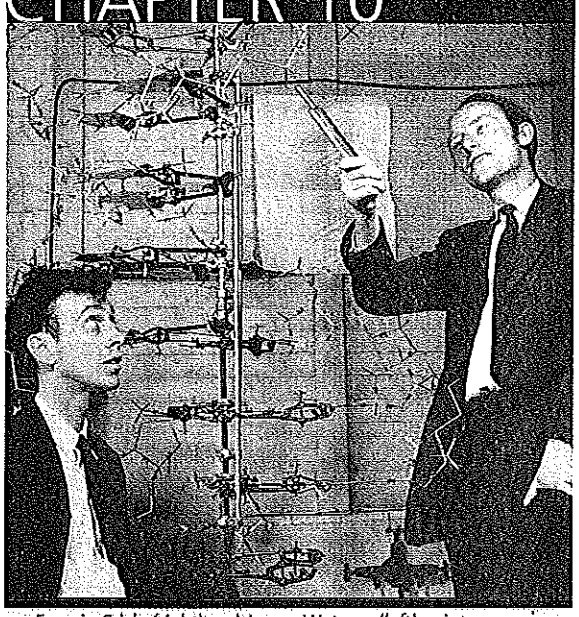
Nucleotides and Nucleic Acids

CHAPTER 10



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Francis Crick *(right)* and James Watson *(left)* point out features of their model for the structure of DNA.

Essential Question

Nucleotides and nucleic acids are compounds containing nitrogen bases (aromatic cyclic structures possessing nitrogen atoms) as part of their structure. Nucleotides are essential to cellular metabolism, and nucleic acids are the molecules of genetic information storage and expression. ***What are the structures of the nucleotides? How are nucleotides joined together to form nucleic acids? How is information stored in nucleic acids? What are the biological functions of nucleotides and nucleic acids?***

We have discovered the secret of life!
Proclamation by **Francis H. C. Crick** to patrons of the Eagle, a pub in Cambridge, England (1953)

Nucleotides and **nucleic acids** are biological molecules that possess heterocyclic nitrogenous bases as principal components of their structure. The biochemical roles of nucleotides are numerous; they participate as essential intermediates in virtually all aspects of cellular metabolism. Serving an even more central biological purpose are the nucleic acids, the elements of heredity and the agents of genetic information transfer. Just as proteins are linear polymers of amino acids, nucleic acids are linear polymers of nucleotides. Like the letters in this sentence, the orderly sequence of nucleotide residues in a nucleic acid can encode information. The two basic kinds of nucleic acids are **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA).** Complete hydrolysis of nucleic acids liberates nitrogenous bases, a five-carbon sugar, and phosphoric acid in equal amounts. The five-carbon sugar in DNA is 2-deoxyribose; in RNA, it is ribose. (See Chapter 7 for a detailed discussion of sugars and other carbohydrates.) DNA is the repository of genetic information in cells, whereas RNA serves in the expression of this information through the processes of **transcription** and **translation** (Figure 10.1). An interesting exception to this rule is that some viruses have their genetic information stored as RNA.

This chapter describes the chemistry of nucleotides and the major classes of nucleic acids. Chapter 11 presents methods for determination of nucleic acid primary structure (nucleic acid sequencing) and describes the higher orders of nucleic acid structure. Chapter 12 introduces the *molecular biology of recombinant DNA:* the construction and uses of novel DNA molecules assembled by combining segments from other DNA molecules.

Key Questions

10.1 What Is the Structure and Chemistry of Nitrogenous Bases?

The bases of nucleotides and nucleic acids are derivatives of either **pyrimidine** or **purine.** Pyrimidines are six-membered heterocyclic aromatic rings containing two nitrogen atoms (Figure 10.2a). The atoms are numbered in a clockwise fashion, as shown in Figure 10.2. The purine ring system consists of two rings of atoms: one resembling the pyrimidine ring and another resembling the imidazole ring (Figure 10.2b). The nine atoms in this fused ring system are numbered according to the convention shown.

The pyrimidine ring system is planar, whereas the purine system deviates somewhat from planarity in having a slight pucker between its imidazole and pyrimidine portions. Both are relatively insoluble in water, as might be expected from their pronounced aromatic character.

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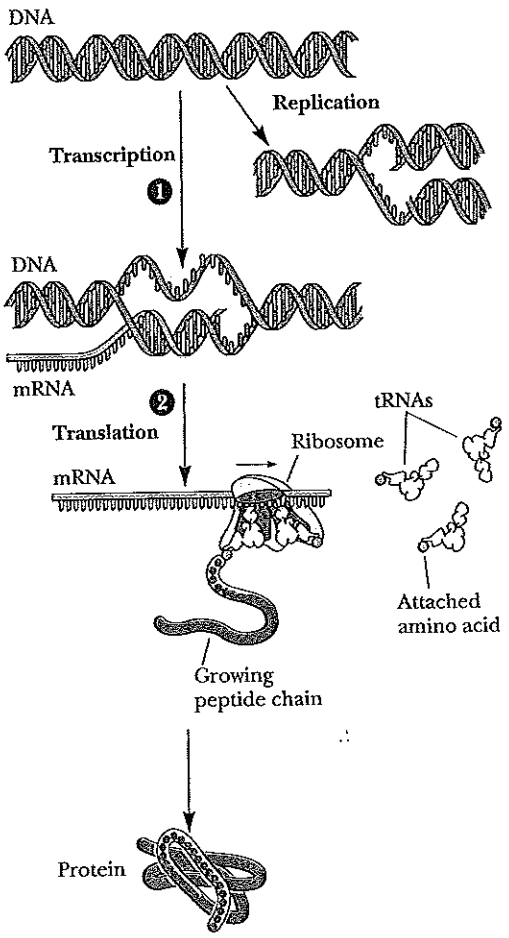


FIGURE 10.1 The fundamental process of information transfer in cells. (1) Information encoded in the nucleotide sequence of DNA is transcribed through synthesis of an RNA molecule whose sequence is dictated by the DNA sequence. (2) As the sequence of this RNA is read (as groups of three consecutive nucleotides) by the protein synthesis machinery, it is translated into the sequence of amino acids in a protein. This information transfer system is encapsulated in the dogma: DNA → RNA → protein.

Three Pyrimidines and Two Purines Are Commonly Found in Cells

The common naturally occurring pyrimidines are **cytosine, uracil,** and **thymine** (5-methyluracil) (Figure 10.3). Cytosine and thymine are the pyrimidines typically found in DNA, whereas cytosine and uracil are common in RNA. To view this generality another way, the uracil component of DNA occurs as the 5-methyl variety thymine. Various pyrimidine derivatives, such as dihydrouracil, are present as minor constituents in certain RNA molecules.

Biochemistry Now™ Go to BiochemistryNow and click BiochemistryInteractive to learn the structures of the common purines and pyrimidines.

Adenine (6-amino purine) and **guanine** (2-amino-6-oxy purine), the two common purines, are found in both DNA and RNA (Figure 10.4). Other naturally occurring purine derivatives include **hypoxanthine, xanthine,** and **uric acid** (Figure 10.5). Hypoxanthine and xanthine are found only rarely as constituents of nucleic acids. Uric acid, the most oxidized state for a purine derivative, is never found in nucleic acids.

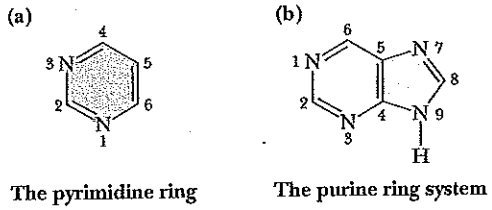


FIGURE 10.2 (a) The pyrimidine ring system; by convention, atoms are numbered as indicated. (b) The purine ring system, atoms numbered as shown.

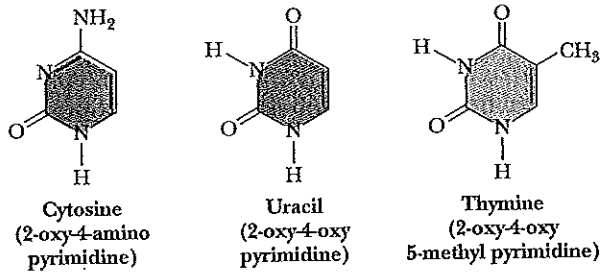


FIGURE 10.3 The common pyrimidine bases—cytosine, uracil, and thymine—in the tautomeric forms predominant at pH 7.

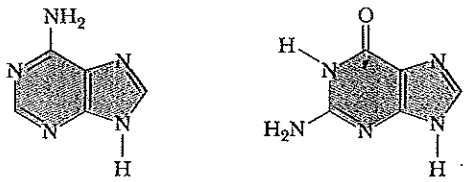


FIGURE 10.4 The common purine bases—adenine and guanine—in the tautomeric forms predominant at pH 7.

The Properties of Pyrimidines and Purines Can Be Traced to Their Electron-Rich Nature

The aromaticity of the pyrimidine and purine ring systems and the electron-rich nature of their —OH and $—NH_2$ substituents endow them with the capacity to undergo **keto–enol tautomeric shifts.** That is, pyrimidines and purines exist as tautomeric pairs, as shown in Figure 10.6 for uracil. The keto tautomer is called a **lactam,** whereas the enol form is a **lactim.** The lactam form vastly predominates at neutral pH. In other words, pK_a values for ring nitrogen atoms 1 and 3 in uracil are greater than 8 (the pK_a value for N-3 is 9.5) (Table 10.1). In contrast, as might be expected from the form of cytosine that predominates at pH 7, the pK_a value for N-3 in this pyrimidine is 4.5. Similarly, keto–enol tautomeric forms can be represented for purines, as given for guanine in Figure 10.7.[1] Here, the pK_a value is 9.4 for N-1 and less than 5 for N-3. These pK_a values specify whether hydrogen atoms are associated with the various ring nitrogens at neutral pH. As such, they are important in determining whether these nitrogens serve as H-bond donors or acceptors. Hydrogen bonding between purine and pyrimidine bases is fundamental to the biological functions of nucleic acids, as in the formation of the double-helix structure of DNA (see Section 10.5). The important functional groups participating in H-bond formation are the amino groups of cytosine, adenine, and guanine; the ring nitrogens at position 3 of pyrimidines and position 1 of purines; and the strongly electronegative oxygen atoms attached at position 4 of uracil and thymine, position 2 of cytosine, and position 6 of guanine (see Figure 10.20).

Another property of pyrimidines and purines is their strong absorbance of ultraviolet (UV) light, which is also a consequence of the aromaticity of their heterocyclic ring structures. Figure 10.8 shows characteristic absorption spectra of several of the common bases of nucleic acids—adenine, uracil, cytosine, and guanine—in their nucleotide forms: AMP, UMP, CMP, and GMP (see Section 10.3). This property is particularly useful in quantitative and qualitative analysis of nucleotides and nucleic acids.

[1]The 2-, 4-, and 6-pyrimidine and purine amino groups can undergo tautomerism as well, changing from amino to imino functions.

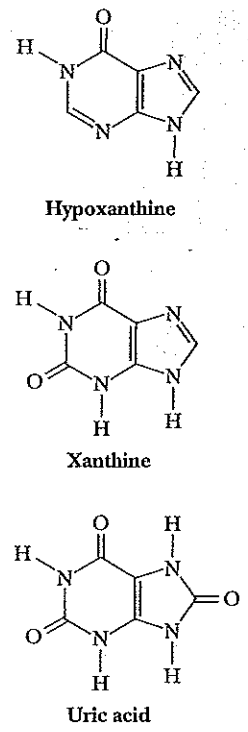


FIGURE 10.5 Other naturally occurring purine derivatives—hypoxanthine, xanthine, and uric acid.

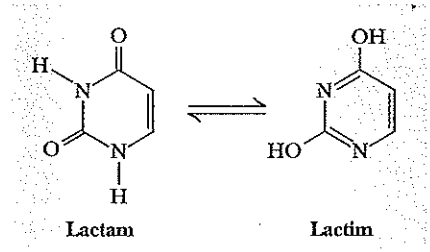


FIGURE 10.6 The keto–enol tautomerization of uracil.

Table 10.1
Proton Dissociation Constants (pK_a Values) for Nucleotides

Nucleotide	pK_a Base-N	pK_1 Phosphate	pK_2 Phosphate
5′-AMP	3.8 (N-1)	0.9	6.1
5′-GMP	9.4 (N-1)	0.7	6.1
	2.4 (N-7)		
5′-CMP	4.5 (N-3)	0.8	6.3
5′-UMP	9.5 (N-3)	1.0	6.4

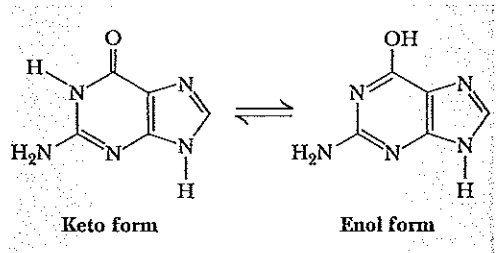


FIGURE 10.7 The tautomerization of the purine guanine.

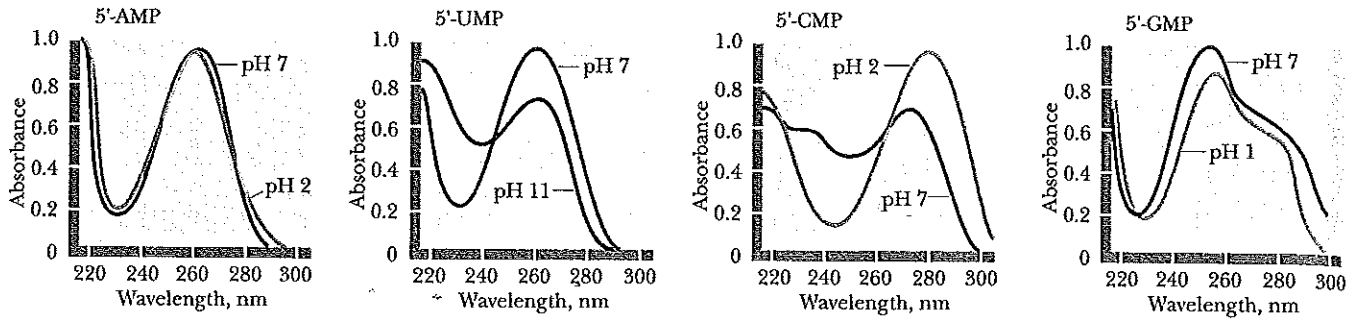


FIGURE 10.8 The UV absorption spectra of the common ribonucleotides.

10.2 What Are Nucleosides?

Nucleosides are compounds formed when a base is linked to a sugar. The sugars of nucleosides are **pentoses** (five-carbon sugars, see Chapter 7). **Ribonucleosides** contain the pentose D-ribose, whereas 2-deoxy-D-ribose is found in **deoxyribonucleosides.** In both instances, the pentose is in the five-membered ring form known as furanose: D-ribofuranose for ribonucleosides and 2-deoxy-D-ribofuranose for deoxyribonucleosides (Figure 10.9). In nucleosides, these ribofuranose atoms are numbered as 1′, 2′, 3′, and so on to distinguish them from the ring atoms of the nitrogenous bases. (As we shall see, the seemingly minor difference of a hydroxyl group at the 2′-position has far-reaching effects on the secondary structures available to RNA and DNA, as well as their relative susceptibilities to chemical and enzymatic hydrolysis.)

In nucleosides, the base is linked to the sugar via a **glycosidic bond** (Figure 10.10). Glycosidic bonds by definition involve the carbonyl carbon atom of the sugar, which in cyclic structures is joined to the ring O atom. As discussed in Chapter 7, such carbon atoms are called **anomeric.** In nucleosides, the bond is an *N*-glycoside because it connects the anomeric C-1′ to N-1 of a pyrimidine or to N-9 of a purine. Recall that glycosidic bonds can be either α or β, depending on their orientation relative to the anomeric C atom. Glycosidic bonds in nucleosides (and nucleotides, see following discussion) are always of the β-configuration, as represented in Figure 10.10. Nucleosides are named by adding the ending *-idine* to the root name of a pyrimidine or *-osine* to the root name of a purine. The common nucleosides are thus **cytidine, uridine, thymidine, adenosine,** and **guanosine.** Structures of the common ribonucleosides are shown in Figure 10.11. The nucleoside formed by hypoxanthine and ribose is **inosine.**

Nucleosides Usually Adopt an Anti Conformation About the Glycosidic Bond

In nucleosides, rotation of the base about the glycosidic bond is sterically hindered, principally by the hydrogen atom on the C-2′ carbon of the furanose. (This hindrance is most easily seen and appreciated by manipulating accurate

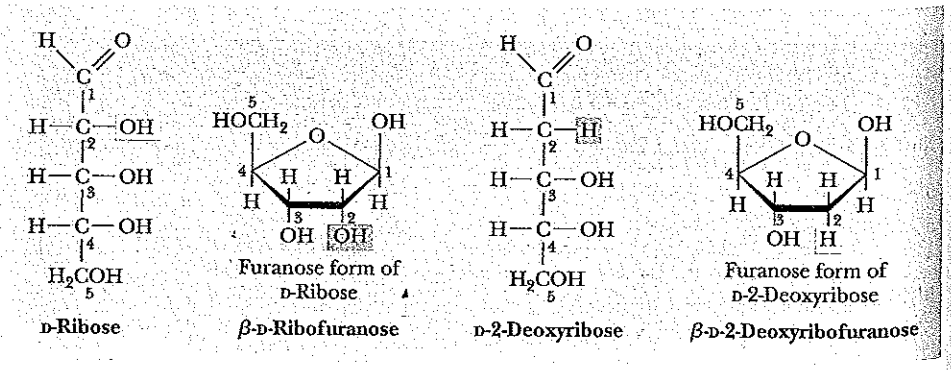
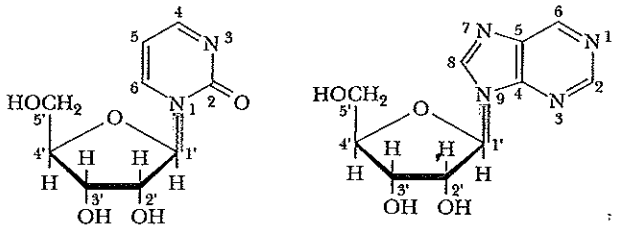


FIGURE 10.9 Furanose structures—ribose and deoxyribose.



β-N_1-glycosidic bond in pyrimidine ribonucleosides

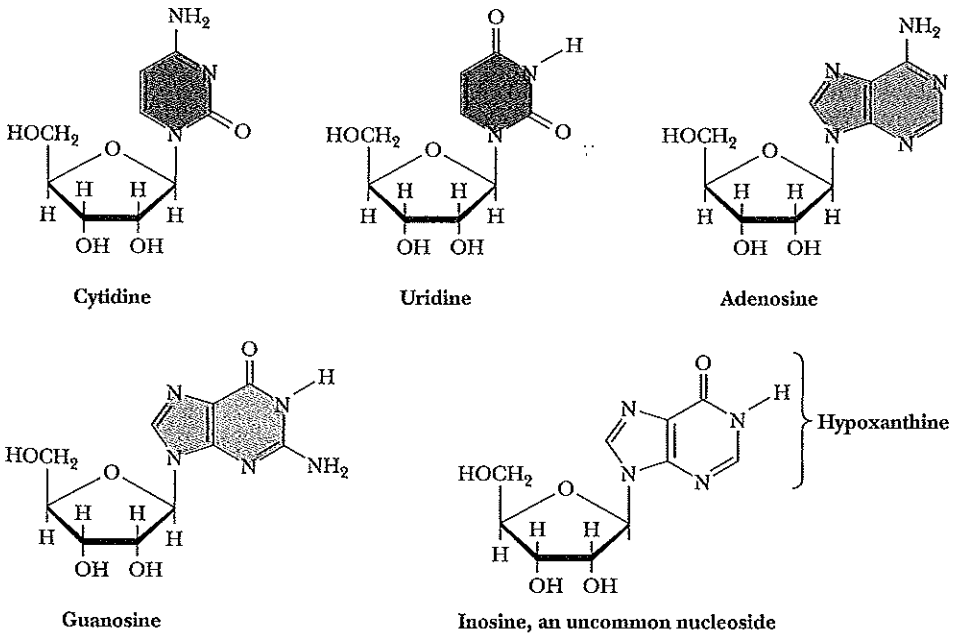
β-N_9-glycosidic bond in purine ribonucleosides

FIGURE 10.10 β-Glycosidic bonds link nitrogenous bases and sugars to form nucleosides.

molecular models of these structures.) Consequently, nucleosides (and nucleotides, see next section) exist in either of two conformations, designated *syn* and *anti* (Figure 10.12). For pyrimidines in the syn conformation, the oxygen substituent at position C-2 would lie in a sterically hindered position immediately above the furanose ring; in the anti conformation, this steric interference is avoided. Consequently, pyrimidine nucleosides adopt the anti conformation. Purine nucleosides can assume either the syn or anti conformation, although the anti conformation is favored. In either conformation, the roughly planar furanose and base rings are not coplanar but lie at approximately right angles to one another.

Nucleosides Are More Water Soluble Than Free Bases

Nucleosides are much more water soluble than the free bases because of the hydrophilicity of the sugar moiety. Like glycosides (see Chapter 7), nucleosides are relatively stable in alkali. Pyrimidine nucleosides are also resistant to acid hydrolysis, but purine nucleosides are easily hydrolyzed in acid to yield the free base and pentose.



