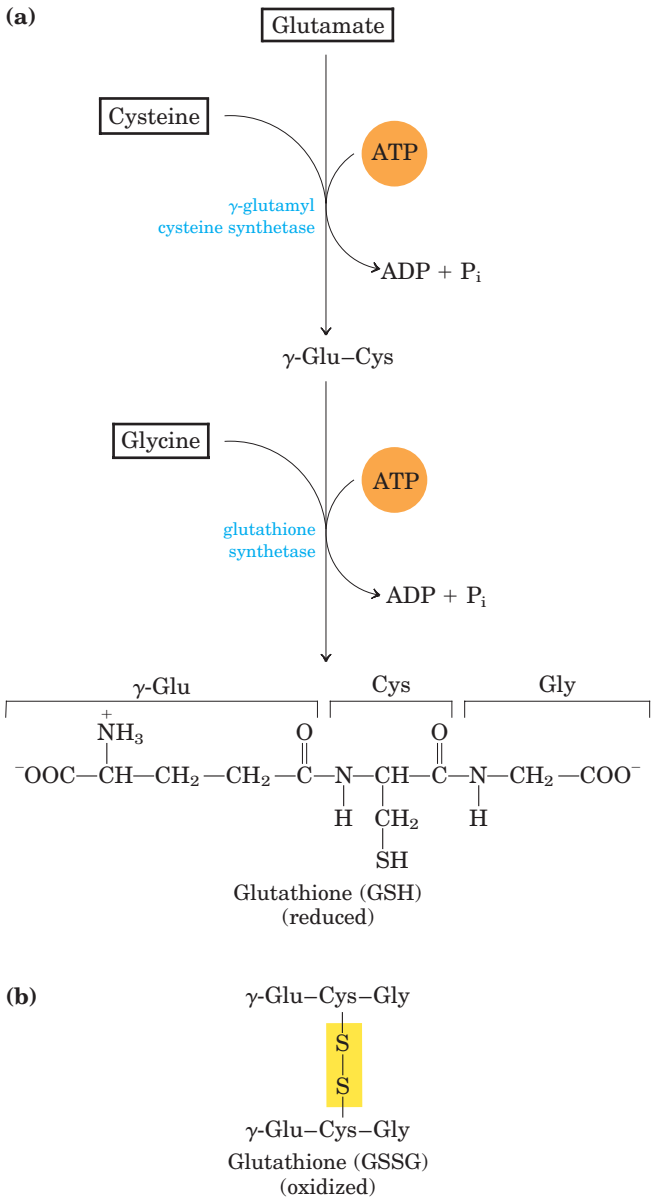


FIGURE 22–27 Glutathione metabolism. (a) Biosynthesis of glutathione. **(b)** Oxidized form of glutathione.

α-amino group of cysteine. A second condensation reaction follows, with the α-carboxyl group of cysteine activated to an acyl phosphate to permit reaction with glycine. The oxidized form of glutathione (GSSG), produced in the course of its redox activities, contains two glutathione molecules linked by a disulfide bond.

Glutathione probably helps maintain the sulfhydryl groups of proteins in the reduced state and the iron of heme in the ferrous (Fe^{2+}) state, and it serves as a reducing agent for glutaredoxin in deoxyribonucleotide synthesis (see Fig. 22–39). Its redox function is also used to remove toxic peroxides formed in the normal course of growth and metabolism under aerobic conditions:

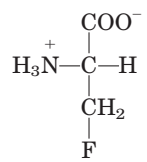
$$2\ GSH + R{-}O{-}O{-}H \longrightarrow GSSG + H_2O + R{-}OH$$

This reaction is catalyzed by **glutathione peroxidase**, a remarkable enzyme in that it contains a covalently bound selenium (Se) atom in the form of selenocysteine (see Fig. 3–8a), which is essential for its activity.

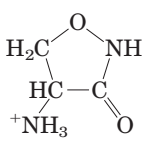
D-Amino Acids Are Found Primarily in Bacteria

Although D-amino acids do not generally occur in proteins, they do serve some special functions

in the structure of bacterial cell walls and peptide antibiotics. Bacterial peptidoglycans (see Fig. 20–31) contain both D-alanine and D-glutamate. D-Amino acids arise directly from the L isomers by the action of amino acid racemases, which have pyridoxal phosphate as cofactor (see Fig. 18–6). Amino acid racemization is uniquely important to bacterial metabolism, and enzymes such as alanine racemase are prime targets for pharmaceutical agents. One such agent, **L-fluoroalanine**, is being tested as an antibacterial drug. Another, **cycloserine**, is used to treat tuberculosis. Because these inhibitors also affect some PLP-requiring human enzymes, however, they have potentially undesirable side effects. ■



L-Fluoroalanine



Cycloserine

Aromatic Amino Acids Are Precursors of Many Plant Substances

Phenylalanine, tyrosine, and tryptophan are converted to a variety of important compounds in plants. The rigid polymer **lignin**, derived from phenylalanine and tyrosine, is second only to cellulose in abundance in plant tissues. The structure of the lignin polymer is complex and not well understood. Tryptophan is also the precursor of the plant growth hormone indole-3-acetate, or **auxin (Fig. 22–28a)**, which is important in the regulation of a wide range of biological processes in plants.

Phenylalanine and tyrosine also give rise to many commercially significant natural products, including the tannins that inhibit oxidation in wines; alkaloids such as morphine, which have potent physiological effects; and the flavoring of cinnamon oil (Fig. 22–28b), nutmeg, cloves, vanilla, cayenne pepper, and other products.

Biological Amines Are Products of Amino Acid Decarboxylation

Many important neurotransmitters are primary or secondary amines, derived from amino acids in simple pathways. In addition, some polyamines that form complexes with DNA are derived from the amino acid ornithine, a component of the urea cycle. A common denominator of many of these pathways is amino acid decarboxylation, another PLP-requiring reaction (see Fig. 18–6).