Cytidine **Uridine** **Adenosine**

Guanosine **Inosine, an uncommon nucleoside**

FIGURE 10.11 The common ribonucleosides—cytidine, uridine, adenosine, and guanosine. Also, inosine drawn in anti conformation.

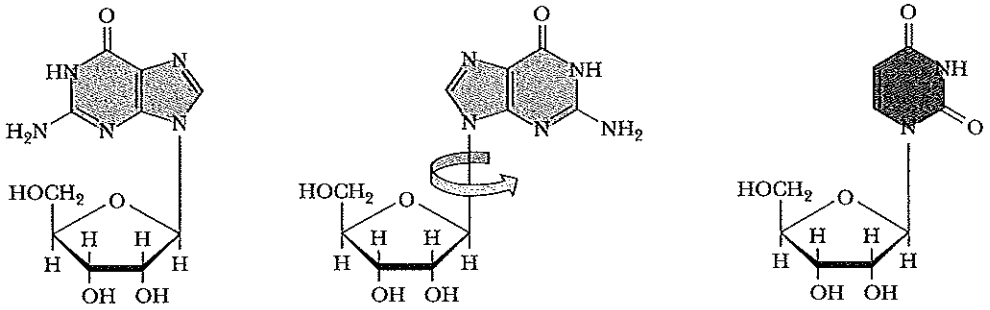


FIGURE 10.12 Rotation around the glycosidic bond is sterically hindered; *syn* versus *anti* conformations in nucleosides are shown.

10.3 What Is the Structure and Chemistry of Nucleotides?

A **nucleotide** results when phosphoric acid is esterified to a sugar —OH group of a nucleoside. The nucleoside ribose ring has three —OH groups available for esterification, at C-2′, C-3′, and C-5′ (although 2′-deoxyribose has only two). The vast majority of monomeric nucleotides in the cell are **ribonucleotides** having 5′-phosphate groups. Figure 10.13 shows the structures of the common four *ribonucleotides,* whose formal names are **adenosine 5′-monophosphate, guanosine 5′-monophosphate, cytidine 5′-monophosphate,** and **uridine 5′-monophosphate.** These compounds are more often referred to by their abbreviations: **5′-AMP, 5′-GMP, 5′-CMP,** and **5′-UMP,** or even more simply as **AMP, GMP, CMP,** and **UMP.** Nucleoside 3′-phosphates and nucleoside 2′-phosphates (3′-NMP and 2′-NMP, where N is a generic designation for "nucleoside") are uncommon, except as products of nucleic acid hydrolysis. Because the pK_a value for the first dissociation of a proton from the phosphoric acid moiety is 1.0 or less (Table 10.1), the nucleotides have acidic properties. This acidity is implicit in the other names by which these substances are known—**adenylic acid, guanylic acid, cytidylic acid,**

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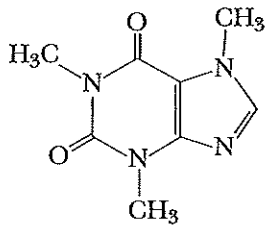
Adenosine: A Nucleoside with Physiological Activity

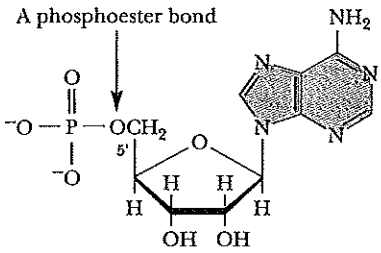
For the most part, nucleosides have no biological role other than to serve as component parts of nucleotides. Adenosine is a rare exception. In mammals, adenosine functions as an **autocoid,** or "local hormone," and as a neuromodulator. This nucleoside circulates in the bloodstream, acting locally on specific cells to influence such diverse physiological phenomena as blood vessel dilation, smooth muscle contraction, neuronal discharge, neurotransmitter release, and metabolism of fat. For example, when muscles work hard, they release adenosine, causing the surrounding blood vessels to dilate, which in turn increases the flow of blood and its delivery of O_2 and nutrients to the muscles. In a different autocoid role, adenosine acts in regulating heartbeat. The natural rhythm of the heart is controlled by a pacemaker, the sinoatrial node, which cyclically sends a wave of electrical excitation to the heart muscles. By blocking the flow of electrical current, adenosine slows the heart rate. *Supraventricular tachycardia* is a heart condition characterized by a rapid heartbeat. Intravenous injection of adenosine causes a momentary interruption of the rapid cycle of contraction and restores a normal heart rate. Adenosine is licensed and marketed as *Adenocard* to treat supraventricular tachycardia.

In addition, adenosine is implicated in sleep regulation. During periods of extended wakefulness, extracellular adenosine levels rise as a result of metabolic activity in the brain, and this increase promotes sleepiness. During sleep, adenosine levels fall. Caffeine promotes wakefulness by blocking the interaction of extracellular adenosine with its neuronal receptors.*

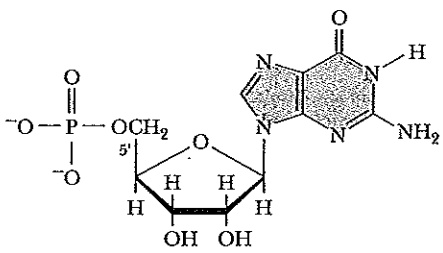


Caffeine

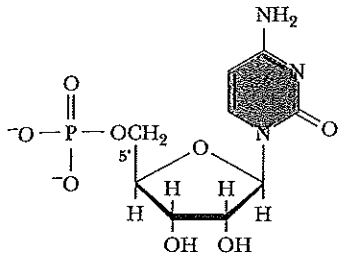
*Porrka-Heiskanen, T., et al., 1997. Adenosine: A mediator of the sleep-inducing effects of prolonged wakefulness. *Science* **276**:1265–1268; and Vaugeois, J-M., 2002. Positive feedback from caffeine. *Nature* **418**:734–726.



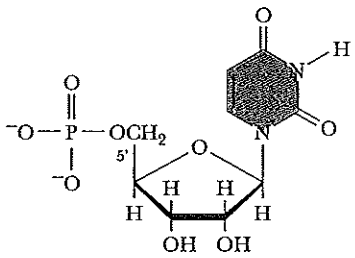
Adenosine 5'-monophosphate (or AMP or adenylic acid)



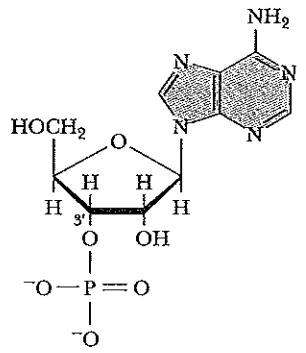
Guanosine 5'-monophosphate (or GMP or guanylic acid)



Cytidine 5'-monophosphate (or CMP or cytidylic acid)



Uridine 5'-monophosphate (or UMP or uridylic acid)



A nucleoside 3'-monophosphate 3'-AMP

FIGURE 10.13 Structures of the four common ribonucleotides—AMP, GMP, CMP, and UMP—together with their two sets of full names, for example, adenosine 5'-monophosphate and adenylic acid. Also shown is the nucleoside 3'-AMP.

and **uridylic acid.** The pK_a value for the second dissociation, pK_2, is about 6.0, so at neutral pH or above, the net charge on a nucleoside monophosphate is −2. Nucleic acids, which are polymers of nucleoside monophosphates, derive their name from the acidity of these phosphate groups.

Cyclic Nucleotides Are Cyclic Phosphodiesters

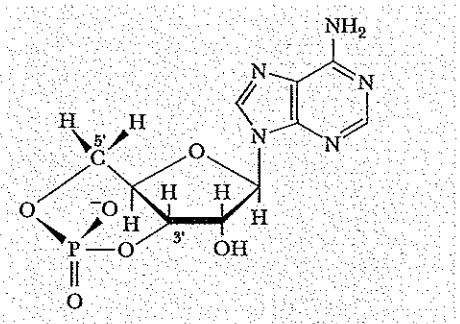
Nucleoside monophosphates in which the phosphoric acid is esterified to *two* of the available ribose hydroxyl groups (Figure 10.14) are found in all cells. Forming two such ester linkages with one phosphate results in a cyclic phosphodiester structure. **3',5'-cyclic AMP,** often abbreviated **cAMP,** and its guanine analog **3',5'-cyclic GMP,** or **cGMP,** are important regulators of cellular metabolism (see Parts 3 and 4).

Nucleoside Diphosphates and Triphosphates Are Nucleotides with Two or Three Phosphate Groups

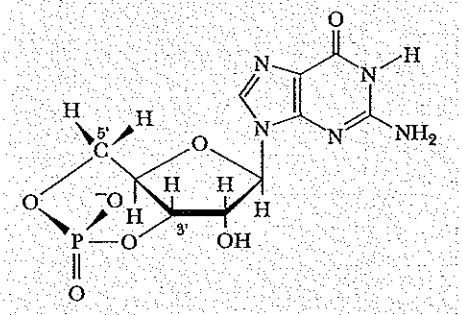
Additional phosphate groups can be linked to the phosphoryl group of a nucleotide through the formation of phosphoric anhydride linkages, as shown in Figure 10.15. Addition of a second phosphate to AMP creates **adenosine 5'-diphosphate,** or **ADP,** and adding a third yields **adenosine 5'-triphosphate,** or **ATP.** The respective phosphate groups are designated by the Greek letters α, β, and γ, starting with the α-phosphate as the one linked directly to the pentose. The abbreviations **GTP, CTP,** and **UTP** represent the other corresponding nucleoside 5'-triphosphates. Like the nucleoside 5'-monophosphates, the nucleoside 5'-diphosphates and 5'-triphosphates all occur in the free state in the cell, as do their deoxyribonucleoside phosphate counterparts, represented as dAMP, dADP, and dATP; dGMP, dGDP, and dGTP; dCMP, dCDP, and dCTP; dUMP, dUDP, and dUTP; and dTMP, dTDP, and dTTP.

NDPs and NTPs Are Polyprotic Acids

Nucleoside 5'-diphosphates (NDPs) and **nucleoside 5'-triphosphates (NTPs)** are relatively strong *polyprotic acids* in that they dissociate three and four protons, respectively, from their phosphoric acid groups. The resulting phosphate



3',5'-Cyclic AMP



3',5'-Cyclic GMP

FIGURE 10.14 Structures of the cyclic nucleotides cAMP and cGMP.

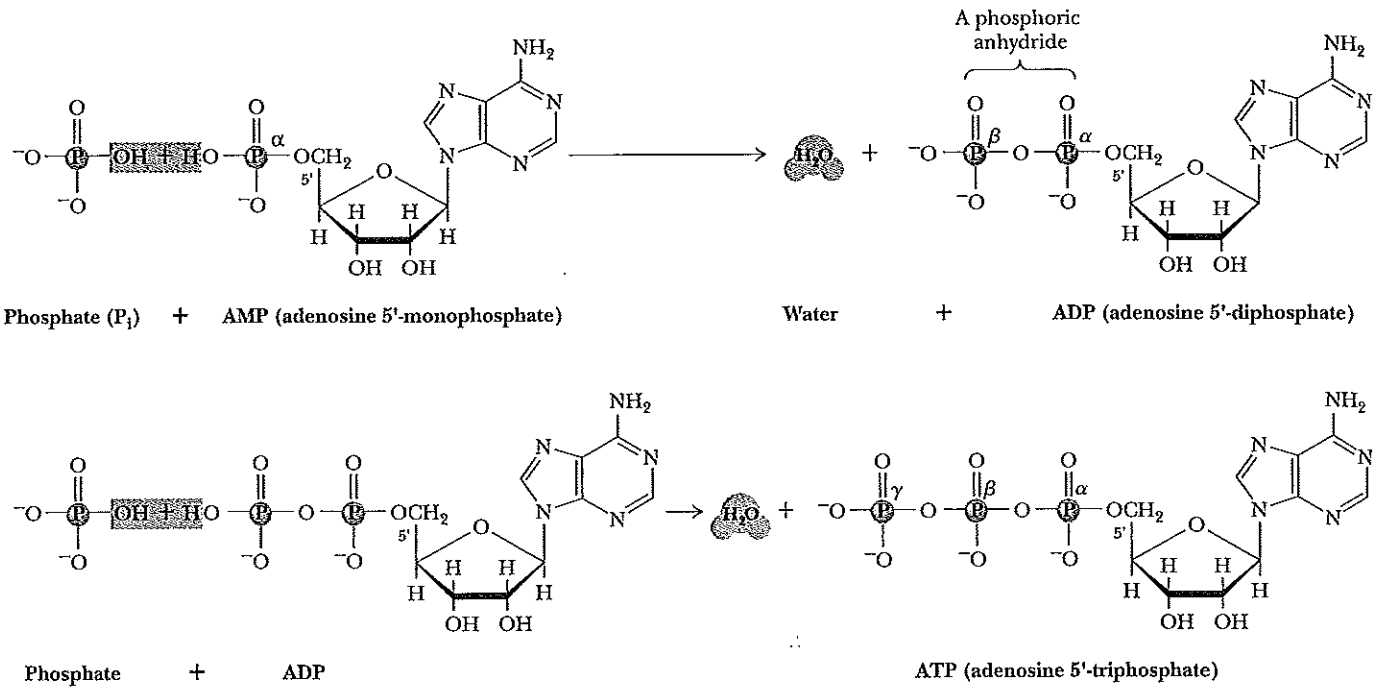


FIGURE 10.15 Formation of ADP and ATP by the successive addition of phosphate groups via phosphoric anhydride linkages. Note the removal of equivalents of H_2O in these dehydration synthesis reactions.

anions on NDPs and NTPs form stable complexes with divalent cations such as Mg^{2+} and Ca^{2+}. Because Mg^{2+} is present at high concentrations (as much as 40 m*M*) intracellularly, NDPs and NTPs occur primarily as Mg^{2+} complexes in the cell. The phosphoric anhydride linkages in NDPs and NTPs are readily hydrolyzed by acid, liberating inorganic phosphate (often symbolized as P_i) and the corresponding NMP. A diagnostic test for NDPs and NTPs is quantitative liberation of P_i upon treatment with 1 *N* HCl at 100°C for 7 minutes.

Nucleoside 5′-Triphosphates Are Carriers of Chemical Energy

Nucleoside 5′-triphosphates are indispensable agents in metabolism because the phosphoric anhydride bonds they possess are a prime source of chemical energy to do biological work. ATP has been termed the energy currency of the cell (see Chapter 3). GTP is the major energy source for protein synthesis (see Chapter 30), CTP is an essential metabolite in phospholipid synthesis (see Chapter 24), and UTP forms activated intermediates with sugars that go on to serve as substrates in the biosynthesis of complex carbohydrates and polysaccharides (see Chapter 22). The evolution of metabolism has led to the dedication of one of these four NTPs to each of these major branches of metabolism. To complete the picture, the four NTPs and their dNTP counterparts are the substrates for the synthesis of the remaining great class of biomolecules—the nucleic acids.

The Bases of Nucleotides Serve as "Information Symbols"

Are the bases of nucleotides directly involved in the biochemistry of metabolism? Not really. Virtually all of the biochemical reactions of nucleotides involve either *phosphate* or *pyrophosphate group transfer:* the release of a phosphoryl group from an NTP to give an NDP, the release of a pyrophosphoryl group to give an NMP unit, or the acceptance of a phosphoryl group by an NMP or an NDP to give an NDP or an NTP (Figure 10.16). Interestingly, the pentose and the base are *not* directly involved in this chemistry. However, as noted, a "division of labor" directs ATP to serve as the primary nucleotide in central pathways of energy metabolism, while GTP, for example, is used to drive protein synthesis. Thus, the various nucleotides are channeled in appropriate metabolic

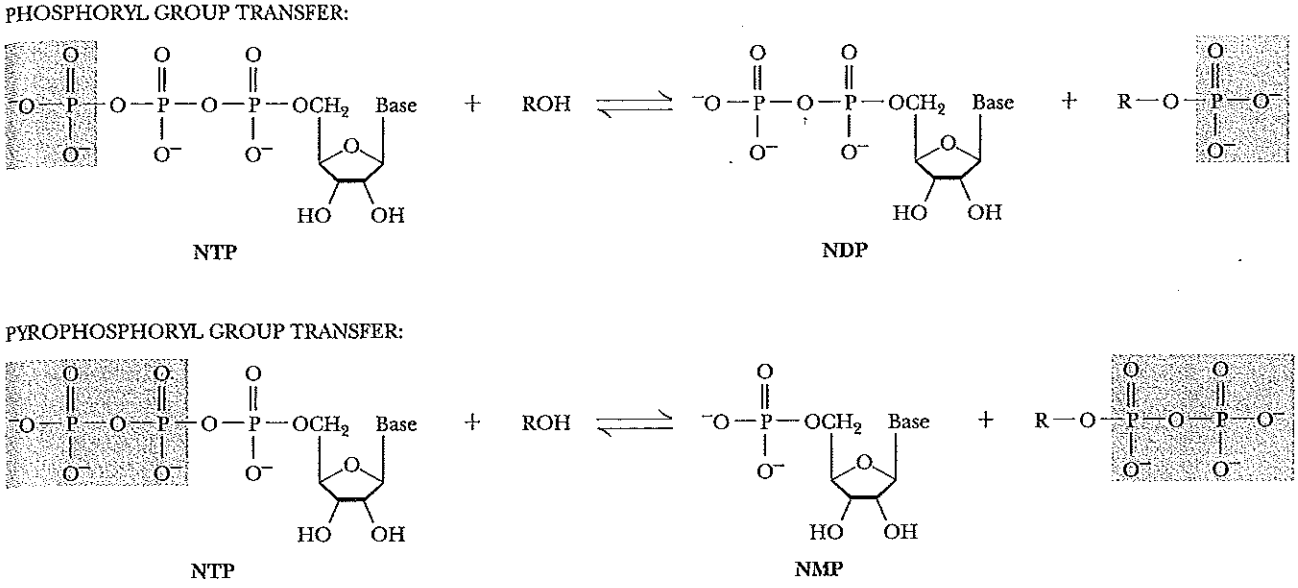


FIGURE 10.16 Phosphoryl and pyrophosphoryl group transfer, the major biochemical reactions of nucleotides.

directions through specific recognition of the base of the nucleotide. That is, the bases of nucleotides serve as *information symbols,* never participating directly in the covalent bond chemistry that goes on. This role as information symbols extends to nucleotide polymers, the nucleic acids, where the bases serve as the information symbols for the code of genetic information.

10.4 What Are Nucleic Acids?

Nucleic acids are **polynucleotides:** linear polymers of nucleotides linked 3′ to 5′ by **phosphodiester bridges** (Figure 10.17). They are formed as 5′-nucleoside monophosphates are successively added to the 3′-OH group of the preceding nucleotide, a process that gives the polymer a directional sense. Polymers of ribonucleotides are named **ribonucleic acid,** or **RNA.** Deoxyribonucleotide polymers are called **deoxyribonucleic acid,** or **DNA.** Because C-1′ and C-4′ in deoxyribonucleotides are involved in furanose ring formation and because there is no 2′-OH, only the 3′- and 5′-hydroxyl groups are available for internucleotide phosphodiester bonds. In the case of DNA, a polynucleotide chain may contain hundreds of millions of nucleotide units. *The convention in all notations of nucleic acid structure is to read the polynucleotide chain from the 5′-end of the polymer to the 3′-end.* Note that this reading direction actually passes through each phosphodiester from 3′ to 5′ (Figure 10.18). A repetitious uniformity exists in the covalent backbone of polynucleotides.

The Base Sequence of a Nucleic Acid Is Its Distinctive Characteristic

The only significant variation that commonly occurs in the chemical structure of nucleic acids is the nature of the base at each nucleotide position. These bases are not part of the sugar–phosphate backbone but instead serve as distinctive side chains, much like the R groups of amino acids along a polypeptide backbone. They give the polymer its unique identity. A simple notation for nucleic acid structures is merely to list the order of bases in the polynucleotide using single capital letters—A, G, C, and U (or T). Occasionally, a lowercase "p" is written between each successive base to indicate the phosphodiester bridge, as in GpApCpGpUpA. A "p" preceding the sequence indicates that the nucleic acid carries a PO_4 on its 5′-end, as in pGpApCpGpUpA; a "p" terminating the sequence connotes the presence of a phosphate on the 3′-OH end, as in GpApCpGpUpAp.

The Synthesis and Degradation of Nucleotides

Essential Question

Virtually all cells are capable of synthesizing purine and pyrimidine nucleotides. These compounds then serve as essential intermediates in metabolism and as the building blocks for DNA and RNA synthesis. ***How do cells synthesize purines and pyrimidines?***

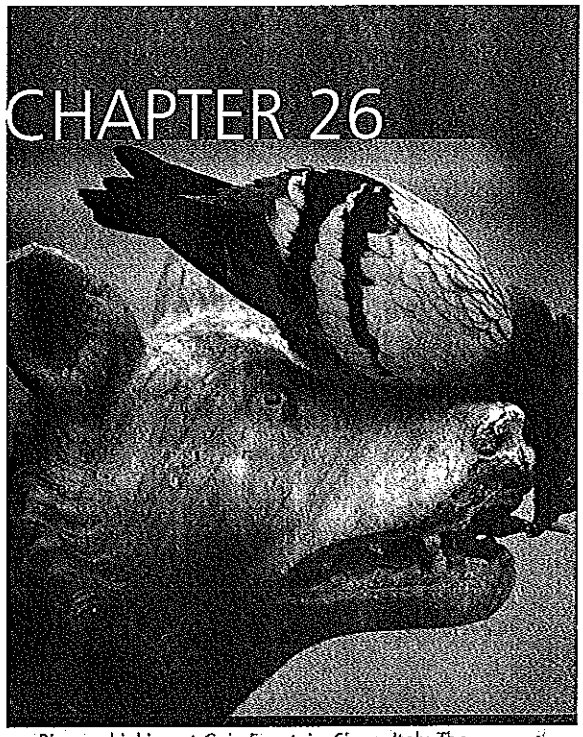
Nucleotides are ubiquitous constituents of life, actively participating in the majority of biochemical reactions. Recall that ATP is the "energy currency" of the cell, that uridine nucleotide derivatives of carbohydrates are common intermediates in cellular transformations of carbohydrates (see Chapter 22), and that biosynthesis of phospholipids proceeds via cytosine nucleotide derivatives (see Chapter 24). In Chapter 30, we will see that GTP serves as the immediate energy source driving the endergonic reactions of protein synthesis. Many of the coenzymes (such as coenzyme A, NAD, NADP, and FAD) are derivatives of nucleotides. Nucleotides also act in metabolic regulation, as in the response of key enzymes of intermediary metabolism to the relative concentrations of AMP, ADP, and ATP (PFK is a prime example here; see also Chapter 18). Furthermore, cyclic derivatives of purine nucleotides such as cAMP and cGMP have no other role in metabolism than regulation. Last but not least, nucleotides are the monomeric units of nucleic acids. Deoxynucleoside triphosphates (dNTPs) and nucleoside triphosphates (NTPs) serve as the immediate substrates for the biosynthesis of DNA and RNA, respectively (see Part 4).

26.1 Can Cells Synthesize Nucleotides?

Nearly all organisms can make the purine and pyrimidine nucleotides via so-called de novo biosynthetic pathways. (*De novo* means "anew"; a less literal but more apt translation might be "from scratch" because de novo pathways are metabolic sequences that form complex end products from rather simple precursors.) Many organisms also have salvage pathways to recover purine and pyrimidine compounds obtained in the diet or released during nucleic acid turnover and degradation. Whereas the ribose of nucleotides can be catabolized to generate energy, the nitrogenous bases do *not* serve as energy sources; their catabolism does not lead to products used by pathways of energy conservation. Compared to slowly dividing cells, rapidly proliferating cells synthesize larger amounts of DNA and RNA per unit time. To meet the increased demand for nucleic acid synthesis, substantially greater quantities of nucleotides must be produced. The pathways of nucleotide biosynthesis thus become attractive targets for the clinical control of rapidly dividing cells such as cancers or infectious bacteria. Many antibiotics and anticancer drugs are inhibitors of purine or pyrimidine nucleotide biosynthesis.

26.2 How Do Cells Synthesize Purines?

Substantial insight into the de novo pathway for purine biosynthesis was provided in 1948 by John Buchanan, who cleverly exploited the fact that birds excrete excess nitrogen principally in the form of uric acid, a water-insoluble purine analog. Buchanan fed isotopically labeled compounds to pigeons and then examined the distribution of the labeled atoms in *uric acid* (Figure 26.1). By tracing the metabolic source of the various atoms in this end product, he

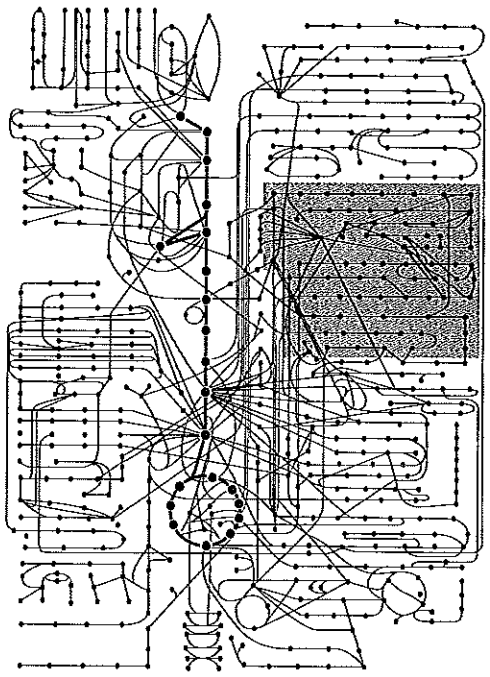


Pigeon drinking at Gaia Fountain, Siena, Italy. The basic features of purine biosynthesis were elucidated initially from metabolic studies of nitrogen metabolism in pigeons. Pigeons excrete excess N as uric acid, a purine analog.

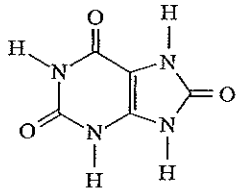
Guano, a substance found on some coasts frequented by sea birds, is composed chiefly of the birds' partially decomposed excrement.... The name for the purine guanine derives from the abundance of this base in guano.

J. C. Nesbit, *On Agricultural Chemistry and the Nature and Properties of Peruvian Guano* (1850)

Key Questions



The synthesis and degradation of nucleotides



Uric acid

FIGURE 26.1 Nitrogen waste is excreted by birds principally as the purine analog, uric acid.

showed that the nine atoms of the purine ring system (Figure 26.2) are contributed by aspartic acid (N-1), glutamine (N-3 and N-9), glycine (C-4, C-5, and N-7), CO_2 (C-6), and THF one-carbon derivatives (C-2 and C-8). The coenzyme THF and its role in one-carbon metabolism were introduced in Chapter 17.

Inosinic Acid (IMP) Is the Immediate Precursor to GMP and AMP

The de novo synthesis of purines occurs in an interesting manner: The atoms forming the purine ring are successively added to *ribose-5-phosphate;* thus, purines are directly synthesized as nucleotide derivatives by assembling the atoms that comprise the purine ring system directly on the ribose. In step 1, ribose-5-phosphate is activated via the direct transfer of a pyrophosphoryl group from ATP to C-1 of the ribose, yielding *5-phosphoribosyl-α-pyrophosphate (PRPP)* (Figure 26.3). The enzyme is **ribose-5-phosphate pyrophosphokinase.** PRPP is the limiting substance in purine biosynthesis. The two major purine nucleoside diphosphates, ADP and GDP, are negative effectors of ribose-5-phosphate pyrophosphokinase. However, because PRPP serves additional metabolic needs, the next reaction is actually the committed step in the pathway.

Step 2 (Figure 26.3) is catalyzed by **glutamine phosphoribosyl pyrophosphate amidotransferase.** The anomeric carbon atom of the substrate PRPP is in the α-configuration; the product is a β-glycoside (recall that all the biologically important nucleotides are β-glycosides). The N atom of this *N*-glycoside becomes N-9 of the nine-membered purine ring; it is the first atom added in the construction of this ring. Glutamine phosphoribosyl pyrophosphate amidotransferase is subject to feedback inhibition by GMP, GDP, and GTP as well as AMP, ADP, and ATP. The G series of nucleotides interacts at a guanine-specific allosteric site on the enzyme, whereas the adenine nucleotides act at an A-specific site. The pattern of inhibition by these nucleotides is competitive, thus ensuring that residual enzyme activity is expressed until sufficient amounts of both adenine and guanine nucleotides are synthesized. Glutamine phosphoribosyl pyrophosphate amidotransferase is also sensitive to inhibition by the glutamine analog **azaserine** (Figure 26.4). Azaserine has been used as an antitumor agent because it causes inactivation of glutamine-dependent enzymes in the purine biosynthetic pathway.

Step 3 is carried out by **glycinamide ribonucleotide synthetase** *(GAR synthetase)* via its ATP-dependent condensation of the glycine carboxyl group with the amine of *5-phosphoribosyl-β-amine* (Figure 26.3). The reaction proceeds in two stages. First, the glycine carboxyl group is activated via ATP-dependent phosphorylation. Next, an amide bond is formed between the activated carboxyl group of glycine and the β-amine. Glycine contributes C-4, C-5, and N-7 of the purine.

Step 4 is the first of two THF-dependent reactions in the purine pathway. **GAR transformylase** transfers the N^{10}-formyl group of N^{10}-formyl-THF to the free amino group of GAR to yield *α-N-formylglycinamide ribonucleotide (FGAR).* Thus, C-8 of the purine is "formyl-ly" introduced. Although all of the atoms of the imidazole portion of the purine ring are now present, the ring is not closed until Reaction 6.

Step 5 is catalyzed by **FGAR amidotransferase** (also known as *FGAM synthetase*). ATP-dependent transfer of the glutamine amido group to the C-4-carbonyl of FGAR yields *formylglycinamidine ribonucleotide (FGAM).* As a glutamine-dependent

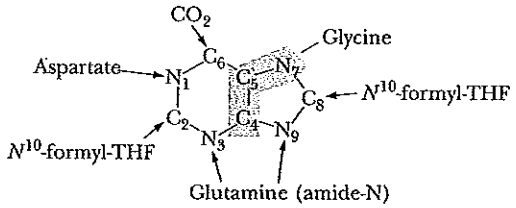


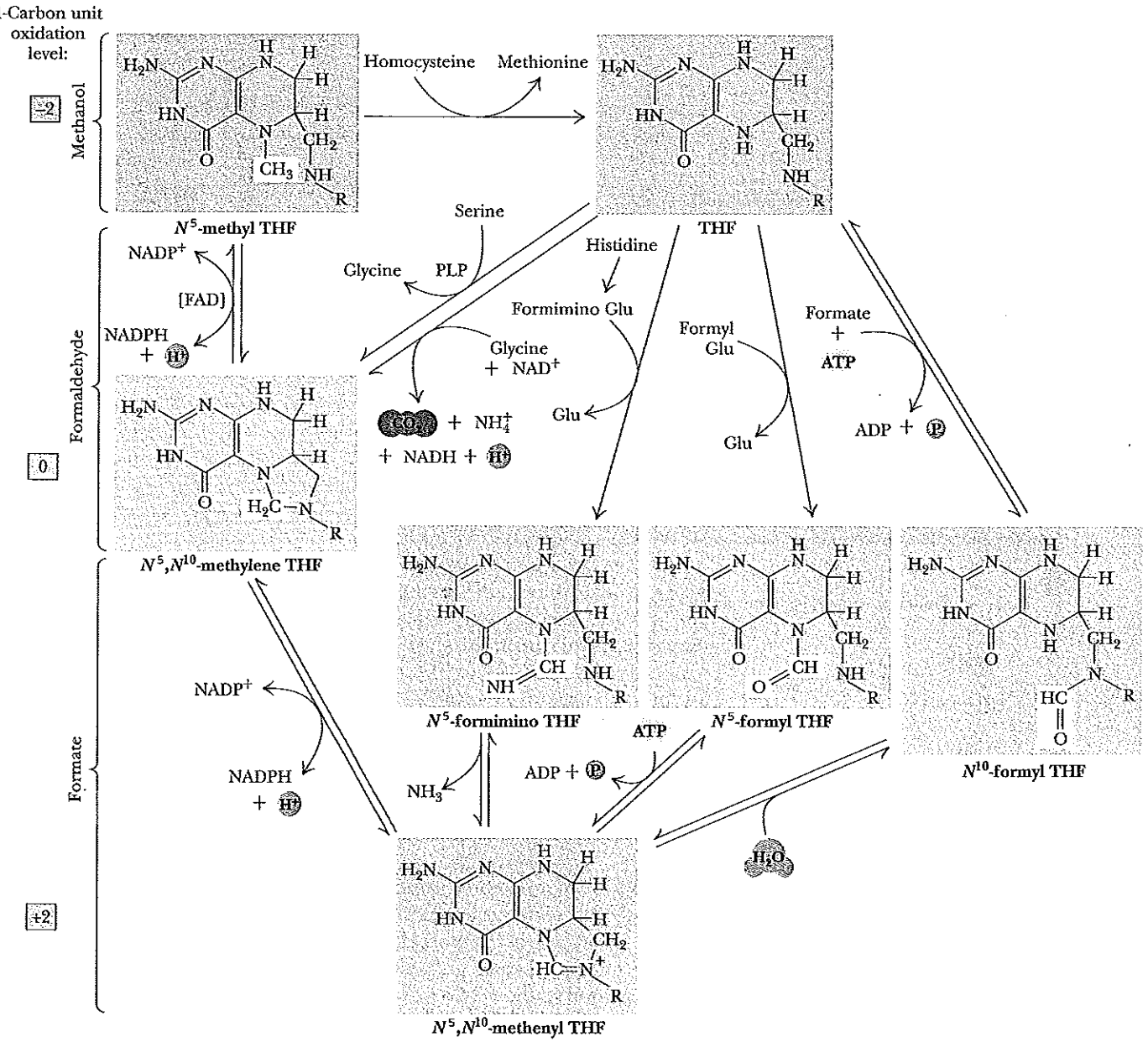
FIGURE 26.2 The metabolic origin of the nine atoms in the purine ring system.

A Deeper Look

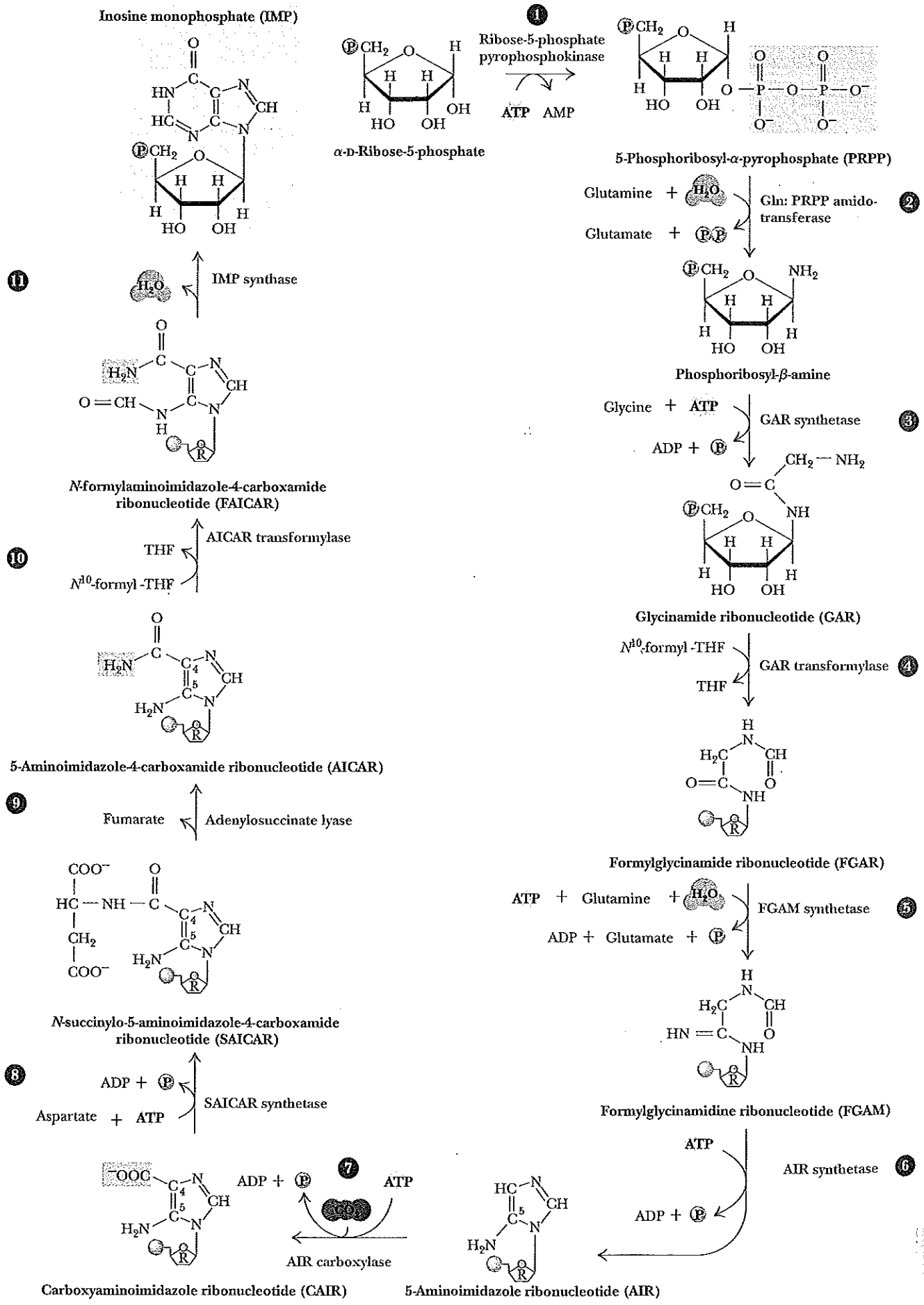
Tetrahydrofolate (THF) and One-Carbon Units

An elaborate ensemble of enzymatic reactions serves to introduce one-carbon units into THF and to interconvert the various oxidation states (see accompanying figure and Table 17.6). N^5-methyltetrahydrofolate can be oxidized directly to N^5,N^{10}-methylenetetrahydrofolate, which can be further oxidized to N^5,N^{10}-methenyltetrahydrofolate. The N^5-formimino-, N^5-formyl-, and N^{10}-formyltetrahydrofolates can be formed from N^5,N^{10}-methenyltetrahydrofolate (all of these being at the same oxidation level), or they can be formed by one-carbon addition reactions from tetrahydrofolate itself. The principal pathway for incorporation of one-carbon units into tetrahydrofolate is the **serine hydroxymethyltransferase** reaction (see Figure 25.32), which converts serine to glycine and forms N^5,N^{10}-methylenetetrahydrofolate.

The biosynthetic pathways for methionine, purines (Figure 26.3), and the pyrimidine thymine (see Figure 26.27) all rely on the incorporation of one-carbon units from tetrahydrofolate derivatives. N^{10}-formyl-THF is the source of carbons 2 and 8 of the purine ring and N^5,N^{10}-methylene-THF, the 5-CH_3 group of the pyrimidine thymine.



▲ The reactions that introduce one-carbon units into tetrahydrofolate (THF) link seven different folate intermediates that carry one-carbon units in three different oxidation states (−2, 0, and +2). *(Adapted from Brody, T., et al., in Machlin, L. J., 1984.* Handbook of Vitamins. *New York: Marcel Dekker.)*


Inosine monophosphate (IMP)
α-D-Ribose-5-phosphate
1
Ribose-5-phosphate pyrophosphokinase
ATP AMP
5-Phosphoribosyl-α-pyrophosphate (PRPP)
Glutamine + H2O
Glutamate + PP
Gln: PRPP amido-transferase
2
Phosphoribosyl-β-amine
Glycine + ATP
ADP + P
GAR synthetase
3
Glycinamide ribonucleotide (GAR)
N10-formyl -THF
THF
GAR transformylase
4
Formylglycinamide ribonucleotide (FGAR)
ATP + Glutamine + H2O
ADP + Glutamate + P
FGAM synthetase
5
Formylglycinamidine ribonucleotide (FGAM)
ATP
ADP + P
AIR synthetase
6
5-Aminoimidazole ribonucleotide (AIR)
ADP + P
ATP
AIR carboxylase
7
Carboxyaminoimidazole ribonucleotide (CAIR)
Aspartate + ATP
ADP + P
SAICAR synthetase
8
N-succinylo-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR)
Fumarate
Adenylosuccinate lyase
9
5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)
N10-formyl -THF
THF
AICAR transformylase
10
N-formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)
H2O
IMP synthase
11

Biochemistry Now™ ACTIVE FIGURE 26.3 The de novo pathway for purine synthesis. The first purine product of this pathway, IMP (inosinic acid or inosine monophosphate), serves as a precursor to AMP and GMP. **Step 1:** PRPP synthesis from ribose-5-phosphate and ATP by ribose-5-phosphate pyrophosphokinase. **Step 2:** 5-Phosphoribosyl-β-1-amine synthesis from α-PRPP, glutamine, and H_2O by glutamine phosphoribosyl pyrophosphate amidotransferase. **Step 3:** Glycinamide ribonucleotide (GAR) synthesis from glycine, ATP, and 5-phosphoribosyl-β-amine by glycinamide ribonucleotide synthetase. **Step 4:** Formylglycinamide ribonucleotide synthesis from N^{10}-formyl-THF and GAR by GAR transformylase. **Step 5:** Formylglycinamidine ribonucleotide (FGAM) synthesis from FGAR, ATP, glutamine, and H_2O by FGAM synthetase (FGAR amidotransferase). The other products are ADP, P_i, and glutamate. **Step 6:** 5-Aminoimidazole ribonucleotide (AIR) synthesis is achieved via the ATP-dependent closure of the imidazole ring, as catalyzed by FGAM cyclase (AIR synthetase). (Note that the ring closure changes the numbering system.) **Step 7:** Carboxyaminoimidazole ribonucleotide (CAIR) synthesis from CO_2, ATP, and AIR by AIR carboxylase. **Step 8:** *N*-succinylo-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR) synthesis from aspartate, CAIR, and ATP by SAICAR synthetase. **Step 9:** 5-Aminoimidazole carboxamide ribonucleotide (AICAR) formation by the nonhydrolytic removal of fumarate from SAICAR. The enzyme is adenylosuccinase. **Step 10:** 5-Formylaminoimidazole carboxamide ribonucleotide (FAICAR) formation from AICAR and N^{10}-formyl-THF by AICAR transformylase. **Step 11:** Dehydration/ring closure yields the authentic purine ribonucleotide IMP. The enzyme is IMP synthase. **Test yourself on the concepts in this figure at http://chemistry.brookscole.com/ggb3**

enzyme, FGAR amidotransferase is, like glutamine phosphoribosyl pyrophosphate amidotransferase (Reaction 2), irreversibly inactivated by azaserine. The imino-N becomes N-3 of the purine.