The synthesis of some neurotransmitters is illustrated in **Figure 22–29**. Tyrosine gives rise to a family of catecholamines that includes **dopamine**, **norepinephrine**, and **epinephrine**. Levels of catecholamines

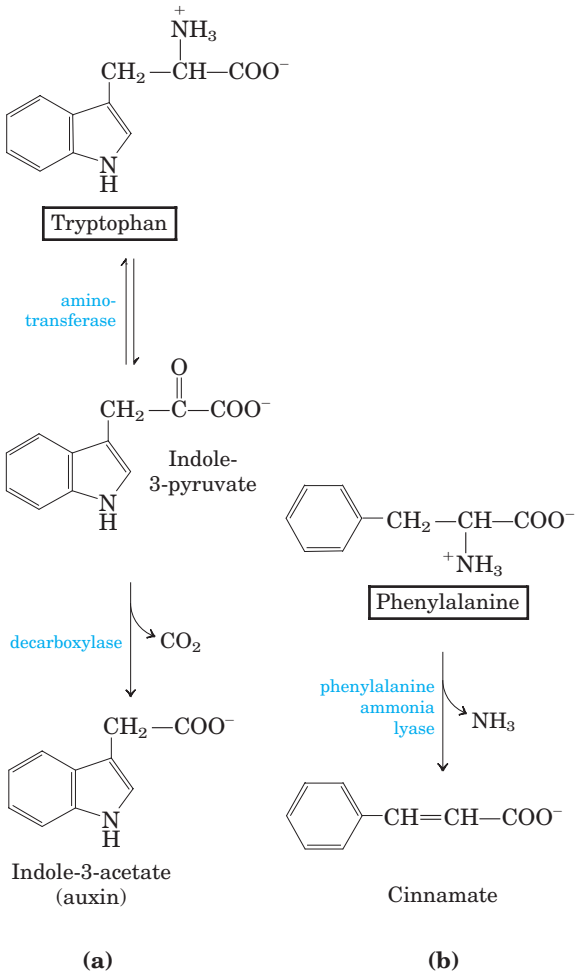


FIGURE 22–28 **Biosynthesis of two plant substances from amino acids.** **(a)** Indole-3-acetate (auxin) and **(b)** cinnamate (cinnamon flavor).

are correlated with, among other things, changes in blood pressure. The neurological disorder Parkinson's disease is associated with an underproduction of dopamine, and it has traditionally been treated by administering L-dopa. Overproduction of dopamine in the brain may be linked to psychological disorders such as schizophrenia.

Glutamate decarboxylation gives rise to ***γ*-aminobutyrate (GABA)**, an inhibitory neurotransmitter. Its underproduction is associated with epileptic seizures. GABA analogs are used in the treatment of epilepsy and hypertension. Levels of GABA can also be increased by administering inhibitors of the GABA-degrading enzyme GABA aminotransferase. Another important neurotransmitter, **serotonin**, is derived from tryptophan in a two-step pathway.

Histidine undergoes decarboxylation to **histamine**, a powerful vasodilator in animal tissues. Histamine is released in large amounts as part of the allergic response, and it also stimulates acid secretion in the stomach. A growing array of pharmaceutical agents are being

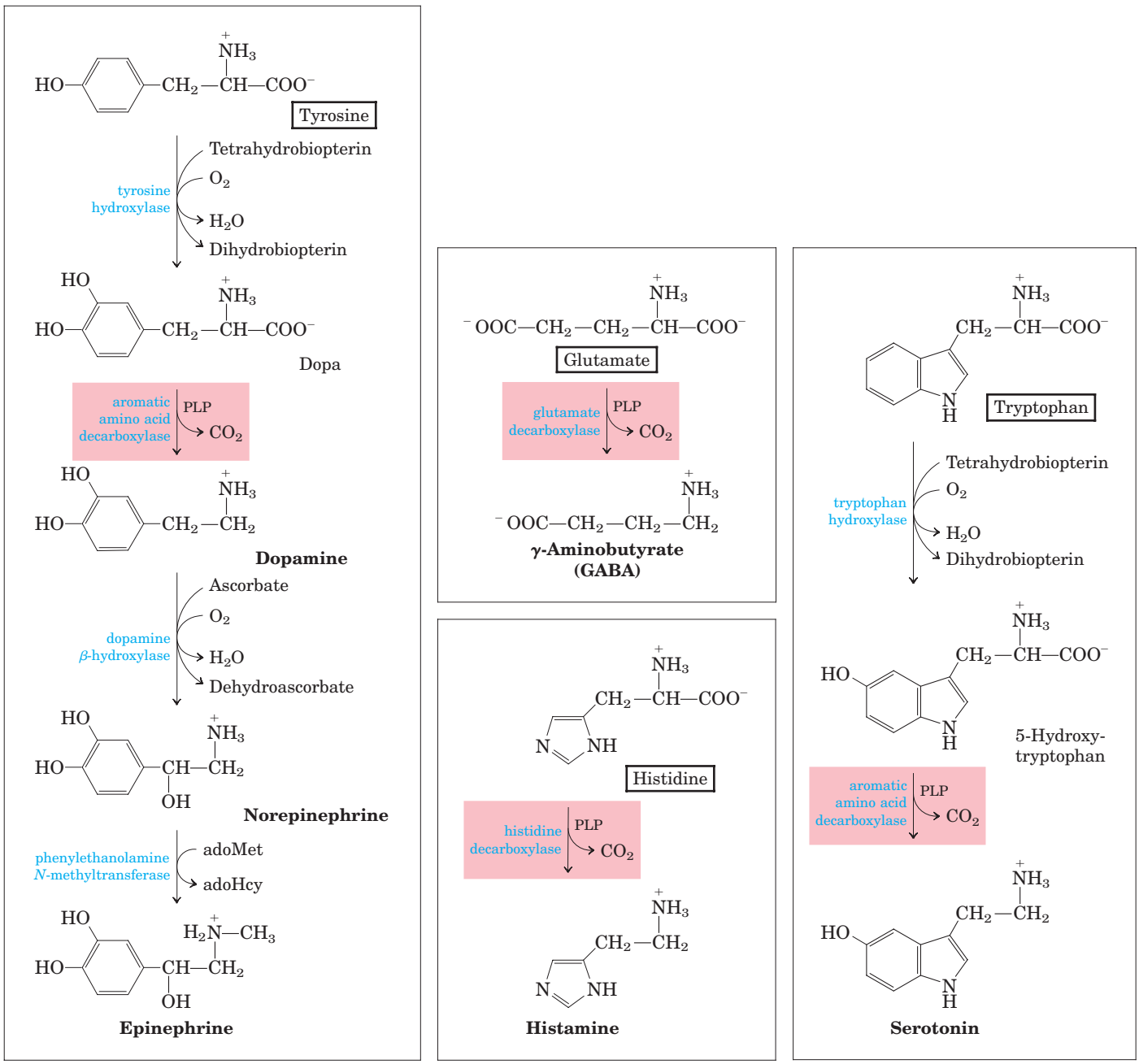
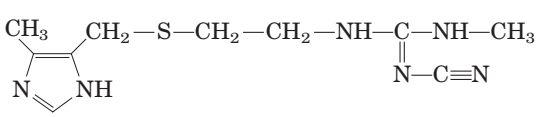


FIGURE 22–29 **Biosynthesis of some neurotransmitters from amino acids.** The key step is the same in each case: a PLP-dependent decarboxylation (shaded in pink).

designed to interfere with either the synthesis or the action of histamine. A prominent example is the histamine receptor antagonist **cimetidine** (Tagamet), a structural analog of histamine:



It promotes the healing of duodenal ulcers by inhibiting secretion of gastric acid.