Step 6 is an ATP-dependent dehydration that leads to formation of the imidazole ring. ATP is used to phosphorylate the oxygen atom of the formyl group, activating it for the ring closure step that follows. Because the product is *5-aminoimidazole ribonucleotide,* or *AIR,* this enzyme is called **AIR synthetase.** In avian liver, the enzymatic activities for steps 3, 4, and 6 (GAR synthetase, GAR transformylase, and AIR synthetase) reside on a single, 110-kD multifunctional polypeptide.

In step 7, carbon dioxide is added at the C-4 position of the imidazole ring by **AIR carboxylase** in an ATP-dependent reaction; the carbon of CO_2 will become C-6 of the purine ring. The product is *carboxyaminoimidazole ribonucleotide (CAIR).*

In step 8, the amino-N of aspartate provides N-1 through linkage to the C-6 carboxyl function of CAIR. ATP hydrolysis drives the condensation of Asp with CAIR. The product is *N-succinylo-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR).* **SAICAR synthetase** catalyzes the reaction. The enzymatic activities for steps 7 and 8 reside on a single, bifunctional polypeptide in avian liver.

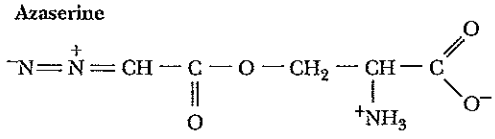
Step 9 removes the four carbons of Asp as fumarate in a nonhydrolytic cleavage. The product is *5-aminoimidazole-4-carboxamide ribonucleotide (AICAR);* the enzyme is **adenylosuccinase** *(adenylosuccinate lyase).* Adenylosuccinase acts again in that part of the purine pathway leading from IMP to AMP and takes its name from this latter reaction (see following). AICAR is also an intermediate in the histidine biosynthetic pathway (see Chapter 25), but because ATP is the precursor to AICAR in that pathway, no net purine synthesis is achieved.

Step 10 adds the formyl carbon of N^{10}-formyl-THF as the ninth and last atom necessary for forming the purine nucleus. The enzyme is called **AICAR transformylase;** the products are THF and *N-formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR).*

Step 11 involves dehydration and ring closure and completes the initial phase of purine biosynthesis. The enzyme is **IMP cyclohydrolase** (also known as *IMP synthase* and *inosinicase*). Unlike step 6, this ring closure does not require ATP. In avian liver, the enzymatic activities catalyzing steps 10 and 11 (AICAR transformylase and inosinicase) activities reside on 67-kD bifunctional polypeptides organized into 135-kD dimers.

Note that 6 ATPs are required in the purine biosynthetic pathway from ribose-5-phosphate to IMP: one each at steps 1, 3, 5, 6, 7, and 8. However, 7 high-energy phosphate bonds (equal to 7 ATP equivalents) are consumed because α-PRPP formation in Reaction 1 followed by PP_i release in Reaction 2 represents the loss of 2 ATP equivalents.

Azaserine



Glutamine

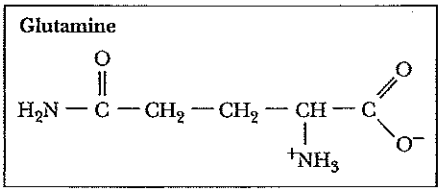
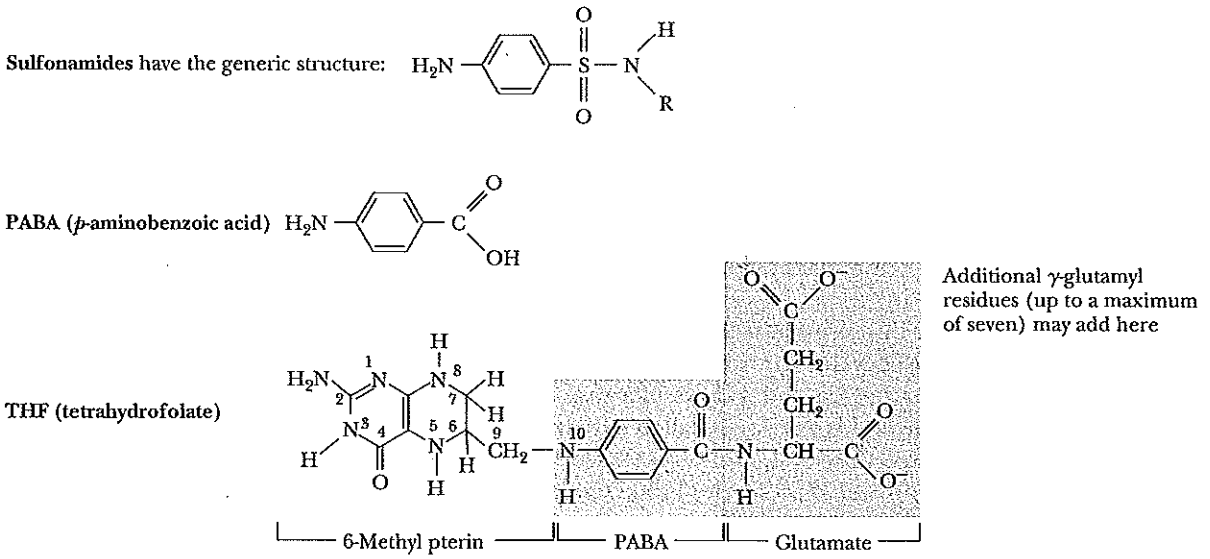


FIGURE 26.4 The structure of azaserine. Azaserine acts as an irreversible inhibitor of glutamine-dependent enzymes by covalently attaching to nucleophilic groups in the glutamine-binding site.

Human Biochemistry

Folate Analogs as Anticancer and Antimicrobial Agents

The dependence of de novo purine biosynthesis on folic acid compounds at steps 4 and 10 means that antagonists of folic acid metabolism (for example, methotrexate; see Figure 26.28) indirectly inhibit purine formation and, in turn, nucleic acid synthesis, cell growth, and cell division. Clearly, rapidly dividing cells such as malignancies or infective bacteria are more susceptible to these antagonists than slower-growing normal cells. Also among the folic acid antagonists are *sulfonamides* (see accompanying figure). Folic acid is a vitamin for animals and is obtained in the diet. In contrast, bacteria synthesize folic acid from precursors, including *p-aminobenzoic acid (PABA),* and thus are more susceptible to sulfonamides than are animal cells.



▲ Sulfa drugs, or sulfonamides, owe their antibiotic properties to their similarity to *p*-aminobenzoate (PABA), an important precursor in folic acid synthesis. Sulfonamides block folic acid formation by competing with PABA.

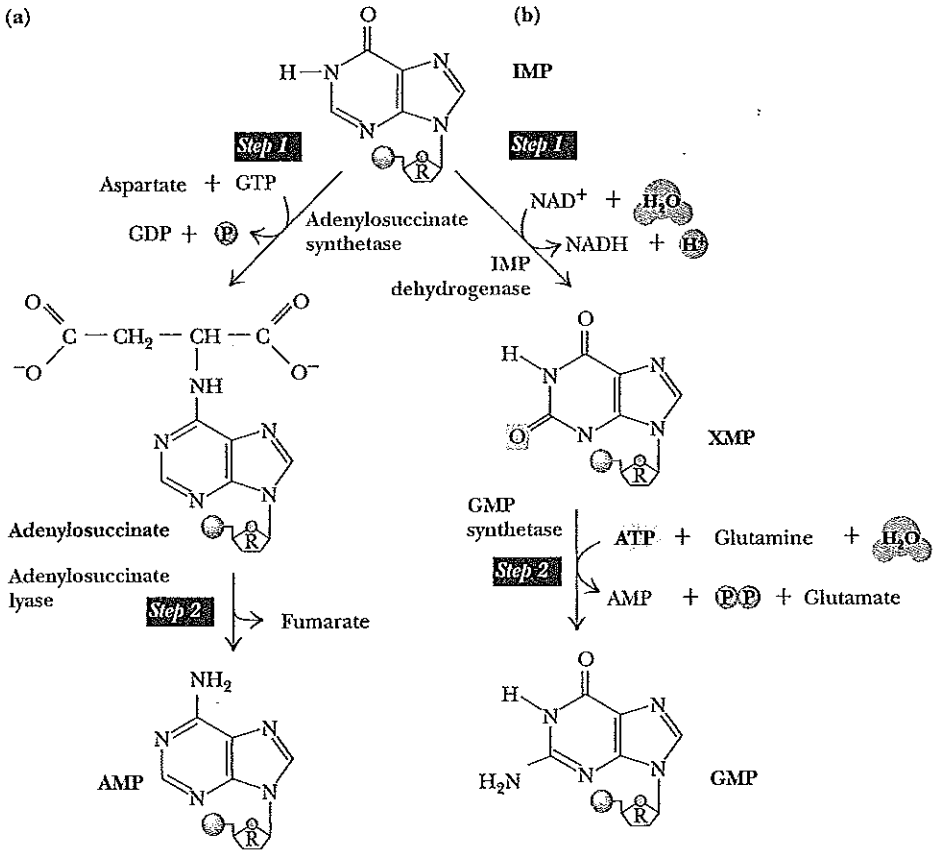
AMP and GMP Are Synthesized from IMP

IMP is the precursor to both AMP and GMP. These major purine nucleotides are formed via distinct two-step metabolic pathways that diverge from IMP. The branch leading to AMP (adenosine 5′-monophosphate) involves the displacement of the 6-O group of inosine with aspartate (Figure 26.5) in a GTP-dependent reaction, followed by the nonhydrolytic removal of the four-carbon skeleton of Asp as fumarate; the Asp amino group remains as the 6-amino group of AMP. **Adenylosuccinate synthetase** and **adenylosuccinase** are the two enzymes. Recall that adenylosuccinase also acted at step 9 in the pathway from ribose-5-phosphate to IMP. Fumarate production provides a connection between purine synthesis and the citric acid cycle.

The formation of GMP from IMP requires oxidation at C-2 of the purine ring, followed by a glutamine-dependent amidotransferase reaction that replaces the oxygen on C-2 with an amino group to yield *2-amino,6-oxy purine nucleoside monophosphate,* or as this compound is commonly known, *guanosine monophosphate.* The enzymes in the GMP branch are **IMP dehydrogenase** and **GMP synthetase.** Note that, starting from ribose-5-phosphate, 8 ATP equivalents are consumed in the synthesis of AMP and 9 in the synthesis of GMP.

The Purine Biosynthetic Pathway Is Regulated at Several Steps

The regulatory network that controls purine synthesis is schematically represented in Figure 26.6. To recapitulate, the purine biosynthetic pathway from ribose-5-phosphate to IMP is allosterically regulated at the first two steps. Ribose-



Biochemistry Now™ ANIMATED FIGURE 26.5
The synthesis of AMP and GMP from IMP. **(a)** AMP synthesis: The two reactions of AMP synthesis mimic steps 8 and 9 in the purine pathway leading to IMP. In **step 1**, the 6-*O* of inosine is displaced by aspartate to yield adenylosuccinate. The energy required to drive this reaction is derived from GTP hydrolysis. The enzyme is adenylosuccinate synthetase. AMP is a competitive inhibitor (with respect to the substrate IMP) of adenylosuccinate synthetase. In **step 2**, adenylosuccinase (also known as adenylosuccinate lyase, the same enzyme catalyzing step 9 in the purine pathway) carries out the nonhydrolytic removal of fumarate from adenylosuccinate, leaving AMP. **(b)** GMP synthesis: The two reactions of GMP synthesis are an NAD^+-dependent oxidation followed by an amidotransferase reaction. In **step 1**, IMP dehydrogenase employs the substrates NAD^+ and H_2O in catalyzing oxidation of IMP at C-2. The products are xanthylic acid (XMP or xanthosine monophosphate), NADH, and H^+. GMP is a competitive inhibitor (with respect to IMP) of IMP dehydrogenase. In **step 2**, transfer of the amido-N of glutamine to the C-2 position of XMP yields GMP. This ATP-dependent reaction is catalyzed by GMP synthetase. Besides GMP, the products are glutamate, AMP, and PP_i. Hydrolysis of PP_i to two P_i by ubiquitous pyrophosphatases pulls this reaction to completion. **See this figure animated at http://chemistry.brookscole.com/ggb3**

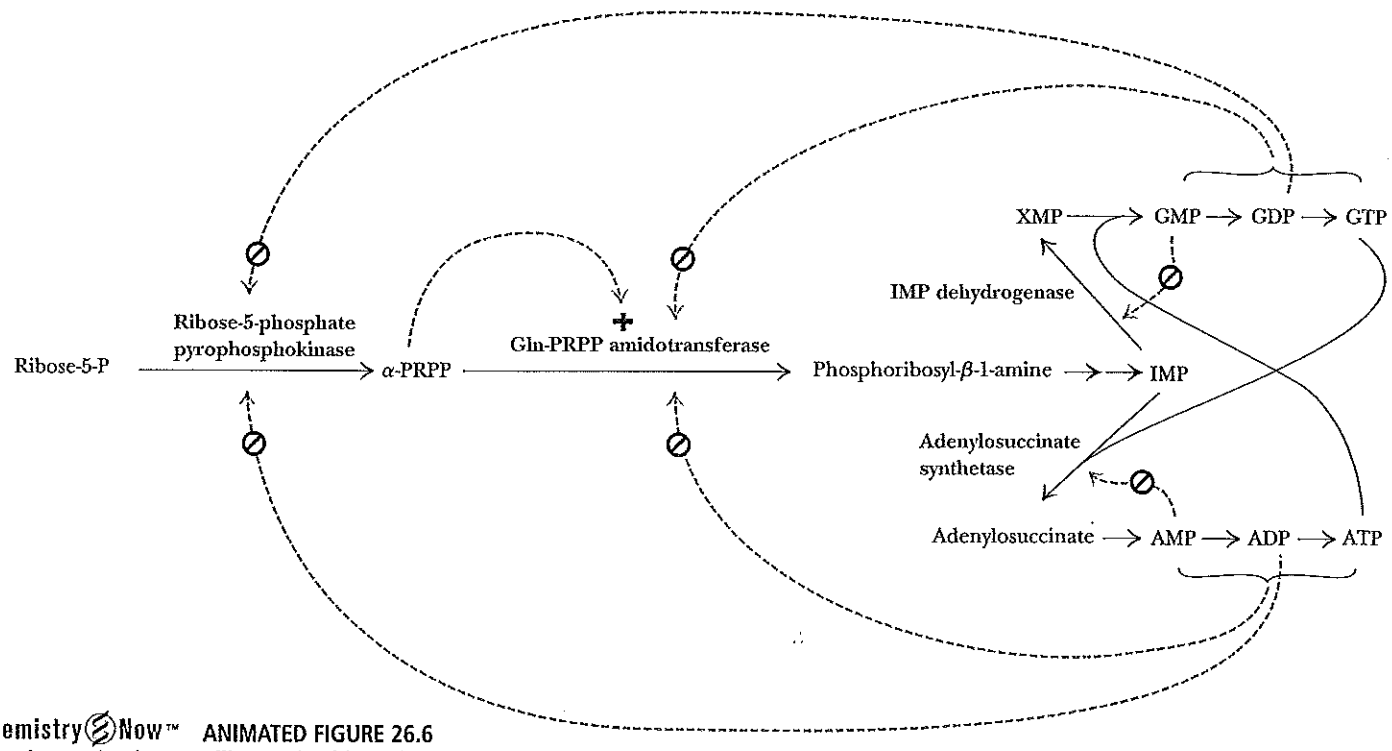
5-phosphate pyrophosphokinase, although not the committed step in purine synthesis, is subject to feedback inhibition by ADP and GDP. The enzyme catalyzing the next step, glutamine phosphoribosyl pyrophosphate amidotransferase, has two allosteric sites, one where the "A" series of nucleoside phosphates (AMP, ADP, and ATP) binds and feedback-inhibits, and another where the corresponding "G" series binds and inhibits. Furthermore, PRPP is a "feed-forward" activator of this enzyme. Thus, the rate of IMP formation by this pathway is governed by the levels of the final end products, the adenine and guanine nucleotides.

The purine pathway splits at IMP. The first enzyme in the AMP branch, adenylosuccinate synthetase, is competitively inhibited by AMP. Its counterpart in the GMP branch, IMP dehydrogenase, is inhibited in a similar fashion by GMP. Thus, the fate of IMP is determined by the relative levels of AMP and GMP, so any deficiency in the amount of either of the principal purine nucleotides is self-correcting. This reciprocity of regulation is an effective mechanism for balancing the formation of AMP and GMP to satisfy cellular needs. Note also that reciprocity is even manifested at the level of energy input: GTP provides the energy to drive AMP synthesis, whereas ATP serves this role in GMP synthesis (Figure 26.6).

ATP-Dependent Kinases Form Nucleoside Diphosphates and Triphosphates from the Nucleoside Monophosphates

The products of de novo purine biosynthesis are the nucleoside monophosphates AMP and GMP. These nucleotides are converted by successive phosphorylation reactions into their metabolically prominent triphosphate forms, ATP and GTP. The first phosphorylation, to give the nucleoside diphosphate forms, is carried out by two base-specific, ATP-dependent kinases, **adenylate kinase** and **guanylate kinase.**

Adenylate kinase: $AMP + ATP \longrightarrow 2\ ADP$
Guanylate kinase: $GMP + ATP \longrightarrow GDP + ADP$



Biochemistry Now™ ANIMATED FIGURE 26.6
The regulatory circuit controlling purine biosynthesis. ADP and GDP are feedback inhibitors of ribose-5-phosphate pyrophosphokinase, the first reaction in the pathway. The second enzyme, glutamine phosphoribosyl pyrophosphate amidotransferase, has two distinct feedback inhibition sites, one for A nucleotides and one for G nucleotides. Also, this enzyme is allosterically activated by PRPP. In the branch leading from IMP to AMP, the first enzyme is feedback-inhibited by AMP, while the corresponding enzyme in the branch from IMP to GMP is feedback-inhibited by GMP. Last, ATP is the energy source for GMP synthesis, whereas GTP is the energy source for AMP synthesis. **See this figure animated at http://chemistry.brookscole.com/ggb3**

These nucleoside monophosphate kinases also act on deoxynucleoside monophosphates to give dADP or dGDP.

Oxidative phosphorylation (see Chapter 20) is primarily responsible for the conversion of ADP into ATP. ATP then serves as the phosphoryl donor for synthesis of the other nucleoside triphosphates from their corresponding NDPs in a reaction catalyzed by **nucleoside diphosphate kinase,** a nonspecific enzyme. For example,

$$GDP + ATP \rightleftharpoons GTP + ADP$$

Because this enzymatic reaction is readily reversible and nonspecific with respect to both phosphoryl acceptor and donor, in effect any NDP can be phosphorylated by any NTP, and vice versa. The preponderance of ATP over all other nucleoside triphosphates means that, in quantitative terms, it is the principal nucleoside diphosphate kinase substrate. The enzyme does not discriminate between the ribose moieties of nucleotides and thus functions in phosphoryl transfers involving deoxy-NDPs and deoxy-NTPs as well.

26.3 Can Cells Salvage Purines?

Nucleic acid turnover (synthesis and degradation) is an ongoing metabolic process in most cells. Messenger RNA in particular is actively synthesized and degraded. These degradative processes can lead to the release of free purines in the form of adenine, guanine, and hypoxanthine (the base in IMP). These substances represent a metabolic investment by cells. So-called salvage pathways exist to recover them in useful form. Salvage reactions involve resynthesis of nucleotides from bases via **phosphoribosyltransferases.**

$$Base + PRPP \rightleftharpoons nucleoside\text{-}5'\text{-}phosphate + PP_i$$

The subsequent hydrolysis of PP_i to inorganic phosphate by pyrophosphatases renders the phosphoribosyltransferase reaction effectively irreversible.

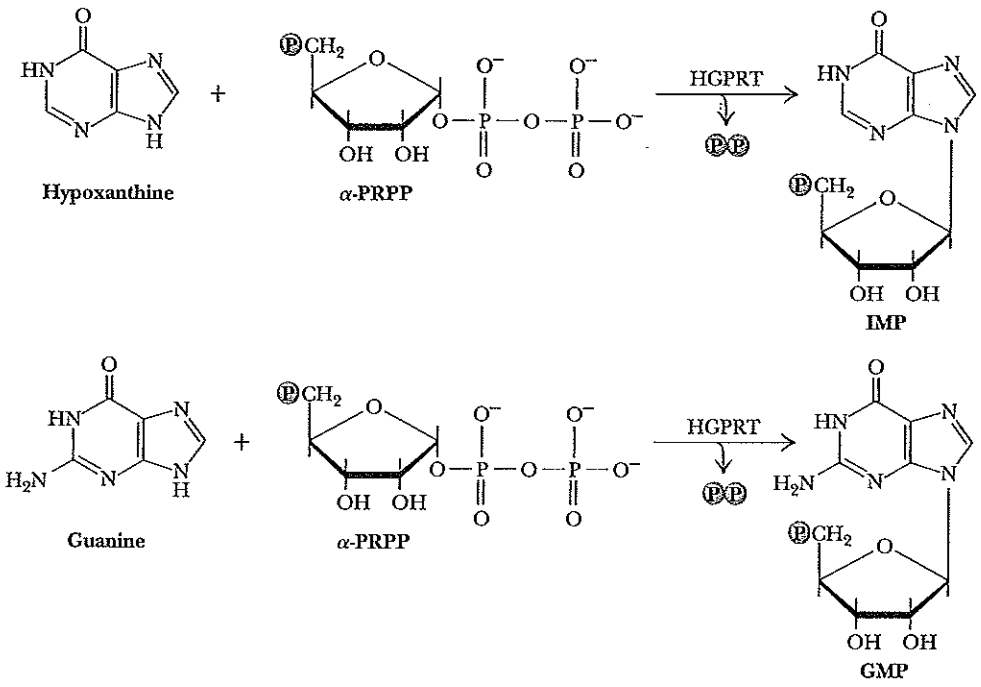


FIGURE 26.7 Purine salvage by the HGPRT reaction.