Polyamines such as **spermine** and **spermidine**, involved in DNA packaging, are derived from methionine and ornithine by the pathway shown in **Figure 22–30**. The first step is decarboxylation of ornithine, a precursor of arginine (Fig. 22–10). **Ornithine decarboxylase**, a PLP-requiring enzyme, is the target of several powerful inhibitors used as pharmaceutical agents (Box 22–3). ■

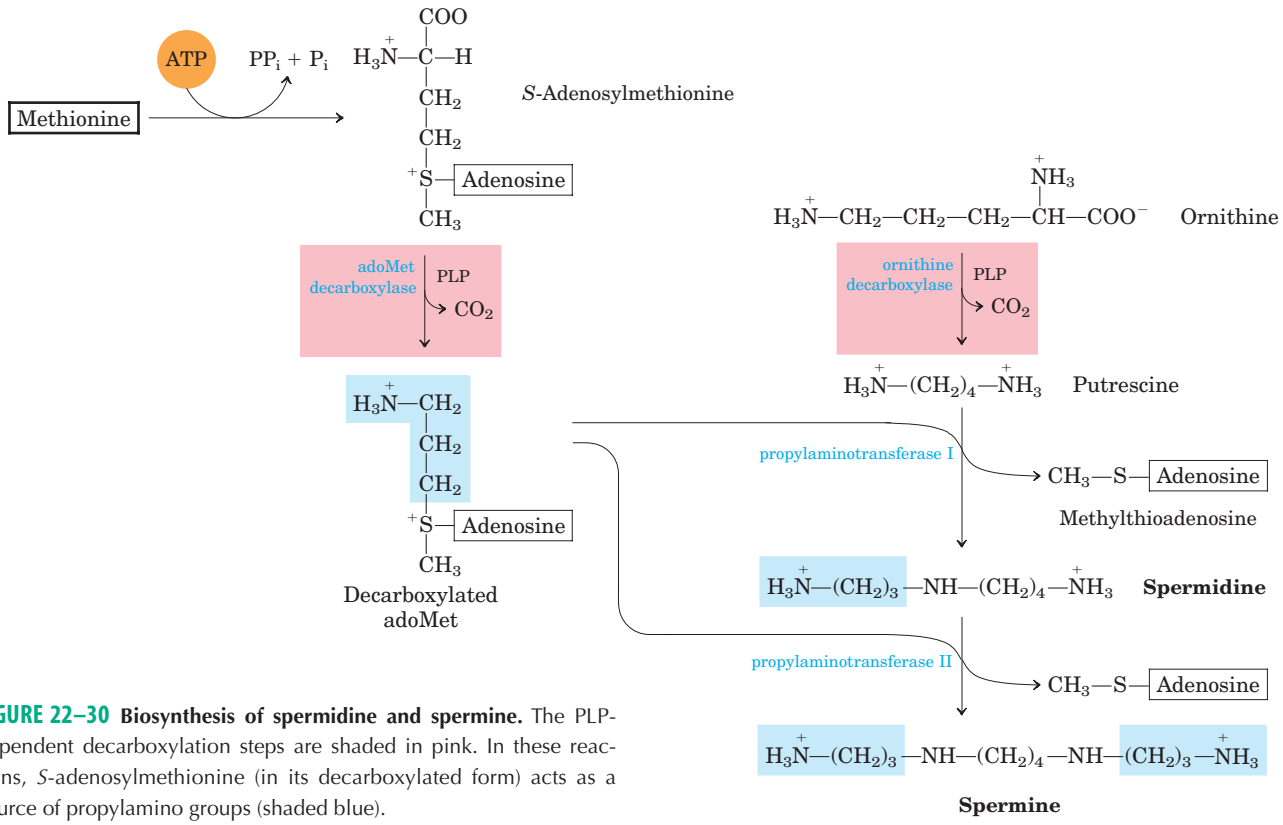


FIGURE 22–30 Biosynthesis of spermidine and spermine. The PLP-dependent decarboxylation steps are shaded in pink. In these reactions, *S*-adenosylmethionine (in its decarboxylated form) acts as a source of propylamino groups (shaded blue).

BOX 22–3 MEDICINE Curing African Sleeping Sickness with a Biochemical Trojan Horse

African sleeping sickness, or African trypanosomiasis, is caused by protists (single-celled eukaryotes) called trypanosomes (Fig. 1). This disease (and related trypanosome-caused diseases) is medically and economically significant in many developing nations. Until the late twentieth century, the disease was virtually incurable. Vaccines are ineffective because the parasite has a novel mechanism to evade the host immune system.

The cell coat of trypanosomes is covered with a single protein, which is the antigen to which the immune system responds. Every so often, however, by a process of genetic recombination (see Table 28–1), a few cells in the population of infecting trypanosomes switch to a new protein coat, not recognized by the immune system. This process of "changing coats" can occur hundreds of times. The result is a chronic cyclic infection: the human host develops a fever, which subsides as the immune system beats back the first infection; trypanosomes with changed coats then become the seed for a second infection, and the fever recurs. This cycle can repeat for weeks, and the weakened person eventually dies.

Some modern approaches to treating African sleeping sickness have been based on an understanding of enzymology and metabolism. In at least one such approach, this involves pharmaceutical agents designed as mechanism-based enzyme inactivators (suicide inactivators; p. 204). A vulnerable point in trypanosome metabolism is the pathway of polyamine biosynthesis. The polyamines spermine and spermidine, involved in DNA packaging, are required in large amounts in rapidly dividing cells. The first step in their synthesis is catalyzed by ornithine decarboxylase, a PLP-requiring enzyme (see Fig. 22–30). In mammalian cells, ornithine decarboxylase undergoes rapid turnover—that is, a constant round of enzyme degradation and synthesis. In some trypanosomes, however, the enzyme (for reasons not well understood) is stable, not readily replaced by newly synthesized enzyme. An inhibitor of ornithine

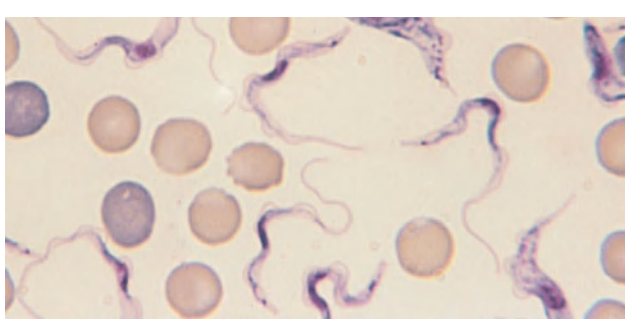


FIGURE 1 *Trypanosoma brucei rhodesiense,* one of several trypanosomes known to cause African sleeping sickness.

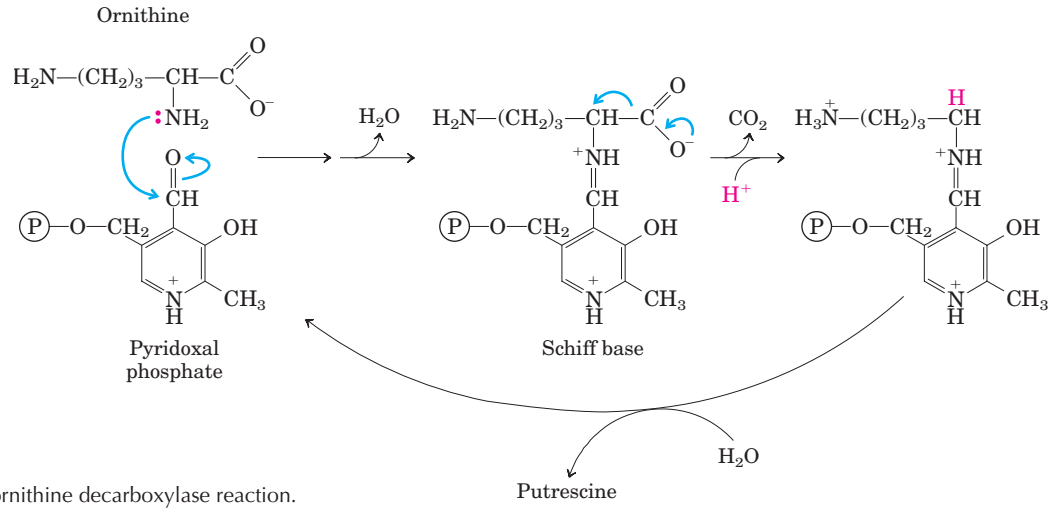


FIGURE 2 Mechanism of ornithine decarboxylase reaction.

decarboxylase that binds permanently to the enzyme would thus have little effect on human cells, which could rapidly replace inactivated enzyme, but would adversely affect the parasite.

The first few steps of the normal reaction catalyzed by ornithine decarboxylase are shown in Figure 2. Once CO_2 is released, the electron movement is reversed and putrescine is produced (see Fig. 22–30). Based on this mechanism, several suicide inactivators have been designed, one of which is difluoromethylornithine (DFMO). DFMO is relatively inert in solution. When it binds to ornithine decarboxylase, however, the enzyme is quickly inactivated (Fig. 3). The inhibitor acts by providing an alternative electron sink in the form of two strategically placed fluorine atoms, which are excellent leaving groups. Instead of electrons moving into the ring structure of PLP, the reaction results in displacement of a fluorine atom. The S of a Cys residue at the enzyme's active site then forms a covalent complex with the highly reactive PLP-inhibitor adduct in an essentially irreversible reaction. In this way, the inhibitor makes use of the enzyme's own reaction mechanisms to kill it.

DFMO has proved highly effective against African sleeping sickness in clinical trials and is now used to treat African sleeping sickness caused by *Trypanosoma brucei gambiense*. Approaches such as this show great promise for treating a wide range of diseases. The design of drugs based on enzyme mechanism and structure can complement the more traditional trial-and-error methods of developing pharmaceuticals.

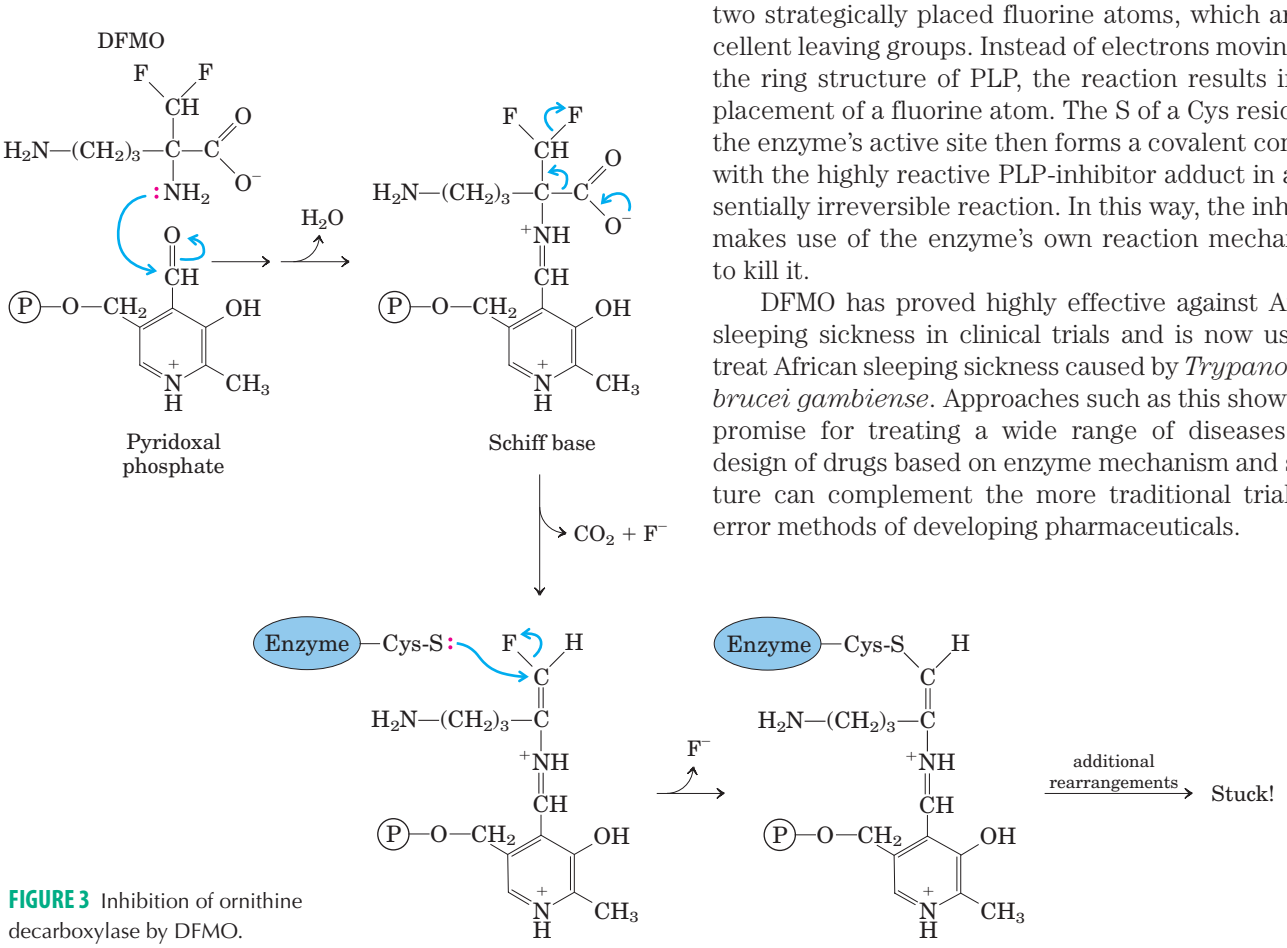


FIGURE 3 Inhibition of ornithine decarboxylase by DFMO.