The purine phosphoribosyltransferases are **adenine phosphoribosyltransferase (APRT)**, which mediates AMP formation, and **hypoxanthine-guanine phosphoribosyltransferase (HGPRT)**, which can act on either hypoxanthine to form IMP or guanine to form GMP (Figure 26.7).

26.4 How Are Purines Degraded?

Because nucleic acids are ubiquitous in cellular material, significant amounts are ingested in the diet. Nucleic acids are degraded in the digestive tract to nucleotides by various nucleases and phosphodiesterases. Nucleotides are then converted to nucleosides by base-specific nucleotidases and nonspecific phosphatases.

$$\text{NMP} + \text{H}_2\text{O} \longrightarrow \text{nucleoside} + \text{P}_\text{i}$$

Nucleosides are hydrolyzed by nucleosidases or nucleoside phosphorylases to release the purine base:

$$\text{Nucleoside} + \text{H}_2\text{O} \xrightarrow{\textit{nucleosidase}} \text{base} + \text{ribose}$$

$$\text{Nucleoside} + \text{P}_\text{i} \xrightarrow{\textit{nucleoside phosphorylase}} \text{base} + \text{ribose-1-P}$$

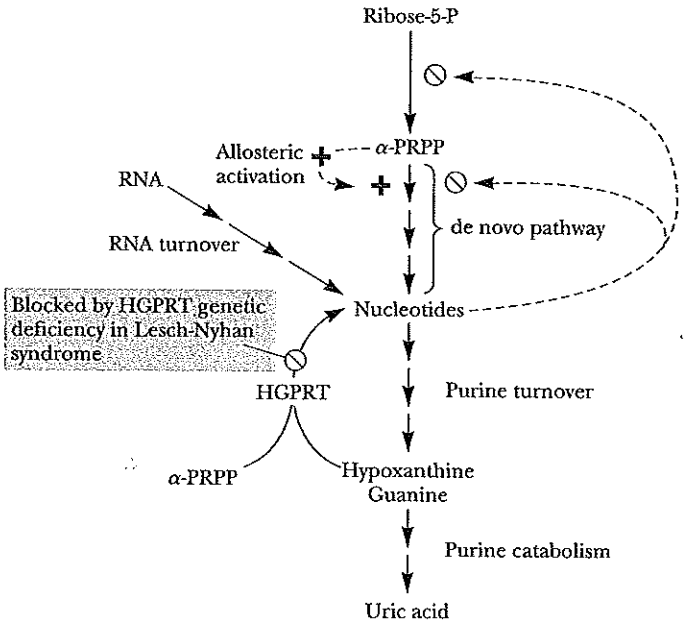
The pentoses liberated in these reactions provide the only source of metabolic energy available from purine nucleotide degradation.

Feeding experiments using radioactively labeled nucleic acids as metabolic tracers have demonstrated that little of the nucleotide ingested in the diet is incorporated into cellular nucleic acids. Dietary purines are converted to uric acid (see following discussion) in the gut and excreted, and pyrimidine nucleosides are inefficiently absorbed into the bloodstream. These findings confirm the de novo pathways of nucleotide biosynthesis as the primary source of nucleic acid precursors. Ingested bases are, for the most part, excreted. Nevertheless, cellular nucleic acids do undergo degradation in the course of the continuous recycling of cellular constituents.

Human Biochemistry

Lesch-Nyhan Syndrome: HGPRT Deficiency Leads to a Severe Clinical Disorder

The symptoms of **Lesch-Nyhan syndrome** are tragic: a crippling gouty arthritis due to excessive uric acid accumulation (uric acid is a purine degradation product, discussed in the next section) and, worse, severe malfunctions in the nervous system that lead to mental retardation, spasticity, aggressive behavior, and self-mutilation. Lesch-Nyhan syndrome results from a complete deficiency in HGPRT activity. The structural gene for HGPRT is located on the X chromosome, and the disease is a congenital, recessive, sex-linked trait manifested only in males. The severe consequences of HGPRT deficiency argue that purine salvage has greater metabolic importance than simply the energy-saving recovery of bases. Although HGPRT might seem to play a minor role in purine metabolism, its absence has profound consequences: De novo purine biosynthesis is dramatically increased, and uric acid levels in the blood are elevated. Presumably, these changes ensue because lack of consumption of PRPP by HGPRT elevates its availability for glutamine-PRPP amidotransferase, enhancing overall de novo purine synthesis and, ultimately, uric acid production (see accompanying figure). Despite these explanations, it remains unclear why deficiency in this single enzyme leads to the particular neurological aberrations characteristic of the syndrome. Fortunately, deficiencies in HGPRT activity in fetal cells can be detected following amniocentesis.

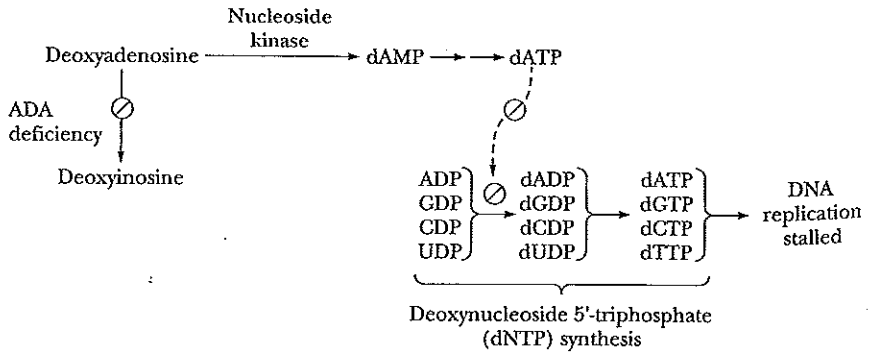


Human Biochemistry

Severe Combined Immunodeficiency Syndrome—A Lack of Adenosine Deaminase Is One Cause of This Inherited Disease

Severe combined immunodeficiency syndrome, or **SCID,** is a group of related inherited disorders characterized by the lack of an immune response to infectious disease. This immunological insufficiency is attributable to the inability of B and T lymphocytes to proliferate and produce antibodies in response to an antigenic challenge. About 30% of SCID patients suffer from a deficiency in the enzyme *adenosine deaminase (ADA).* ADA deficiency is also implicated in a variety of other diseases, including AIDS, anemia, and various lymphomas and leukemias. **Gene therapy,** *the repair of a genetic deficiency through introduction of a functional recombinant version of the gene,* has been attempted on individuals with SCID due to a defective ADA gene (see Section 12.4). ADA is a Zn^{2+}-dependent enzyme, and Zn^{2+} deficiency can also lead to reduced immune function.

In the absence of ADA, deoxyadenosine is not degraded but instead is converted into dAMP and then into dATP. dATP is a potent feedback inhibitor of deoxynucleotide biosynthesis (discussed later in this chapter). Without deoxyribonucleotides, DNA cannot be replicated and cells cannot divide (see accompanying figure). Rapidly proliferating cell types such as lymphocytes are particularly susceptible if DNA synthesis is impaired.



▷ The effect of elevated levels of deoxyadenosine on purine metabolism. If ADA is deficient or absent, deoxyadenosine is not converted into deoxyinosine as normal (see Figure 26.8). Instead, it is salvaged by a nucleoside kinase, which converts it to dAMP, leading to accumulation of dATP and inhibition of deoxynucleotide synthesis (see Figure 26.24). Thus, DNA replication is stalled.

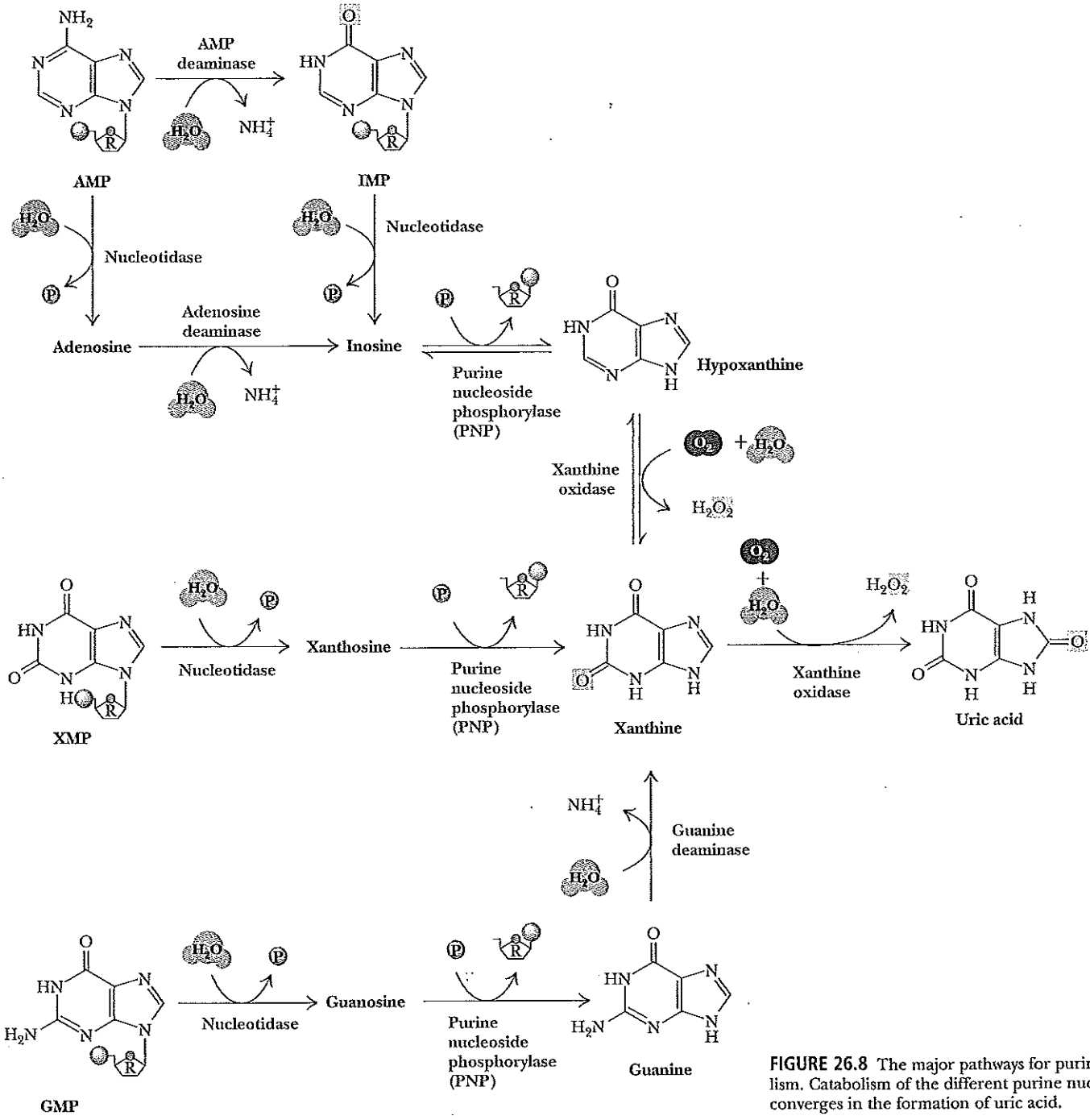


FIGURE 26.8 The major pathways for purine catabolism. Catabolism of the different purine nucleotides converges in the formation of uric acid.

The Major Pathways of Purine Catabolism Lead to Uric Acid

The major pathways of purine catabolism in animals are outlined in Figure 26.8. The various nucleotides are first converted to nucleosides by **intracellular nucleotidases.** These nucleotidases are under strict metabolic regulation to ensure that their substrates, which act as intermediates in many vital processes, are not depleted below critical levels. Nucleosides are then degraded by the enzyme **purine nucleoside phosphorylase (PNP)** to release the purine base and ribose-1-P. Note that neither adenosine nor deoxyadenosine is a substrate for PNP. Instead, these nucleosides are first converted to inosine by **adenosine deaminase.** The PNP products are merged into *xanthine* by **guanine deaminase** and **xanthine oxidase,** and xanthine is then oxidized to uric acid by this latter enzyme.

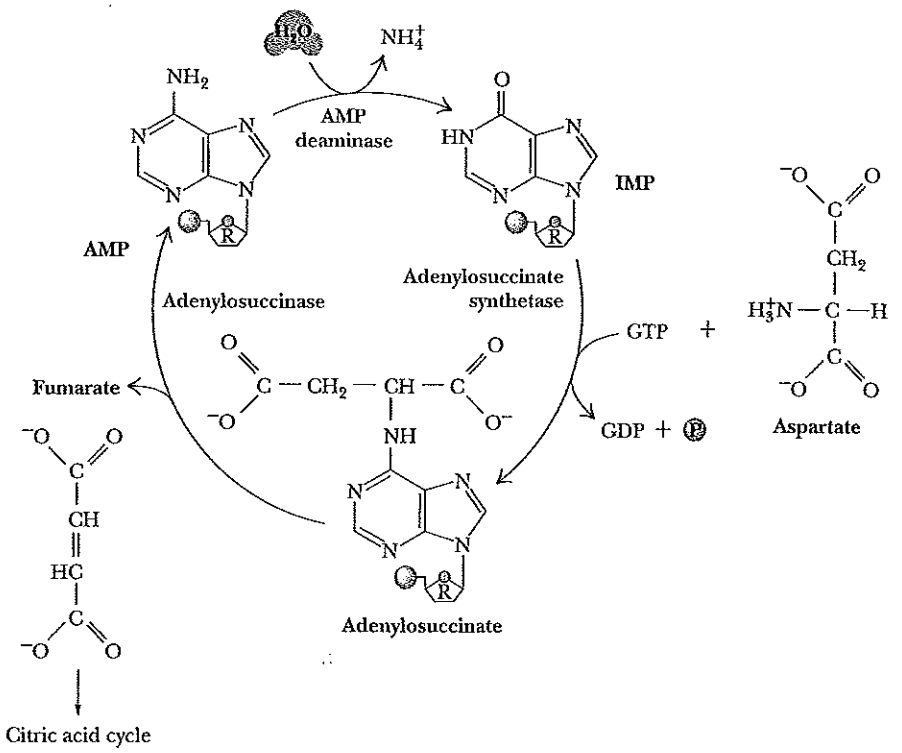


FIGURE 26.9 The purine nucleoside cycle for anaplerotic replenishment of citric acid cycle intermediates in skeletal muscle.

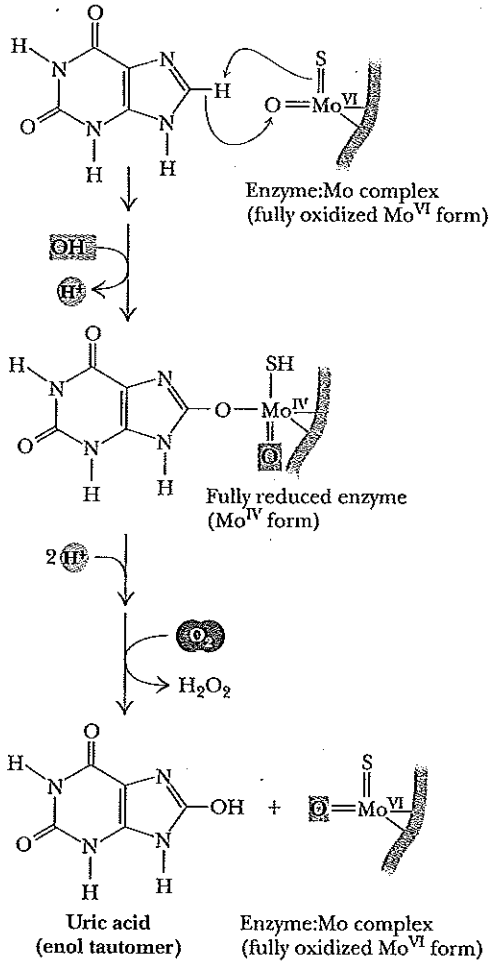


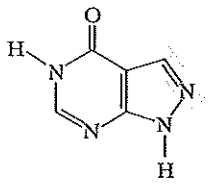
FIGURE 26.10 Xanthine oxidase catalyzes a hydroxylase-type reaction.

The Purine Nucleoside Cycle in Skeletal Muscle Serves as an Anaplerotic Pathway

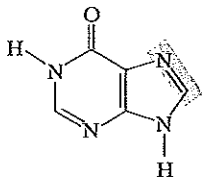
Deamination of AMP to IMP by **AMP deaminase** (Figure 26.8) followed by resynthesis of AMP from IMP by the de novo purine pathway enzymes, *adenylosuccinate synthetase* and *adenylosuccinate lyase,* constitutes a purine nucleoside cycle (Figure 26.9). This cycle has the net effect of converting aspartate to fumarate plus NH_4^+. Although this cycle might seem like senseless energy consumption, it plays an important role in energy metabolism in skeletal muscle: The fumarate that it generates replenishes the levels of citric acid cycle intermediates lost in amphibolic side reactions (see Chapter 19). Skeletal muscle lacks the usual complement of anaplerotic enzymes and relies on enhanced levels of AMP deaminase, adenylosuccinate synthetase, and adenylosuccinate lyase to compensate.

Xanthine Oxidase

Xanthine oxidase (Figure 26.8) is present in large amounts in liver, intestinal mucosa, and milk. It oxidizes hypoxanthine to xanthine and xanthine to uric acid. Xanthine oxidase is a rather indiscriminate enzyme, using molecular oxygen to oxidize a wide variety of purines, pteridines, and aldehydes, producing H_2O_2 as a product. Xanthine oxidase possesses FAD, nonheme Fe-S centers, and *molybdenum cofactor* (a molybdenum-containing pterin complex) as electron-transferring prosthetic groups. Its mechanism of action is diagrammed in Figure 26.10. In humans and other primates, uric acid is the end product of purine catabolism and is excreted in the urine. Birds, terrestrial reptiles, and many insects also excrete uric acid, but in these organisms, uric acid represents the major nitrogen excretory compound, because, unlike mammals, they do not also produce urea (see Chapter 25). Instead, the catabolism of all nitrogenous compounds, including amino acids, is channeled into uric acid. This route of nitrogen catabolism allows these animals to conserve water by excreting crystals of uric acid in pastelike solid form.



Allopurinol



Hypoxanthine

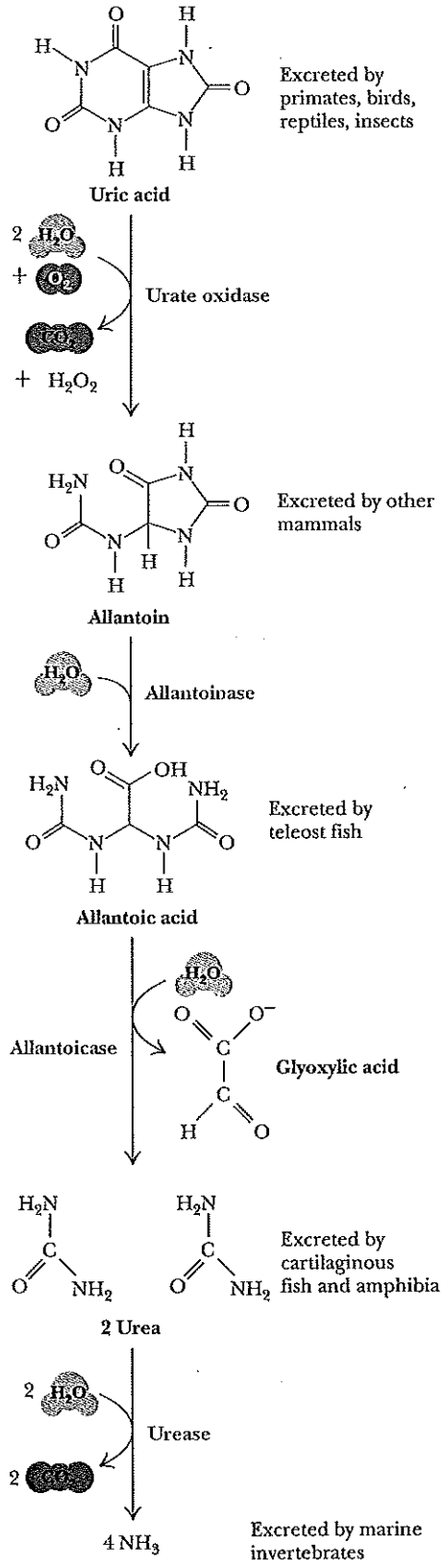
FIGURE 26.11 Allopurinol, an analog of hypoxanthine, is a potent inhibitor of xanthine oxidase.

Gout Is a Disease Caused by an Excess of Uric Acid

Gout is the clinical term describing the physiological consequences accompanying excessive uric acid accumulation in body fluids. Uric acid and urate salts are rather insoluble in water and tend to precipitate from solution if produced in excess. The most common symptom of gout is arthritic pain in the joints as a result of urate deposition in cartilaginous tissue. The joint of the big toe is particularly susceptible. Urate crystals may also appear as kidney stones and lead to painful obstruction of the urinary tract. **Hyperuricemia,** chronic elevation of blood uric acid levels, occurs in about 3% of the population as a consequence of impaired excretion of uric acid or overproduction of purines. Purine-rich foods such as caviar (fish eggs rich in nucleic acids) may exacerbate the condition. The biochemical causes of gout are varied. However, a common treatment is *allopurinol* (Figure 26.11). This hypoxanthine analog binds tightly to xanthine oxidase, thereby inhibiting its activity and preventing uric acid formation. Hypoxanthine and xanthine do not accumulate to harmful concentrations because they are more soluble and thus more easily excreted.