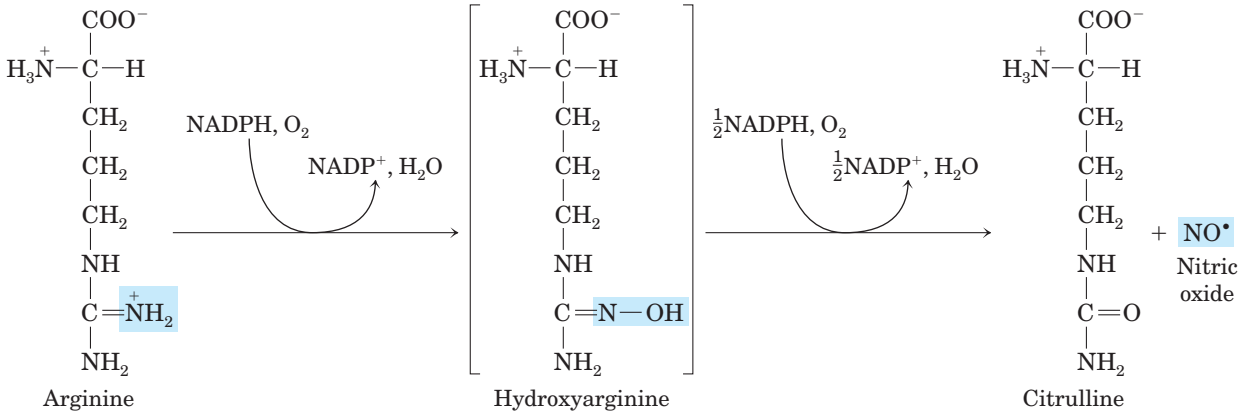


FIGURE 22–31 Biosynthesis of nitric oxide. Both steps are catalyzed by nitric oxide synthase. The nitrogen of the NO is derived from the guanidinium group of arginine.

Arginine Is the Precursor for Biological Synthesis of Nitric Oxide

A surprise finding in the mid-1980s was the role of nitric oxide (NO)—previously known mainly as a component of smog—as an important biological messenger. This simple gaseous substance diffuses readily through membranes, although its high reactivity limits its range of diffusion to about a 1 mm radius from the site of synthesis. In humans NO plays a role in a range of physiological processes, including neurotransmission, blood clotting, and the control of blood pressure. Its mode of action is described in Chapter 12 (p. 446).

Nitric oxide is synthesized from arginine in an NADPH-dependent reaction catalyzed by nitric oxide synthase **(Fig. 22–31)**, a dimeric enzyme structurally related to NADPH cytochrome P-450 reductase (see Box 21–1). The reaction is a five-electron oxidation. Each subunit of the enzyme contains one bound molecule of each of four different cofactors: FMN, FAD, tetrahydrobiopterin, and Fe^{3+} heme. NO is an unstable molecule and cannot be stored. Its synthesis is stimulated by interaction of nitric oxide synthase with Ca^{2+}-calmodulin (see Fig. 12–11).

SUMMARY 22.3 Molecules Derived from Amino Acids

- Many important biomolecules are derived from amino acids. Glycine is a precursor of porphyrins. Degradation of iron-porphyrin (heme) generates bilirubin, which is converted to bile pigments, with several physiological functions.
- Glycine and arginine give rise to creatine and phosphocreatine, an energy buffer. Glutathione, formed from three amino acids, is an important cellular reducing agent.
- Bacteria synthesize D-amino acids from L-amino acids in racemization reactions requiring pyridoxal phosphate. D-Amino acids are commonly found in certain bacterial walls and certain antibiotics.
- The aromatic amino acids give rise to many plant substances. The PLP-dependent decarboxylation of some amino acids yields important biological amines, including neurotransmitters.
- Arginine is the precursor of nitric oxide, a biological messenger.

22.4 Biosynthesis and Degradation of Nucleotides

As discussed in Chapter 8, nucleotides have a variety of important functions in all cells. They are the precursors of DNA and RNA. They are essential carriers of chemical energy—a role primarily of ATP and to some extent GTP. They are components of the cofactors NAD, FAD, *S*-adenosylmethionine, and coenzyme A, as well as of activated biosynthetic intermediates such as UDP-glucose and CDP-diacylglycerol. Some, such as cAMP and cGMP, are also cellular second messengers.

Two types of pathways lead to nucleotides: the **de novo pathways** and the **salvage pathways**. De novo synthesis of nucleotides begins with their metabolic precursors: amino acids, ribose 5-phosphate, CO_2, and NH_3. Salvage pathways recycle the free bases and nucleosides released from nucleic acid breakdown. Both types of pathways are important in cellular metabolism and both are discussed in this section.

The de novo pathways for purine and pyrimidine biosynthesis seem to be nearly identical in all living organisms. Notably, the free bases guanine, adenine, thymine, cytidine, and uracil are *not* intermediates in these pathways; that is, the bases are not synthesized and then attached to ribose, as might be expected. The purine ring structure is built up one or a few atoms at a time, attached to ribose throughout the process. The pyrimidine ring is synthesized as **orotate**, attached to

ribose phosphate, and then converted to the common pyrimidine nucleotides required in nucleic acid synthesis. Although the free bases are not intermediates in the de novo pathways, they are intermediates in some of the salvage pathways.

Several important precursors are shared by the de novo pathways for synthesis of pyrimidines and purines. Phosphoribosyl pyrophosphate (PRPP) is important in both, and in these pathways the structure of ribose is retained in the product nucleotide, in contrast to its fate in the tryptophan and histidine biosynthetic pathways discussed earlier. An amino acid is an important precursor in each type of pathway: glycine for purines and aspartate for pyrimidines. Glutamine again is the most important source of amino groups—in five different steps in the de novo pathways. Aspartate is also used as the source of an amino group in the purine pathways, in two steps.

Two other features deserve mention. First, there is evidence, especially in the de novo purine pathway, that the enzymes are present as large, multienzyme complexes in the cell, a recurring theme in our discussion of metabolism. Second, the cellular pools of nucleotides (other than ATP) are quite small, perhaps 1% or less of the amounts required to synthesize the cell's DNA. Therefore, cells must continue to synthesize nucleotides during nucleic acid synthesis, and in some cases nucleotide synthesis may limit the rates of DNA replication and transcription. Because of the importance of these processes in dividing cells, agents that inhibit nucleotide synthesis have become particularly important in medicine.

We examine here the biosynthetic pathways of purine and pyrimidine nucleotides and their regulation, the formation of the deoxynucleotides, and the degradation of purines and pyrimidines to uric acid and urea. We end with a discussion of chemotherapeutic agents that affect nucleotide synthesis.

De Novo Purine Nucleotide Synthesis Begins with PRPP

The two parent purine nucleotides of nucleic acids are adenosine 5′-monophosphate (AMP; adenylate) and guanosine 5′-monophosphate (GMP; guanylate), containing the purine bases adenine and guanine. **Figure 22–32** shows the origin of the carbon and nitrogen atoms of the purine ring system, as determined by John Buchanan using isotopic tracer experiments in birds. The detailed pathway of purine biosynthesis was worked out primarily by Buchanan and G. Robert Greenberg in the 1950s.

John Buchanan

In the first committed step of the pathway, an amino group donated by glutamine is attached at C-1 of PRPP **(Fig. 22–33)**. The resulting **5-phosphoribosylamine** is highly unstable, with a half-life of 30 seconds at pH 7.5. The purine ring is subsequently built up on this structure. The pathway described here is identical in all organisms, with the exception of one step that differs in higher eukaryotes as noted below.

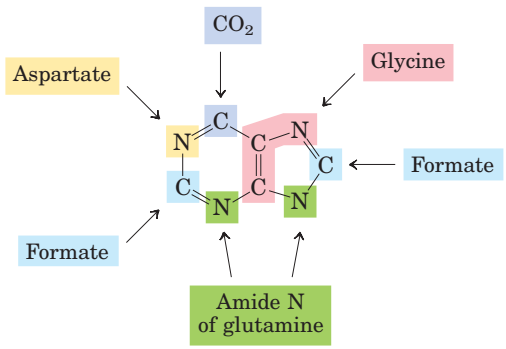


FIGURE 22–32 Origin of the ring atoms of purines. This information was obtained from isotopic experiments with ^{14}C- or ^{15}N-labeled precursors. Formate is supplied in the form of N^{10}-formyltetrahydrofolate.

The second step is the addition of three atoms from glycine (Fig. 22–33, step ②). An ATP is consumed to activate the glycine carboxyl group (in the form of an acyl phosphate) for this condensation reaction. The added glycine amino group is then formylated by N^{10}-formyltetrahydrofolate (step ③), and a nitrogen is contributed by glutamine (step ④), before dehydration and ring closure yield the five-membered imidazole ring of the purine nucleus, as 5-aminoimidazole ribonucleotide (AIR; step ⑤).

At this point, three of the six atoms needed for the second ring in the purine structure are in place. To complete the process, a carboxyl group is first added (step ⑥). This carboxylation is unusual in that it does not require biotin, but instead uses the bicarbonate generally present in aqueous solutions. A rearrangement transfers the carboxylate from the exocyclic amino group to position 4 of the imidazole ring (step ⑦). Steps ⑥ and ⑦ are found only in bacteria and fungi. In higher eukaryotes, including humans, the 5-aminoimidazole ribonucleotide product of step ⑤ is carboxylated directly to carboxyaminoimidazole ribonucleotide in one step instead of two (step ⑥a). The enzyme catalyzing this reaction is AIR carboxylase.

Aspartate now donates its amino group in two steps (⑧ and ⑨): formation of an amide bond, followed by elimination of the carbon skeleton of aspartate (as fumarate). (Recall that aspartate plays an analogous role in two steps of the urea cycle; see Fig. 18–10.) The final carbon is contributed by N^{10}-formyltetrahydrofolate (step ⑩), and a second ring closure takes place to yield the second fused ring of the purine nucleus (step ⑪). The first intermediate with a complete purine ring is **inosinate (IMP)**.