Animals Other Than Humans Oxidize Uric Acid to Form Excretory Products

The subsequent metabolism of uric acid in organisms that don't excrete it is shown in Figure 26.12. In molluscs and in mammals other than primates, uric acid is oxidized by **urate oxidase** to *allantoin* and excreted. In bony fishes (teleosts), uric acid degradation proceeds through yet another step wherein allantoin is hydrolyzed to *allantoic acid* by **allantoinase** before excretion. Cartilaginous fish (sharks and rays) and amphibians further degrade allantoic acid via the enzyme **allantoicase** to liberate glyoxylic acid and 2 equivalents of *urea*. Even simpler animals, such as most marine invertebrates (crustacea and so forth), use **urease** to hydrolyze urea to CO_2 and ammonia. In contrast to animals that must rid themselves of potentially harmful nitrogen waste products, microorganisms often are limited in growth by nitrogen availability. Many possess an identical pathway of uric acid degradation, using it instead to liberate NH_3 from uric acid so that it can be assimilated into organic-N compounds essential to their survival.



➢ **FIGURE 26.12** The catabolism of uric acid to allantoin, allantoic acid, urea, or ammonia in various animals.

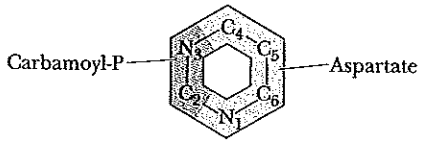


FIGURE 26.13 The metabolic origin of the six atoms of the pyrimidine ring.

26.5 How Do Cells Synthesize Pyrimidines?

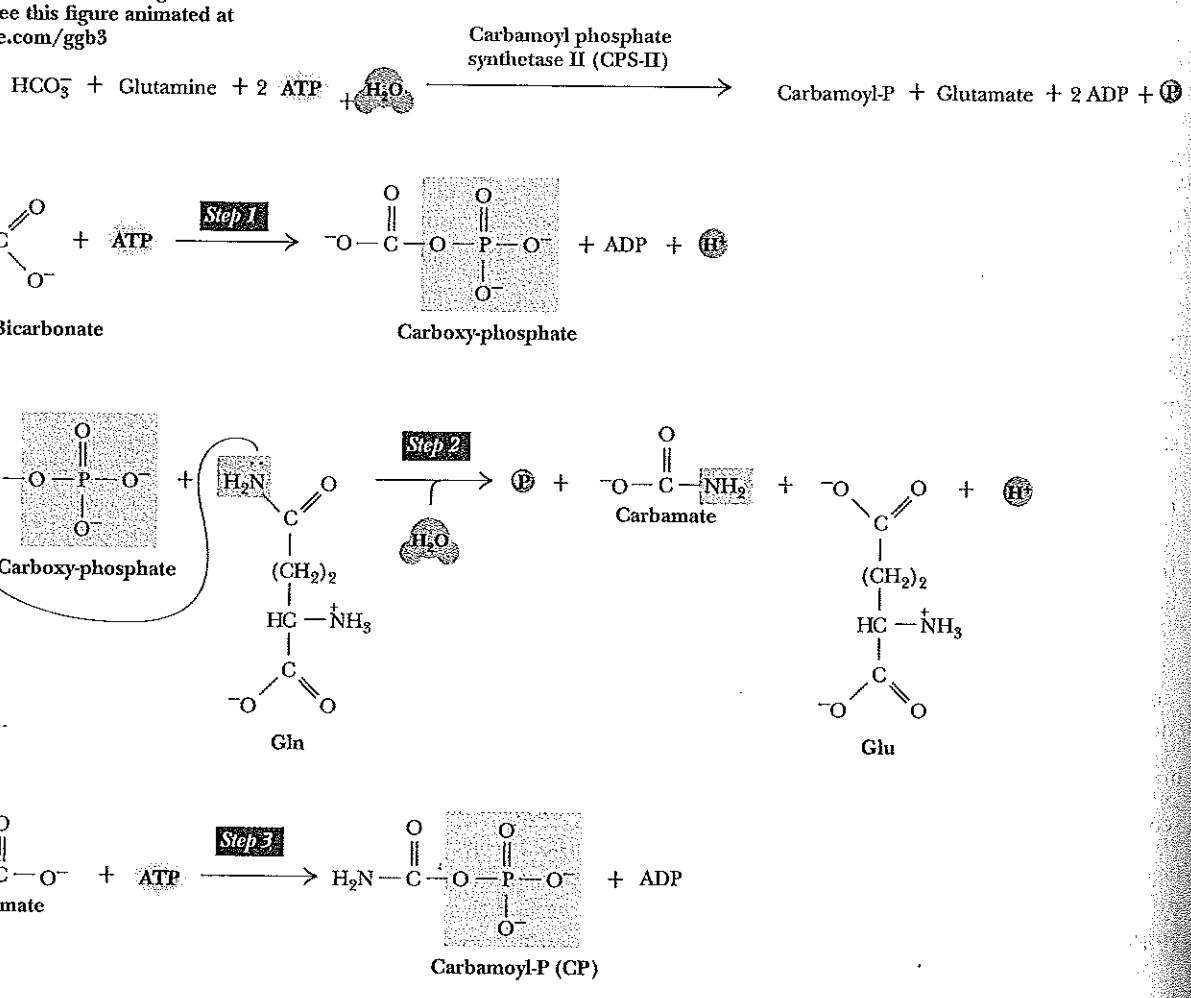
In contrast to purines, pyrimidines are not synthesized as nucleotide derivatives. Instead, the pyrimidine ring system is completed before a ribose-5-P moiety is attached. Also, only two precursors, carbamoyl-P and aspartate, contribute atoms to the six-membered pyrimidine ring (Figure 26.13), compared to seven precursors for the nine purine atoms.

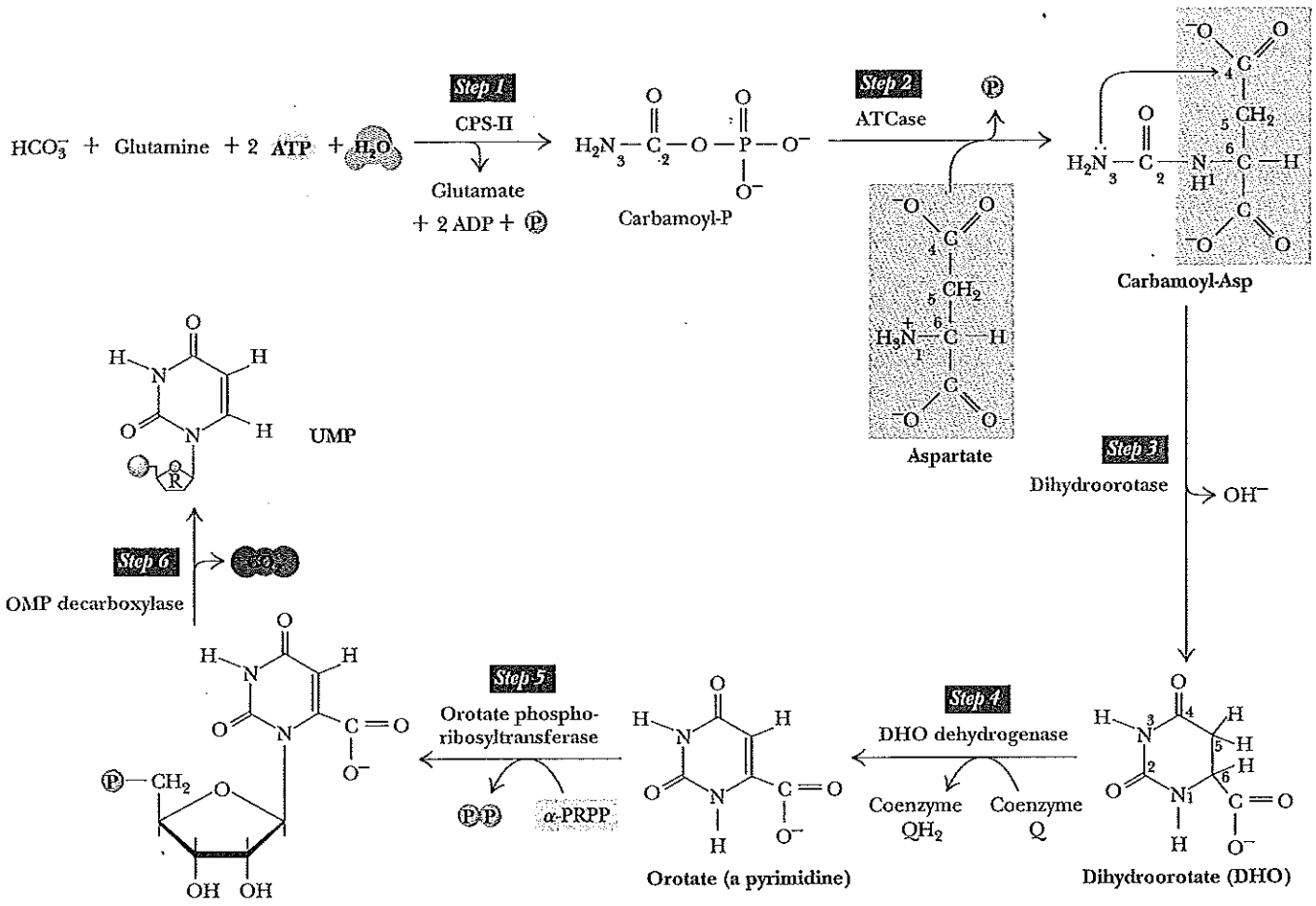
Mammals have two enzymes for carbamoyl phosphate synthesis. Carbamoyl phosphate for pyrimidine biosynthesis is formed by **carbamoyl phosphate synthetase II (CPS-II),** a cytosolic enzyme. Recall that carbamoyl phosphate synthetase I is a mitochondrial enzyme dedicated to the urea cycle and arginine biosynthesis (see Figures 25.22 and 25.23). The substrates of carbamoyl phosphate synthetase II are HCO_3^-, H_2O, glutamine, and 2 ATPs (Figure 26.14). Because carbamoyl phosphate made by CPS-II in mammals has no fate other than incorporation into pyrimidines, mammalian CPS-II can be viewed as the committed step in the pyrimidine de novo pathway. Bacteria have but one CPS, and its carbamoyl phosphate product is incorporated into arginine as well as pyrimidines. Thus, the committed step in bacterial pyrimidine synthesis is the next reaction, which is mediated by **aspartate transcarbamoylase (ATCase).**

ATCase catalyzes the condensation of carbamoyl phosphate with aspartate to form carbamoyl-aspartate (Figure 26.15). No ATP input is required at this step because carbamoyl phosphate represents an "activated" carbamoyl group.

Step 3 of pyrimidine synthesis involves ring closure and dehydration via linkage of the $-NH_2$ group introduced by carbamoyl-P with the former β-COO^- of aspartate; this reaction is mediated by the enzyme **dihydroorotase.** The product of the reaction is *dihydroorotate,* a six-membered ring compound. Dihydroorotate

Biochemistry Now™ ANIMATED FIGURE 26.14 The reaction catalyzed by carbamoyl phosphate synthetase II (CPS-II). Note that, in contrast to carbamoyl phosphate synthetase I, CPS-II uses the amide of glutamine, not NH_4^+, to form carbamoyl-P. **Step 1:** The first ATP consumed in carbamoyl phosphate synthesis is used in forming carboxy-phosphate, an activated form of CO_2. **Step 2:** Carboxy-phosphate (also called carbonylphosphate) then reacts with the glutamine amide to yield carbamate and glutamate. **Step 3:** Carbamate is phosphorylated by the second ATP to give ADP and carbamoyl phosphate. **See this figure animated at http://chemistry.brookscole.com/ggb3**





Biochemistry Now™ ACTIVE FIGURE 26.15
The de novo pyrimidine biosynthetic pathway. **Step 1:** Carbamoyl-P synthesis. **Step 2:** Condensation of carbamoyl phosphate and aspartate to yield carbamoyl-aspartate is catalyzed by aspartate transcarbamoylase (ATCase). **Step 3:** An intramolecular condensation catalyzed by dihydroorotase gives the six-membered heterocyclic ring characteristic of pyrimidines. The product is dihydroorotate (DHO). **Step 4:** The oxidation of DHO by dihydroorotate dehydrogenase gives orotate. (In bacteria, NAD^+ is the electron acceptor from DHO.) **Step 5:** PRPP provides the ribose-5-P moiety that transforms orotate into orotidine-5′-monophosphate, a pyrimidine nucleotide. Note that orotate phosphoribosyltransferase joins N-1 of the pyrimidine to the ribosyl group in appropriate β-configuration. PP_i hydrolysis renders this reaction thermodynamically favorable. **Step 6:** Decarboxylation of OMP by OMP decarboxylase yields UMP. **Test yourself on the concepts in this figure at http://chemistry.brookscole.com/ggb3**

is not a true pyrimidine, but its oxidation yields *orotate,* which is. This oxidation (step 4) is catalyzed by **dihydroorotate dehydrogenase.** Bacterial dihydroorotate dehydrogenases are NAD^+-linked flavoproteins, which are somewhat unusual in possessing both FAD and FMN; these enzymes also have nonheme Fe-S centers as additional redox prosthetic groups. The eukaryotic version of dihydroorotate dehydrogenase is a protein component of the inner mitochondrial membrane; its immediate e^- acceptor is a quinone, and oxidation of the reduced quinone by the mitochondrial e^- transport chain can drive ATP synthesis via oxidative phosphorylation. At this stage, ribose-5-phosphate is joined to N-1 of orotate, giving the pyrimidine nucleotide *orotidine-5′-monophosphate,* or *OMP* (step 5, Figure 26.15). The ribose phosphate donor is PRPP; the enzyme is **orotate phosphoribosyltransferase.** The next reaction is catalyzed by **OMP decarboxylase.** Decarboxylation of OMP gives *UMP* (*uridine-5′-monophosphate,* or *uridylic acid*), one of the two common pyrimidine ribonucleotides.

Pyrimidine Biosynthesis in Mammals Is Another Example of "Metabolic Channeling"

In bacteria, the six enzymes of de novo pyrimidine biosynthesis exist as distinct proteins, each independently catalyzing its specific step in the overall pathway. In contrast, in mammals, the six enzymatic activities are distributed among only three proteins, two of which are **multifunctional polypeptides:** single polypeptide chains having two or more enzymic centers. The first three steps of pyrimidine synthesis, CPS-II, aspartate transcarbamoylase, and dihydroorotase, are all localized on a single 210-kD cytosolic polypeptide. This multifunctional enzyme is the product of a solitary gene, yet it is equipped with the active sites for all

three enzymatic activities. Step 4 (Figure 26.15) is catalyzed by DHO dehydrogenase, a separate enzyme associated with the outer surface of the inner mitochondrial membrane, but the enzymatic activities mediating steps 5 and 6, namely, orotate phosphoribosyltransferase and OMP decarboxylase in mammals, are also found on a single cytosolic polypeptide known as **UMP synthase.**

The purine biosynthetic pathway of avian liver also provides examples of metabolic channeling. Recall that steps 3, 4, and 6 of de novo purine synthesis are catalyzed by three enzymatic activities localized on a single multifunctional polypeptide, and steps 7 and 8 and steps 10 and 11 by respective bifunctional polypeptides (see Figure 26.3).

Such multifunctional enzymes confer an advantage: The product of one reaction in a pathway is the substrate for the next. In multifunctional enzymes, such products remain bound and are channeled directly to the next active site, rather than dissociated into the surrounding medium for diffusion to the next enzyme. This **metabolic channeling** is more efficient for a variety of reasons: Transit time for movement from one active site to the next is shortened, substrates are not diluted into the solvent phase, chemically reactive intermediates are protected from decomposition in the aqueous milieu, no pools of intermediates accumulate, and intermediates are shielded from interactions with other enzymes that might metabolize them.

UMP Synthesis Leads to Formation of the Two Most Prominent Ribonucleotides—UTP and CTP

The two prominent pyrimidine ribonucleotide products are derived from UMP via the same unbranched pathway. First, UDP is formed from UMP via an ATP-dependent *nucleoside monophosphate kinase.*

$$\text{UMP} + \text{ATP} \rightleftharpoons \text{UDP} + \text{ADP}$$

Then, UTP is formed by *nucleoside diphosphate kinase.*

$$\text{UDP} + \text{ATP} \rightleftharpoons \text{UTP} + \text{ADP}$$

Amination of UTP at the 6-position gives CTP. The enzyme **CTP synthetase** is a glutamine amidotransferase (Figure 26.16). ATP hydrolysis provides the energy to drive the reaction.

Pyrimidine Biosynthesis Is Regulated at ATCase in Bacteria and at CPS-II In Animals

Pyrimidine biosynthesis in bacteria is allosterically regulated at aspartate transcarbamoylase (ATCase). *Escherichia coli* ATCase is feedback-inhibited by the end product, CTP. ATP, which can be viewed as a signal of both energy availability and purine sufficiency, is an allosteric activator of ATCase. CTP and ATP compete for a common allosteric site on the enzyme. In many bacteria, UTP, not CTP, acts as the ATCase feedback inhibitor.

In animals, CPS-II catalyzes the committed step in pyrimidine synthesis and serves as the focal point for allosteric regulation. UDP and UTP are feedback

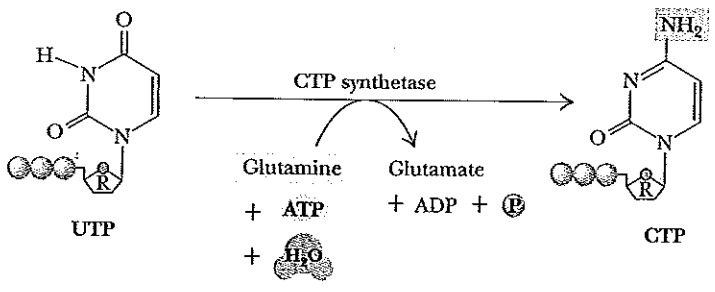
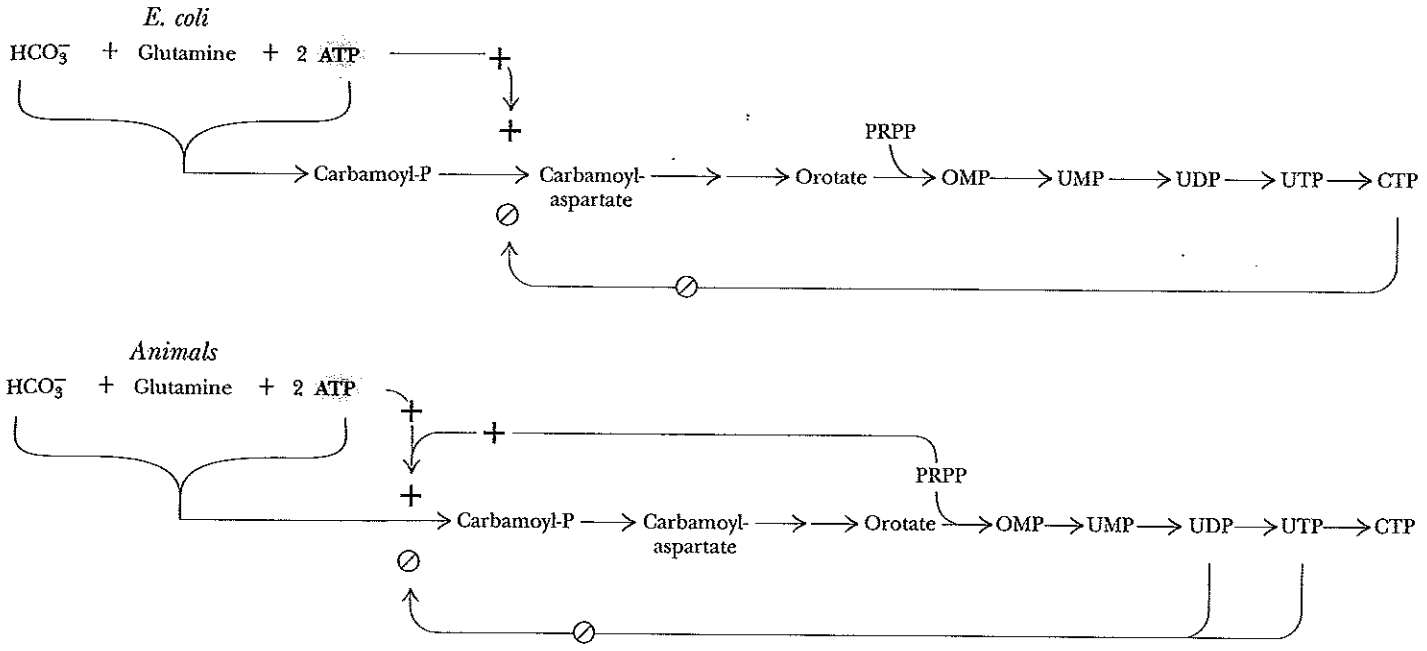


FIGURE 26.16 CTP synthesis from UTP. CTP synthetase catalyzes amination of the 4-position of the UTP pyrimidine ring, yielding CTP. In eukaryotes, this NH_2 comes from the amide-N of glutamine; in bacteria, NH_4^+ serves this role.