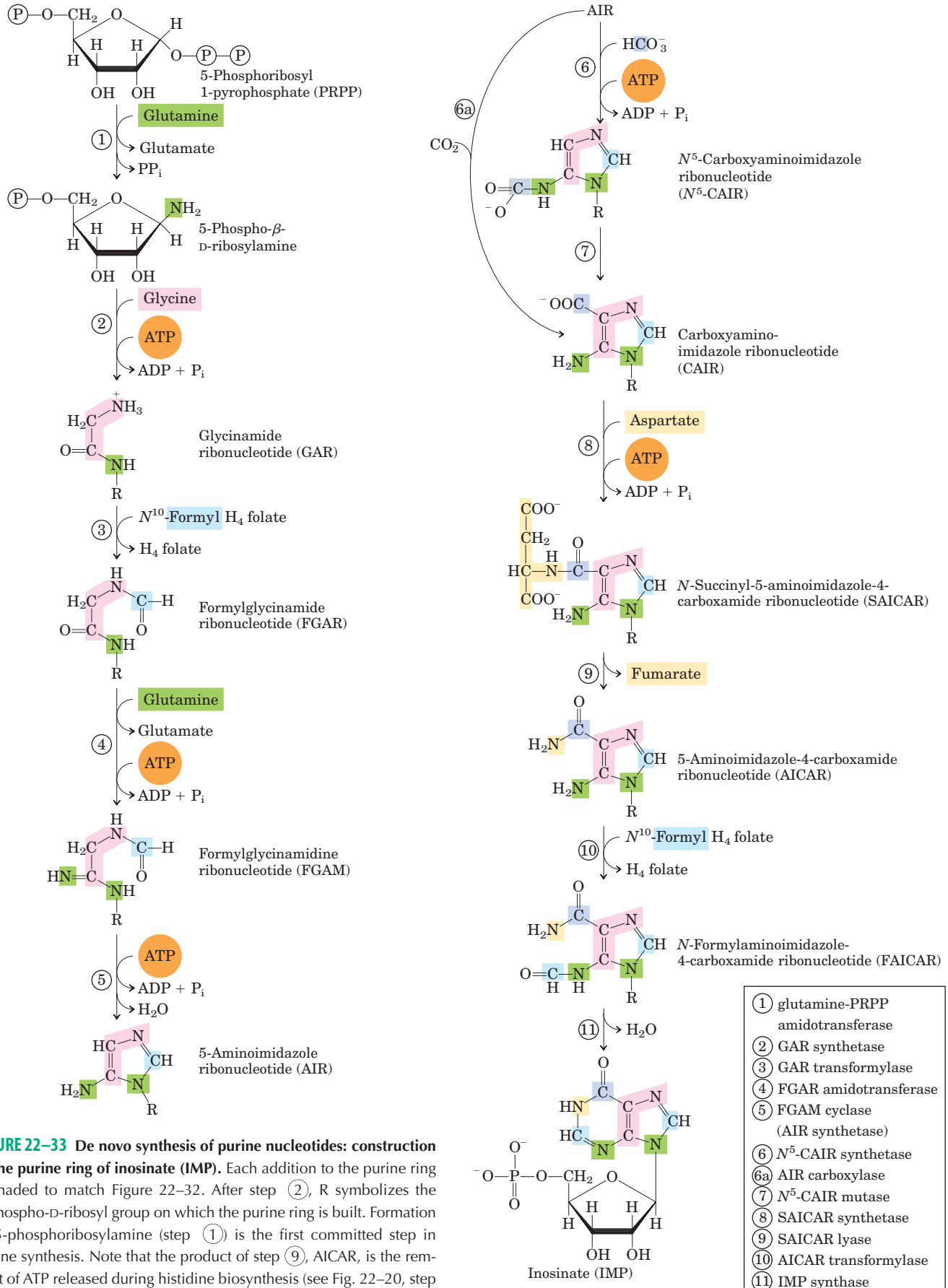


FIGURE 22–33 De novo synthesis of purine nucleotides: construction of the purine ring of inosinate (IMP). Each addition to the purine ring is shaded to match Figure 22–32. After step (2), R symbolizes the 5-phospho-D-ribosyl group on which the purine ring is built. Formation of 5-phosphoribosylamine (step (1)) is the first committed step in purine synthesis. Note that the product of step (9), AICAR, is the remnant of ATP released during histidine biosynthesis (see Fig. 22–20, step (5)). Abbreviations are given for most intermediates to simplify the naming of the enzymes. Step (6a) is the alternative path from AIR to CAIR occurring in higher eukaryotes.

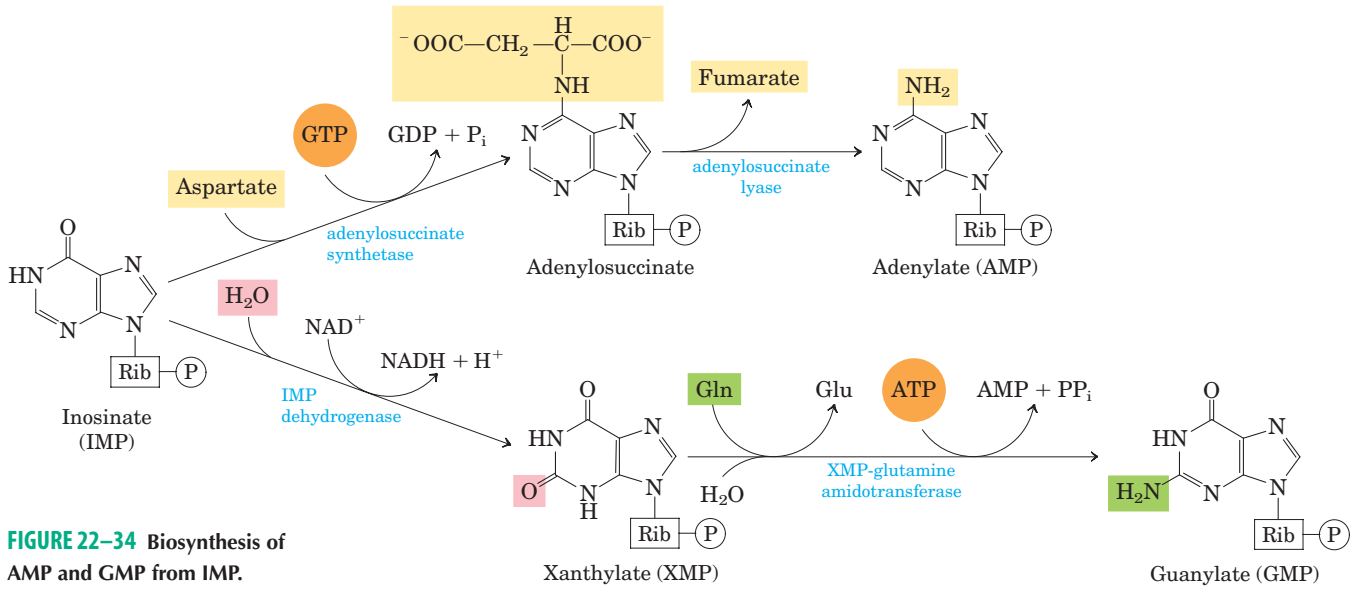


FIGURE 22–34 **Biosynthesis of AMP and GMP from IMP.**

As in the tryptophan and histidine biosynthetic pathways, the enzymes of IMP synthesis seem to be organized as large, multienzyme complexes in the cell. Once again, evidence comes from the existence of single polypeptides with several functions, some catalyzing nonsequential steps in the pathway. In eukaryotic cells ranging from yeast to fruit flies to chickens, steps ①, ③, and ⑤ in Figure 22–33 are catalyzed by a multifunctional protein. An additional multifunctional protein catalyzes steps ⑩ and ⑪. In humans, a multifunctional enzyme combines the activities of AIR carboxylase and SAICAR synthetase (steps ⑥a and ⑧). In bacteria, these activities are found on separate proteins, but the proteins may form a large noncovalent complex. The channeling of reaction intermediates from one enzyme to the next permitted by these complexes is probably especially important for unstable intermediates such as 5-phosphoribosylamine.

Conversion of inosinate to adenylate requires the insertion of an amino group derived from aspartate **(Fig. 22–34)**; this takes place in two reactions similar to those used to introduce N-1 of the purine ring (Fig. 22–33, steps ⑧ and ⑨). A crucial difference is that GTP rather than ATP is the source of the high-energy phosphate in synthesizing adenylosuccinate. Guanylate is formed by the NAD^+-requiring oxidation of inosinate at C-2, followed by addition of an amino group derived from glutamine. ATP is cleaved to AMP and PP_i in the final step (Fig. 22–34).

Purine Nucleotide Biosynthesis Is Regulated by Feedback Inhibition

Three major feedback mechanisms cooperate in regulating the overall rate of de novo purine nucleotide synthesis and the relative rates of formation of the two end products, adenylate and guanylate **(Fig. 22–35)**.

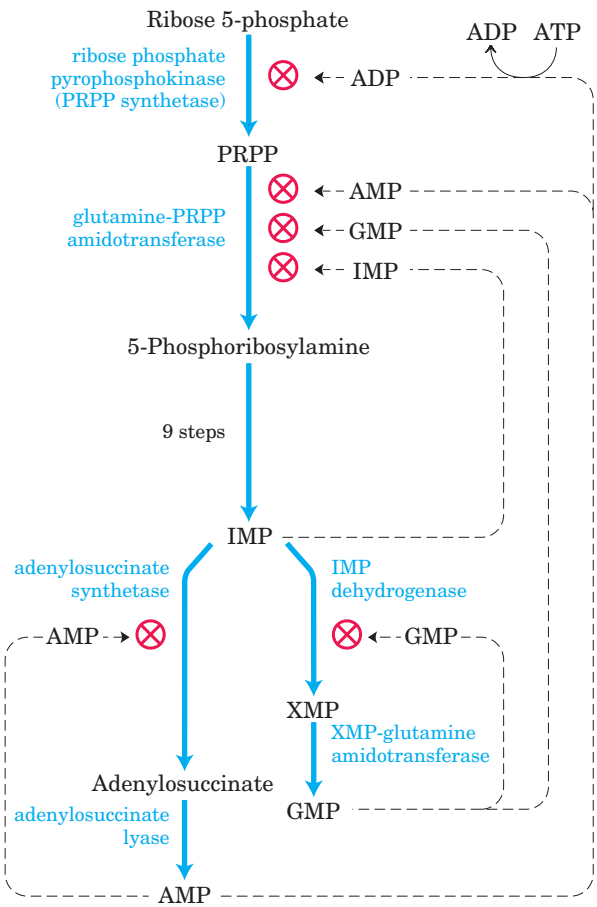


FIGURE 22–35 **Regulatory mechanisms in the biosynthesis of adenine and guanine nucleotides in *E. coli*.** Regulation of these pathways differs in other organisms.

The first mechanism is exerted on the first reaction that is unique to purine synthesis: transfer of an amino group to PRPP to form 5-phosphoribosylamine. This reaction is catalyzed by the allosteric enzyme

glutamine-PRPP amidotransferase, which is inhibited by the end products IMP, AMP, and GMP. AMP and GMP act synergistically in this concerted inhibition. Thus, whenever either AMP or GMP accumulates to excess, the first step in its biosynthesis from PRPP is partially inhibited.

In the second control mechanism, exerted at a later stage, an excess of GMP in the cell inhibits formation of xanthylate from inosinate by IMP dehydrogenase, without affecting the formation of AMP (Fig. 22–35). Conversely, an accumulation of adenylate inhibits formation of adenylosuccinate by adenylosuccinate synthetase, without affecting the biosynthesis of GMP. In the third mechanism, GTP is required in the conversion of IMP to AMP, whereas ATP is required for conversion of IMP to GMP (Fig. 22–34), a reciprocal arrangement that tends to balance the synthesis of the two ribonucleotides.

The final control mechanism is the inhibition of PRPP synthesis by the allosteric regulation of ribose phosphate pyrophosphokinase. This enzyme is inhibited by ADP and GDP, in addition to metabolites from other pathways for which PRPP is a starting point.

Pyrimidine Nucleotides Are Made from Aspartate, PRPP, and Carbamoyl Phosphate

The common pyrimidine ribonucleotides are cytidine 5′-monophosphate (CMP; cytidylate) and uridine 5′-monophosphate (UMP; uridylate), which contain the pyrimidines cytosine and uracil. De novo pyrimidine nucleotide biosynthesis **(Fig. 22–36)** proceeds in a somewhat different manner from purine nucleotide synthesis; the six-membered pyrimidine ring is made first and then attached to ribose 5-phosphate. Required in this process is carbamoyl phosphate, also an intermediate in the urea cycle (see Fig. 18–10). However, as we noted in Chapter 18, in animals the carbamoyl phosphate required in urea synthesis is made in mitochondria by carbamoyl phosphate synthetase I, whereas the carbamoyl phosphate required in pyrimidine biosynthesis is made in the cytosol by a different form of the enzyme, **carbamoyl phosphate synthetase II**. In bacteria, a single enzyme supplies carbamoyl phosphate for the synthesis of arginine and pyrimidines. The bacterial enzyme has three separate active sites, spaced along a channel nearly 100 Å long **(Fig. 22–37)**. Bacterial carbamoyl phosphate synthetase provides a vivid illustration of the channeling of unstable reaction intermediates between active sites.

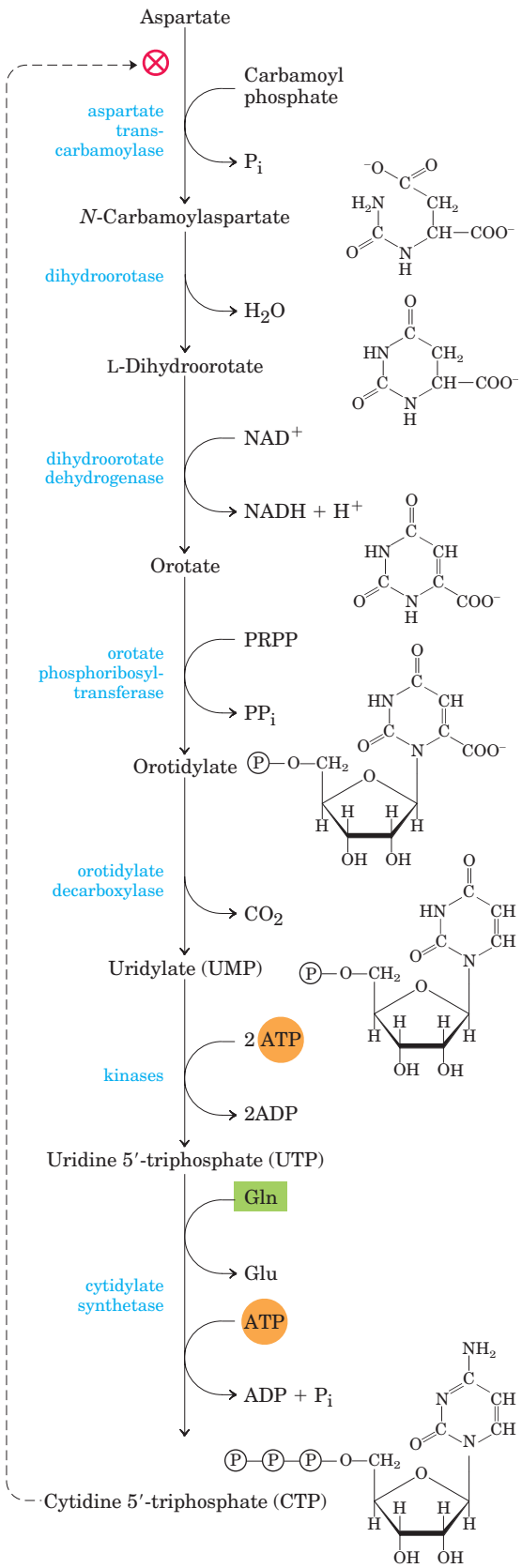


FIGURE 22–36 De novo synthesis of pyrimidine nucleotides: biosynthesis of UTP and CTP via orotidylate. The pyrimidine is constructed from carbamoyl phosphate and aspartate. The ribose 5-phosphate is then added to the completed pyrimidine ring by orotate phosphoribosyltransferase. The first step in this pathway (not shown here; see Fig. 18–11a) is the synthesis of carbamoyl phosphate from CO_2 and NH_4^+, catalyzed in eukaryotes by carbamoyl phosphate synthetase II.