Biochemistry Now™ ANIMATED FIGURE 26.17
A comparison of the regulatory circuits that control pyrimidine synthesis in *E. coli* and animals. **See this figure animated at http://chemistry.brookscole.com/ggb3**

inhibitors of CPS-II, whereas PRPP and ATP are allosteric activators. With the exception of ATP, none of these compounds are substrates of CPS-II or of either of the two other enzymic activities residing with it on the trifunctional polypeptide. Figure 26.17 compares the regulatory circuits governing pyrimidine synthesis in bacteria and animals.

26.6 How Are Pyrimidines Degraded?

In some organisms, free pyrimidines, like purines, are salvaged and recycled to form nucleotides via phosphoribosyltransferase reactions similar to those discussed earlier. In humans, however, pyrimidines are recycled from nucleosides, but free pyrimidine bases are not salvaged. Pyrimidine catabolism results in degradation of the pyrimidine ring to products reminiscent of the original substrates, aspartate, CO_2, and ammonia (Figure 26.18). β-Alanine can be recycled into the synthesis of coenzyme A. Catabolism of the pyrimidine base, thymine (5-methyluracil), yields β-aminoisobutyric acid instead of β-alanine.

Human Biochemistry

Mammalian CPS-II Is Activated In Vitro by MAP Kinase and In Vivo by Epidermal Growth Factor

The rate-limiting step in mammalian de novo pyrimidine synthesis is catalyzed by CPS-II, and proliferating cells require lots of pyrimidine nucleotides for growth and cell division. Normally, CPS-II is feedback-inhibited by UTP, but in vitro phosphorylation of CPS-II by **MAP kinase** *(mitogen-activated protein kinase)* creates a covalently modified (phosphorylated) CPS-II that is no longer sensitive to UTP inhibition. Furthermore, phosphorylated CPS-II is more responsive to PRPP activation. Both of these responses favor enhanced pyrimidine biosynthesis. This regulation occurs in vivo when **epidermal growth factor (EGF)**, a **mitogen**, initiates an intracellular cascade of reactions that culminates in MAP kinase activation. By this action, pyrimidine nucleotides are made available for RNA and DNA synthesis, processes that are central to the cell proliferation that follows EGF activation.

A *mitogen* is a hormone that stimulates mitosis (cell division).

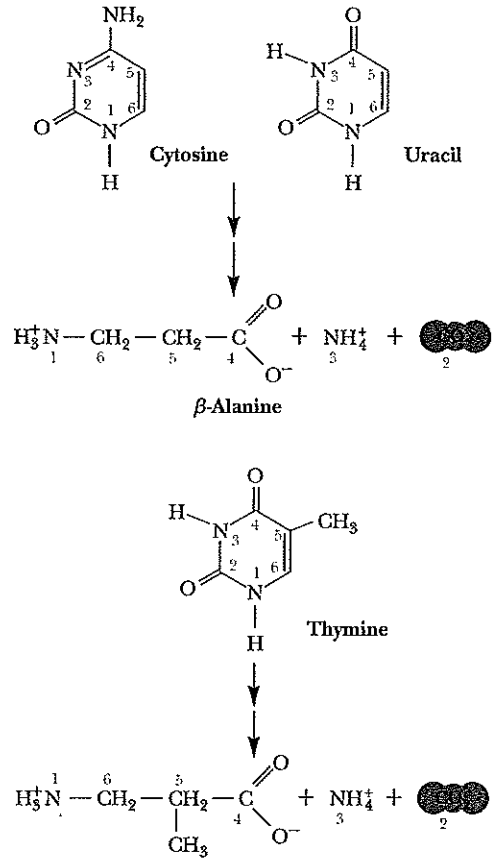


FIGURE 26.18 Pyrimidine degradation. Carbons 4, 5, and 6 plus N-1 are released as β-alanine, N-3 as NH_4^+, and C-2 as CO_2. (The pyrimidine thymine yields β-aminoisobutyric acid.) Recall that aspartate was the source of N-1 and C-4, C-5, and C-6, whereas C-2 came from CO_2 and N-3 from NH_4^+ via glutamine.

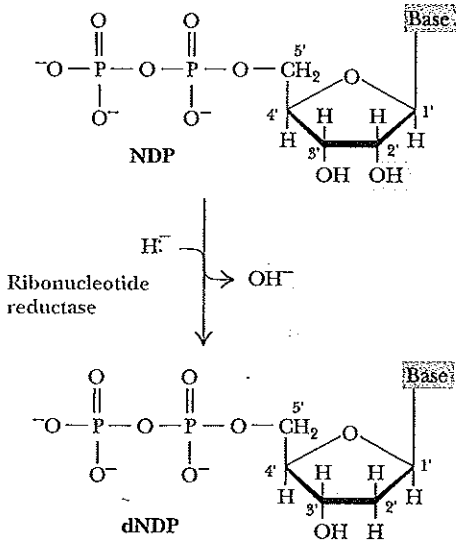


FIGURE 26.19 Deoxyribonucleotide synthesis involves reduction at the 2′-position of the ribose ring of nucleoside diphosphates.

Pathways presented thus far in this chapter account for the synthesis of the four principal ribonucleotides: ATP, GTP, UTP, and CTP. These compounds serve important coenzymic functions in metabolism and are the immediate precursors for ribonucleic acid (RNA) synthesis. Roughly 90% of the total nucleic acid in cells is RNA, with the remainder being deoxyribonucleic acid (DNA). DNA differs from RNA in being a polymer of deoxyribonucleotides, one of which is deoxythymidylic acid. We now turn to the synthesis of these compounds.

26.7 How Do Cells Form the Deoxyribonucleotides That Are Necessary for DNA Synthesis?

The deoxyribonucleotides have only one metabolic purpose: to serve as precursors for DNA synthesis. In most organisms, ribonucleoside diphosphates (NDPs) are the substrates for deoxyribonucleotide formation. Reduction at the 2′-position of the ribose ring in NDPs produces 2′-deoxy forms of these nucleotides (Figure 26.19). This reaction involves replacement of the 2′-OH by a hydride ion ($H:^-$) and is catalyzed by an enzyme known as **ribonucleotide reductase.** Enzymatic ribonucleotide reduction involves a free radical mechanism, and three classes of ribonucleotide reductases are known, differing from each other in their mechanisms of free radical generation. Class I enzymes, found in *E. coli* and virtually all eukaryotes, are Fe-dependent and generate the required free radical on a specific tyrosyl side chain.

E. coli Ribonucleotide Reductase Has Three Different Nucleotide-Binding Sites

The enzyme system for dNDP formation consists of four proteins, two of which constitute the ribonucleotide reductase proper, an enzyme of the $\alpha_2\beta_2$ type. The other two proteins, **thioredoxin** and **thioredoxin reductase,** function in the delivery of reducing equivalents, as we shall see shortly. The two proteins of ribonucleotide reductase are designated **R1** (86 kD) and **R2** (43.5 kD), and each is a homodimer in the holoenzyme (Figure 26.20). The R1 homodimer carries two types of regulatory sites in addition to the **catalytic site** (the active site). Substrates (ADP, CDP, GDP, and UDP) bind at the catalytic site. One regulatory site—the **substrate specificity site**—binds ATP, dATP, dGTP, or dTTP, and which of these nucleotides is bound there determines which nucleoside diphosphate is bound at the catalytic site. The other regulatory site, the **overall activity site,** binds either the activator ATP or the negative effector dATP; the nucleotide bound here determines whether the enzyme is active or inactive. Activity depends also on residues Cys^{439}, Cys^{225}, and Cys^{462} in R1. The 2 Fe atoms within the single active site formed by the R2 homodimer generate the free radical required for ribonucleotide reduction on a specific R2 residue, Tyr^{122}, which in turn generates a thiyl free radical (Cys-S·) on Cys^{439}. Cys^{439}-S· initiates ribonucleotide reduction by abstracting the 3′-H from the ribose ring of the nucleoside diphosphate substrate (Figure 26.21) and forming a free radical on C-3′. Subsequent dehydration forms the deoxyribonucleotide product.

Thioredoxin Provides the Reducing Power for Ribonucleotide Reductase

NADPH is the ultimate source of reducing equivalents for ribonucleotide reduction, but the immediate source is reduced **thioredoxin,** a small (12-kD) protein with reactive Cys-sulfhydryl groups situated next to one another in the sequence Cys-Gly-Pro-Cys. These Cys residues are able to undergo reversible

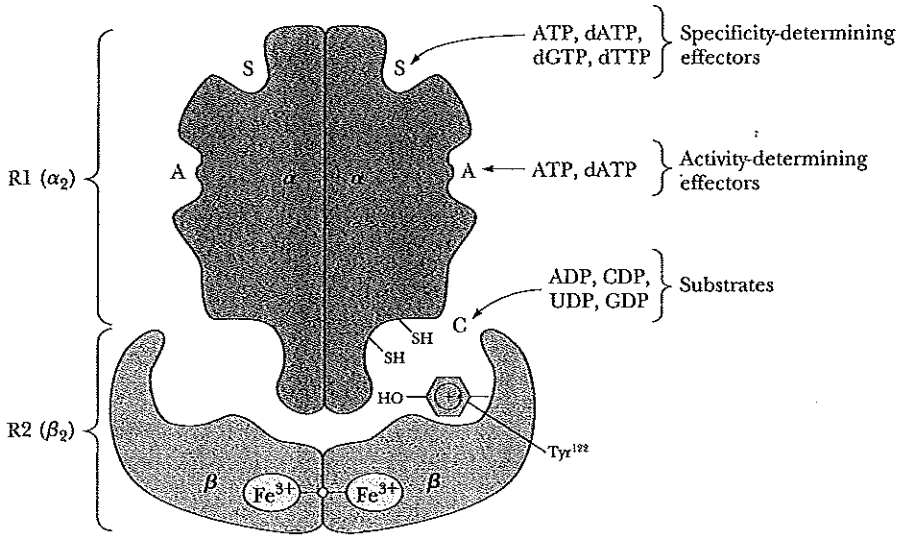
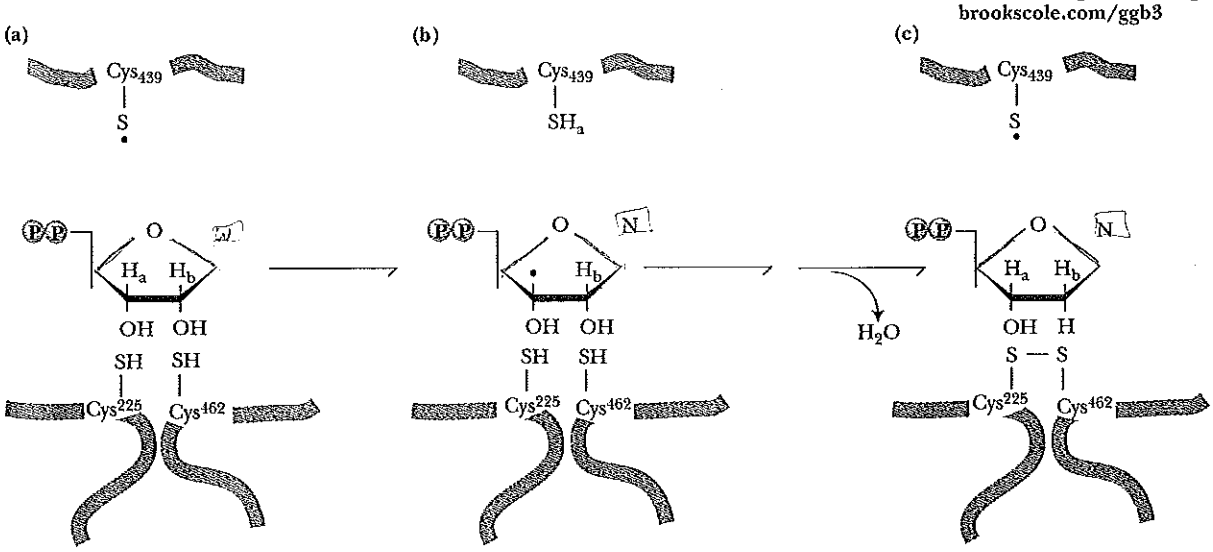


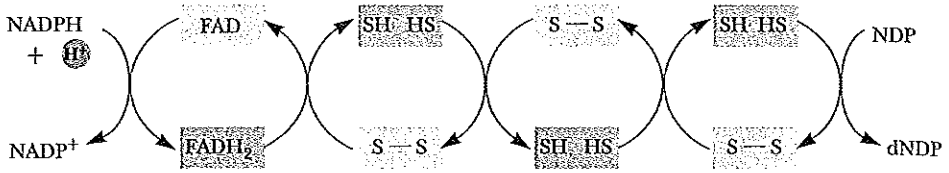
FIGURE 26.20 *E. coli* ribonucleotide reductase: its binding sites and subunit organization. Two proteins, R1 and R2 (each a dimer of identical subunits), combine to form the holoenzyme. The holoenzyme has three classes of nucleotide binding sites: S, the specificity-determining sites; A, the activity-determining sites; and C, the catalytic or active site. These various sites bind different nucleotide ligands. Note that the holoenzyme apparently possesses only one active site formed by interaction between Fe^{3+} atoms in each R2 subunit.

oxidation–reduction between (—S—S—) and (—SH HS—) and, in their reduced form, serve as primary electron donors to regenerate the reactive —SH pair of the ribonucleotide reductase active site (Figure 26.21). In turn, the sulfhydryls of thioredoxin must be restored to the (—SH HS—) state for another catalytic cycle. **Thioredoxin reductase,** an α_2-type enzyme composed of 58-kD flavoprotein subunits, mediates the NADPH-dependent reduction of thioredoxin (Figure 26.22). Thioredoxin functions in a number of metabolic roles besides deoxyribonucleotide synthesis, the common denominator of which is reversible sulfide:sulfhydryl transitions. Another sulfhydryl protein similar to thioredoxin, called **glutaredoxin,** can also function in ribonucleotide reduction. Oxidized glutaredoxin is re-reduced by 2 equivalents of **glutathione** (γ-glutamylcysteinylglycine; Figure 26.23), which in turn is re-reduced by glutathione reductase, another NADPH-dependent flavoenzyme.

The substrates for ribonucleotide reductase are CDP, UDP, GDP, and ADP, and the corresponding products are dCDP, dUDP, dGDP, and dADP. Because CDP is not an intermediate in pyrimidine nucleotide synthesis, it must arise by dephosphorylation of CTP, for instance, via nucleoside diphosphate kinase action. Although uridine nucleotides do not occur in DNA, UDP is a substrate. The formation of dUDP is justified because it is a precursor to dTTP, a necessary substrate for DNA synthesis (see following discussion).

Biochemistry Now™ ACTIVE FIGURE 26.21
The free radical mechanism of ribonucleotide reduction. H_a designates the C-3′ hydrogen and H_b the C-2′ hydrogen atom. Formation of a thiyl radical on Cys^{439} (a) of the *E. coli* ribonucleotide reductase R1 homodimer through reaction with a Tyr^{122} free radical on R2 leads to removal of the H_a hydrogen and creation of a C-3′ radical (b). Dehydration via removal of H_b together with the C-2′—OH group and restoration of H_a to C-3′ forms the dNDP product, accompanied by oxidation of R1 Cys^{225} and Cys^{462} —SH groups to form a disulfide (c). *(Adapted from Reichard, P., 1997. The evolution of ribonucleotide reduction.* Trends in Biochemical Sciences *22:81–85. This free radical mechanism of ribonucleotide reduction was originally proposed by JoAnn Stubbe of MIT.)* **Test yourself on the concepts in this figure at http://chemistry.brookscole.com/ggb3**



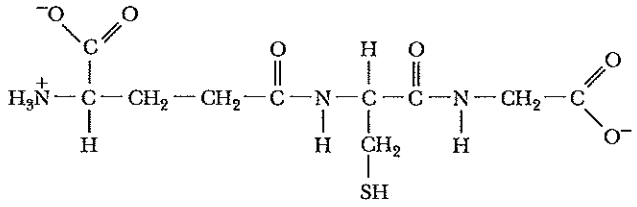


Biochemistry Now™ ANIMATED FIGURE 26.22 The (—S—S—)/(—SH HS—) oxidation–reduction cycle involving ribonucleotide reductase, thioredoxin, thioredoxin reductase, and NADPH. **See this figure animated at http://chemistry.brookscole.com/ggb3**

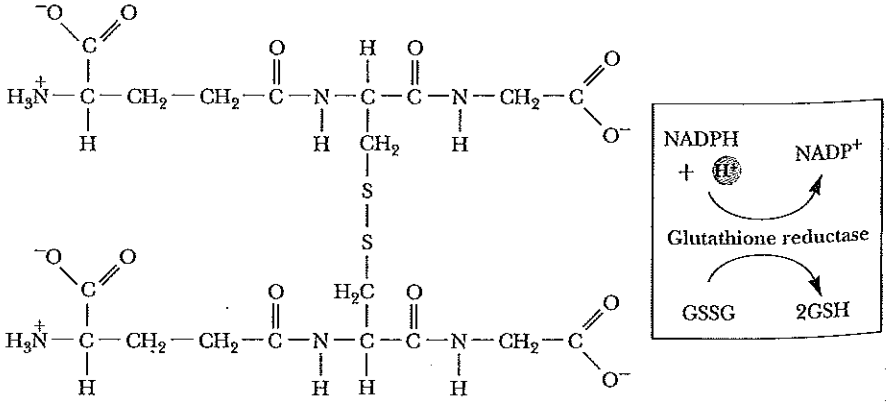
Both the Specificity and the Catalytic Activity of Ribonucleotide Reductase Are Regulated by Nucleotide Binding

Ribonucleotide reductase activity must be modulated in two ways in order to maintain an appropriate balance of the four deoxynucleotides essential to DNA synthesis, namely, dATP, dGTP, dCTP, and dTTP. First, the overall activity of the enzyme must be turned on and off in response to the need for dNTPs. Second, the relative amounts of each NDP substrate transformed into dNDP must be controlled so that the right balance of dATP:dGTP:dCTP:dTTP is produced. The two different effector-binding sites on ribonucleotide reductase, *discrete from the substrate-binding catalytic site,* are designed to serve these purposes. As noted previously, these two regulatory sites are designated the *overall activity site* and the *substrate specificity site.* Only ATP and dATP are able to bind at the *overall activity site.* ATP is an allosteric activator and dATP is an allosteric inhibitor, and they compete for the same site. If ATP is bound, the enzyme is active, whereas if its deoxy counterpart, dATP, occupies this site, the enzyme is inactive. That is, ATP is a positive effector and dATP is a negative effector with respect to enzyme activity, and they compete for the same site.

The second regulatory site, the *substrate specificity site,* can bind either ATP, dTTP, dGTP, or dATP, and the substrate specificity of the enzyme is determined by which of these nucleotides occupies this site. If ATP is in the *sub-*



Reduced glutathione (γ-glutamylcysteinylglycine, GSH)



Oxidized glutathione (GSSG) or glutathione disulfide

FIGURE 26.23 The structure of glutathione.

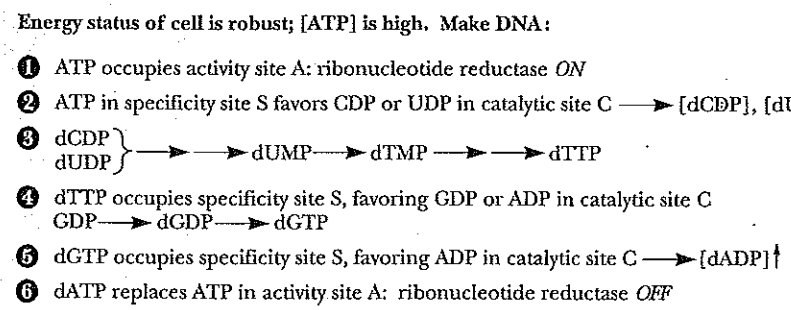


FIGURE 26.24 Regulation of deoxynucleotide biosynthesis: the rationale for the various affinities displayed by the two nucleotide-binding regulatory sites on ribonucleotide reductase.

strate specificity site, ribonucleotide reductase preferentially binds pyrimidine nucleotides (UDP or CDP) at its active site and reduces them to dUDP and dCDP. With dTTP in the specificity-determining site, GDP is the preferred substrate. When dGTP binds to the specificity site, ADP becomes the favored substrate for reduction. The rationale for these varying affinities is as follows (Figure 26.24): High [ATP] is consistent with cell growth and division and, consequently, the need for DNA synthesis. Thus, ATP binds in the *overall activity site* of ribonucleotide reductase, turning it on and promoting production of dNTPs for DNA synthesis. Under these conditions, ATP is also likely to occupy the *substrate specificity site,* so UDP and CDP are bound at the *catalytic site* and reduced to dUDP and dCDP. Both of these pyrimidine deoxynucleoside diphosphates are precursors to dTTP. Thus, elevation of dUDP and dCDP levels leads to an increase in [dTTP]. High dTTP levels increase the likelihood that it will occupy the *substrate specificity site,* in which case GDP becomes the preferred substrate and dGTP levels rise. Upon dGTP association with the *substrate specificity site,* ADP is the favored substrate, leading to ADP reduction and the eventual accumulation of dATP. Binding of dATP to the *overall activity site* then shuts the enzyme down. In summary, the relative affinities of the three classes of nucleotide binding sites in ribonucleotide reductase for the various substrates, activators, and inhibitors are such that the formation of dNDPs proceeds in an orderly and balanced fashion. As these dNDPs are formed in amounts consistent with cellular needs, their phosphorylation by nucleoside diphosphate kinases produces dNTPs, the actual substrates of DNA synthesis.

26.8 How Are Thymine Nucleotides Synthesized?

The synthesis of thymine nucleotides proceeds from other pyrimidine deoxyribonucleotides. Cells have no requirement for free thymine ribonucleotides and do not synthesize them. Small amounts of thymine ribonucleotides do occur in tRNA (tRNA is notable for having unusual nucleotides), but these Ts arise via methylation of U residues already incorporated into the tRNA. Both dUDP and dCDP can lead to formation of dUMP, the immediate precursor for dTMP synthesis (Figure 26.25). Interestingly, formation of

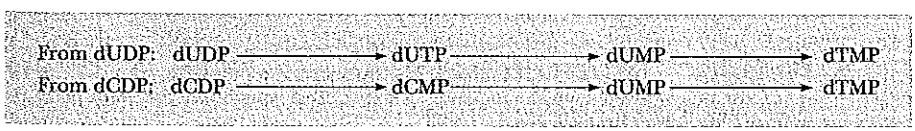


FIGURE 26.25 Pathways of dTMP synthesis. dTMP production is dependent on dUMP formation from dCDP and dUDP synthesis. If the dCDP pathway is traced from the common pyrimidine precursor, UMP, it will proceed as follows:
UMP → UDP → UTP → CTP → CDP → dCDP → dCMP → dUMP → dTMP

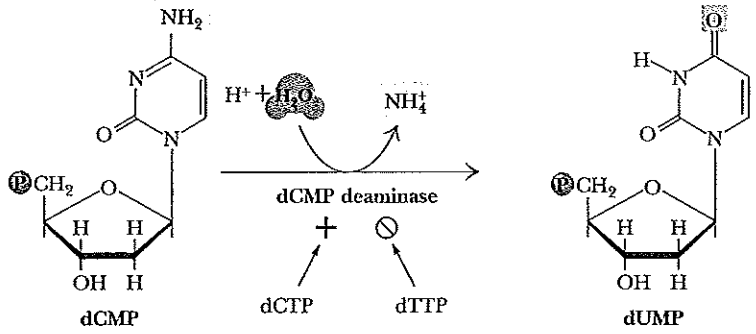
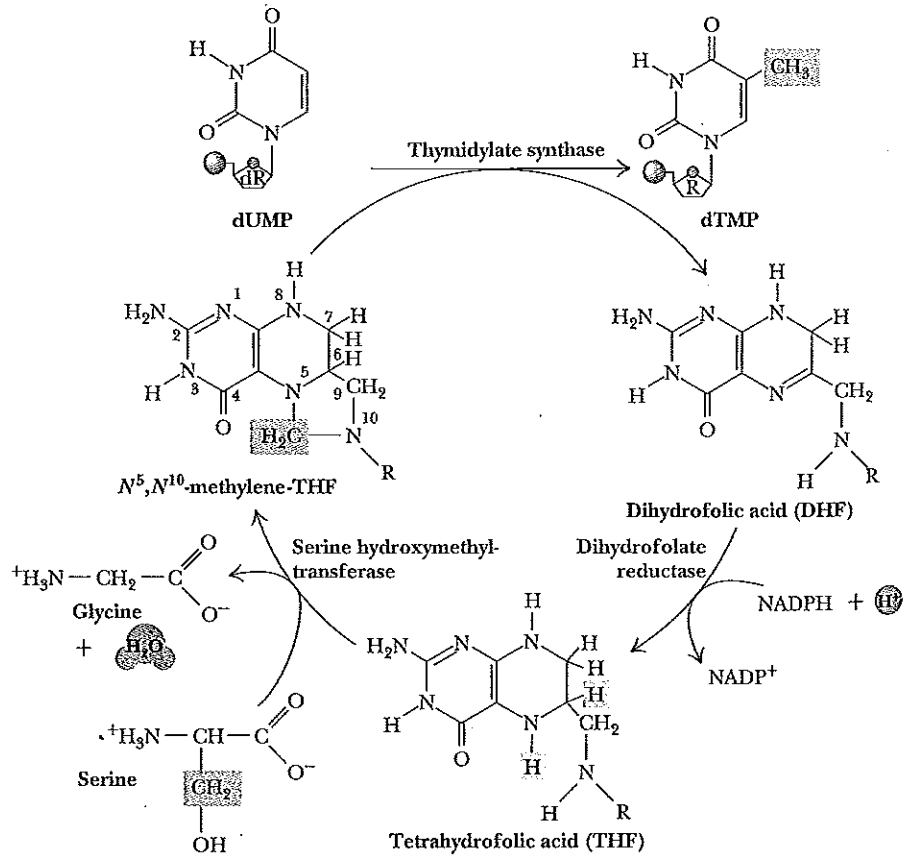


FIGURE 26.26 The dCMP deaminase reaction.

dUMP from dUDP passes through dUTP, which is then cleaved by **dUTPase,** a pyrophosphatase that removes PP_i from dUTP. The action of dUTPase prevents dUTP from serving as a substrate in DNA synthesis. An alternative route to dUMP formation starts with dCDP, which is dephosphorylated to dCMP and then deaminated by **dCMP deaminase** (Figure 26.26), leaving dUMP. dCMP deaminase provides a second point for allosteric regulation of dNTP synthesis; it is allosterically activated by dCTP and feedback-inhibited by dTTP. Of the four dNTPs, only dCTP does not interact with either of the



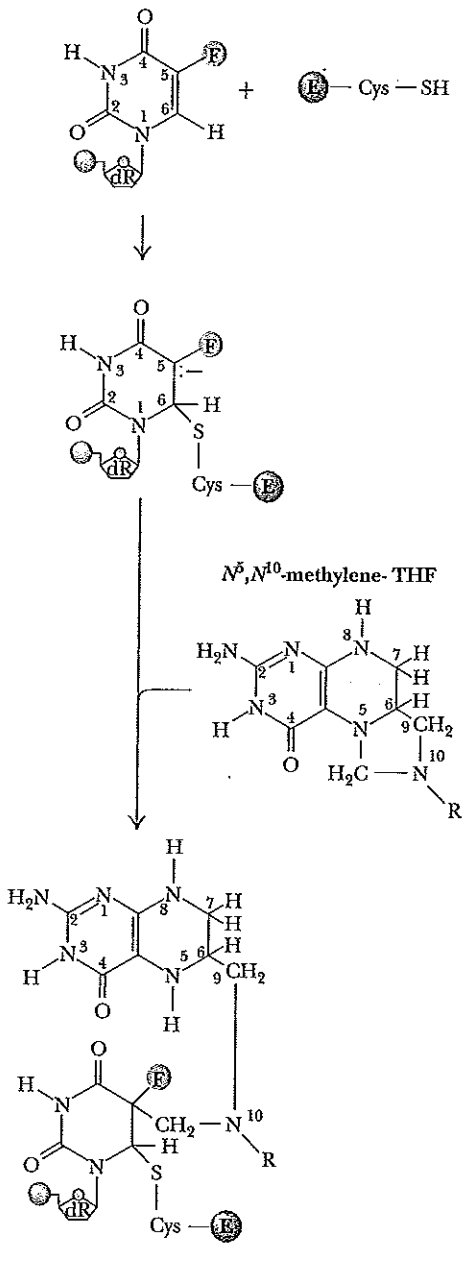
BiochemistryNow™ ACTIVE FIGURE 26.27 The thymidylate synthase reaction. The 5-CH_3 group is ultimately derived from the β-carbon of serine. **Test yourself on the concepts in this figure at http://chemistry.brookscole.com/ggb3**

A Deeper Look

Fluoro-Substituted Analogs as Therapeutic Agents

Carbon–fluorine bonds are exceedingly rare in nature, and fluorine is an uncommon constituent of biological molecules. F has three properties attractive to drug designers: (1) It is the smallest replacement for an H atom in organic synthesis, (2) fluorine is the most electronegative element, and (3) the F—C bond is relatively unreactive. This steric compactness and potential for strong inductive effects through its electronegativity renders F a useful substituent in the construction of inhibitory analogs of enzyme substrates. One interesting strategy is to devise fluorinated precursors that are taken up and processed by normal metabolic pathways to generate a potent antimetabolite. A classic example is *fluoroacetate*. FCH_2COO^- is exceptionally toxic because it is readily converted to fluorocitrate by citrate synthase of the citric acid cycle (see Chapter 19). In turn, fluorocitrate is a powerful inhibitor of aconitase. The metabolic transformation of an otherwise innocuous compound into a poisonous derivative is termed **lethal synthesis.** *5-Fluorouracil* and *5-fluorocytosine* are also examples of this strategy (see text).

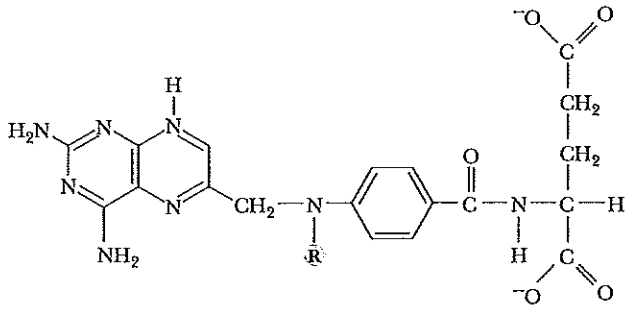
Unlike hydrogen, which is often abstracted from substrates as H^+, electronegative fluorine cannot be readily eliminated as the corresponding F^+. Thus, enzyme inhibitors can be fashioned in which F replaces H at positions where catalysis involves H removal as H^+. Thymidylate synthase catalyzes removal of H from dUMP as H^+ through a covalent catalysis mechanism. A thiol group on this enzyme normally attacks the 6-position of the uracil moiety of 2′-deoxyuridylic acid so that C-5 can act as a carbanion in attack on the methylene carbon of N^5,N^{10}-methylene-THF (see accompanying figure). Regeneration of free enzyme then occurs through loss of the C-5 H atom as H^+ and dissociation of product dTMP. If F replaces H at C-5 as in 2′-deoxy-5-fluorouridylate (dFUMP), the enzyme is immobilized in a very stable ternary [enzyme:dFUMP:methylene-THF] complex and effectively inactivated. Enzyme inhibitors like dFUMP whose adverse properties are elicited only through direct participation in the catalytic cycle are variously called **mechanism-based inhibitors, suicide substrates,** or **Trojan horse substrates.**



> The effect of the 5-fluoro substitution on the mechanism of action of thymidylate synthase. An enzyme thiol group (from a Cys side chain) ordinarily attacks the 6-position of dUMP so that C-5 can react as a carbanion with N^5,N^{10}-methylene-THF. Normally, free enzyme is regenerated following release of the hydrogen at C-5 as a proton. Because release of fluorine as F^+ cannot occur, the ternary (three-part) complex of [enzyme:fluorouridylate:methylene-THF] is stable and persists, preventing enzyme turnover. (The N^5,N^{10}-methylene-THF structure is given in abbreviated form.)

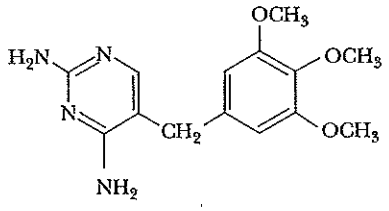
regulatory sites on ribonucleotide reductase (Figure 26.20). Instead, it acts upon dCMP deaminase.

Synthesis of dTMP from dUMP is catalyzed by **thymidylate synthase** (Figure 26.27). This enzyme methylates dUMP at the 5-position to create dTMP; the methyl donor is the one-carbon folic acid derivative N^5,N^{10}-methylene-THF. The reaction is actually a reductive methylation in which the one-carbon unit is transferred at the methylene level of reduction and then reduced to the methyl level. The THF cofactor is oxidized at the expense of



2-Amino, 4-amino analogs of folic acid

R = H Aminopterin
R = CH_3 Amethopterin (methotrexate)



Trimethoprim

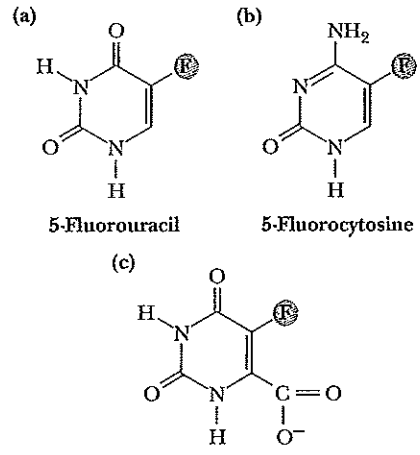
FIGURE 26.28 Precursors and analogs of folic acid employed as antimetabolites include sulfonamides (see Human Biochemistry box on page 858), as well as methotrexate, aminopterin, and trimethoprim, whose structures are shown here. The compounds shown here bind to dihydrofolate reductase with about 1000-fold greater affinity than DHF and thus act as virtually irreversible inhibitors.

methylene reduction to yield dihydrofolate, or DHF. Dihydrofolate reductase then reduces DHF back to THF for service again as a one-carbon vehicle (see Figure 17.35). Thymidylate synthase sits at a junction connecting dNTP synthesis with folate metabolism. It has become a preferred target for inhibitors designed to disrupt DNA synthesis. An indirect approach is to employ folic acid precursors or analogs as antimetabolites of dTMP synthesis (Figure 26.28). Purine synthesis is affected as well because it is also dependent on THF (Figure 26.3).

Human Biochemistry

Fluoro-Substituted Pyrimidines in Cancer Chemotherapy, Fungal Infections, and Malaria

5-Fluorouracil (*5-FU;* see accompanying figure) is a thymine analog. It is converted in vivo to *5′-fluorouridylate* by a PRPP-dependent phosphoribosyltransferase and passes through the reactions of dNTP synthesis, culminating ultimately as *2′-deoxy-5-fluorouridylic acid,* a potent inhibitor of dTMP synthase (see the A Deeper Look box on page 875). 5-FU is used as a chemotherapeutic agent in the treatment of human cancers. Similarly, *5-fluorocytosine* (see figure) is used as an antifungal drug because fungi, unlike mammals, can convert it to 2′-deoxy-5-fluorouridylate. Furthermore, malarial parasites can use exogenous orotate to make pyrimidines for nucleic acid synthesis, whereas mammals cannot. Thus, *5-fluoroorotate* (see figure) is an effective antimalarial drug because it is selectively toxic to these parasites.



5-Fluorouracil 5-Fluorocytosine

5-Fluoroorotate

⊩ The structures of 5-fluorouracil (5-FU), 5-fluorocytosine, and 5-fluoroorotate.

Summary

26.1 Can Cells Synthesize Nucleotides? Nucleotides are ubiquitous constituents of life and nearly all cells are capable of synthesizing them "from scratch" via de novo pathways. Rapidly proliferating cells must make lots of purine and pyrimidine nucleotides to satisfy demands for DNA and RNA synthesis. Nucleotide biosynthetic pathways are attractive targets for the clinical control of rapidly dividing cells such as cancers or infectious bacteria. Many antibiotics and anticancer drugs are inhibitors of purine or pyrimidine nucleotide biosynthesis.

26.2 How Do Cells Synthesize Purines? The nine atoms of the purine ring system are derived from aspartate (N-1), glutamine (N-3 and N-9), glycine (C-4, C-5, and N-7), CO_2 (C-6), and THF one-carbon derivatives (C-2 and C-8). The atoms of the purine ring are successively added to *ribose-5-phosphate*, so purines begin as nucleotide derivatives through assembly of the purine ring system directly on the ribose. Because purine biosynthesis depends on folic acid derivatives, it is sensitive to inhibition by folate analogs. Distinct, two-step metabolic pathways diverge from IMP, one leading to AMP and the other to GMP. Purine biosynthesis is regulated at several stages: Reaction 1 (ribose-5-phosphate pyrophosphokinase) is feedback-inhibited by ADP and GDP; the enzyme catalyzing reaction 2 (glutamine phosphoribosyl pyrophosphate amidotransferase) has two inhibitory allosteric sites, one where adenine nucleotides bind and another where guanine nucleotides bind. PRPP is a "feed-forward" activator of this enzyme. The first reaction in the conversion of IMP to AMP involves adenylosuccinate synthetase, which is inhibited by AMP; the first step in the conversion of IMP to GMP is catalyzed by IMP dehydrogenase and is inhibited by GMP. ATP-dependent kinases form nucleoside diphosphates and triphosphates from AMP and GMP.

26.3 Can Cells Salvage Purines? Purine ring systems represent a metabolic investment by cells, and salvage pathways exist to recover them when degradation of nucleic releases free purines in the form of adenine, guanine, and hypoxanthine (the base in IMP). Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) acts on either hypoxanthine to form IMP or guanine to form GMP; an absence of HGPRT is the basis of Lesch-Nyhan syndrome.

26.4 How Are Purines Degraded? Dietary nucleic acids are digested to nucleotides by various nucleases and phosphodiesterases, the nucleotides are converted to nucleosides by base-specific nucleotidases and nonspecific phosphatases, and then nucleosides are hydrolyzed to release the purine base. Only the pentoses of nucleotides serve as sources of metabolic energy. In humans, the purine ring is oxidized to uric acid by xanthine oxidase and excreted. Gout occurs when bodily fluids accumulate an excess of uric acid. Skeletal muscle operates a purine nucleoside cycle as an anaplerotic pathway.

26.5 How Do Cells Synthesize Pyrimidines? In contrast to formation of the purine ring system, the pyrimidine ring system is completed before a ribose-5-P moiety is attached. Only two precursors, carbamoyl-P and aspartate, contribute atoms to the six-membered pyrimidine ring. The first step in humans is catalyzed by CPS-II. ATCase then links carbamoyl-P with aspartate. Subsequent reactions close the ring and oxidize it, before adding ribose-5-P, using α-PRPP as donor. Decarboxylation gives UMP. In mammals, the six enzymatic activities of pyrimidine biosynthesis are distributed among only three proteins, two of which are multifunctional polypeptides. Purine and pyrimidine synthesis in mammals are two prominent examples of metabolic channeling. UMP leads to UTP, the substrate for formation of CTP via CTP synthetase. Regulation of pyrimidine synthesis in animals occurs at CPS-II. UDP and UTP are feedback inhibitors, whereas PRPP and ATP are allosteric activators. In bacteria, regulation acts at ATCase through feedback inhibition by CTP (or UTP) and activation by ATP.

26.6 How Are Pyrimidines Degraded? Degradation of the pyrimidine ring generates β-alanine, CO_2, and ammonia. In humans, pyrimidines are recycled from nucleosides, but free pyrimidine bases are not salvaged.

26.7 How Do Cells Form the Deoxyribonucleotides That Are Necessary for DNA Synthesis? 2′-Deoxyribonucleotides are formed from ribonucleotides through reduction at the 2′-position of the ribose ring in NDPs. The reaction, catalyzed by ribonucleotide reductase, involves a free radical mechanism that replaces the 2′-OH by a hydride ion ($H:^-$). Thioredoxin provides the reducing power for ribonucleotide reduction. *E. coli* ribonucleotide reductase has three different nucleotide-binding sites: the catalytic site (or active site), which binds substrates (ADP, CDP, GDP, and UDP); the substrate specificity site, which can bind ATP, dATP, dGTP, or dTTP; and the overall activity site, which binds either the activator ATP or the negative effector dATP. The relative affinities of the three classes of nucleotide binding sites in ribonucleotide reductase for the various substrates, activators, and inhibitors are such that the various dNDPs are formed in amounts consistent with cellular needs.

26.8 How Are Thymine Nucleotides Synthesized? Both dUDP and dCDP can lead to formation of dUMP, the immediate precursor for dTMP synthesis. Formation of dTMP from dUMP is catalyzed by thymidylate synthase through reductive methylation of dUMP at the 5-position. The methyl donor is the one-carbon folic acid derivative N^5,N^{10}-methylene-THF. Fluoro-substituted pyrimidine analogs such as 5-fluorouracil (5-FU), 5-fluorocytosine, and 5-fluoroorotate inhibit thymidylate synthase. These fluoro compounds have found a range of therapeutic uses in treating diseases from cancer to malaria.

Problems

1. Draw the purine and pyrimidine ring structures, indicating the metabolic source of each atom in the rings.
2. Starting from glutamine, aspartate, glycine, CO_2 and N^{10}-formyl-THF, how many ATP equivalents are expended in the synthesis of (a) ATP, (b) GTP, (c) UTP, and (d) CTP?
3. Illustrate the key points of regulation in (a) the biosynthesis of IMP, AMP, and GMP; (b) *E. coli* pyrimidine biosynthesis; and (c) mammalian pyrimidine biosynthesis.
4. Indicate which reactions of purine or pyrimidine metabolism are affected by the inhibitors (a) azaserine, (b) methotrexate, (c) sulfonamides, (d) allopurinol, and (e) 5-fluorouracil.
5. Since dUTP is not a normal component of DNA, why do you suppose ribonucleotide reductase has the capacity to convert UDP to dUDP?
6. Describe the underlying rationale for the regulatory effects exerted on ribonucleotide reductase by ATP, dATP, dTTP, and dGTP.
7. (Integrates with Chapters 18–20 and 22.) By what pathway(s) does the ribose released upon nucleotide degradation enter intermediary metabolism and become converted to cellular energy? How many ATP equivalents can be recovered from one equivalent of ribose?
8. (Integrates with Chapter 25.) At which steps does the purine biosynthetic pathway resemble the pathway for biosynthesis of the amino acid histidine?
9. Write reasonable chemical mechanisms for steps 6, 8, and 9 in purine biosynthesis (Figure 26.3).
10. Write a balanced equation for the conversion of aspartate to fumarate by the purine nucleoside cycle in skeletal muscle.
11. Write a balanced equation for the oxidation of uric acid to glyoxylic acid, CO_2, and NH_3, showing each step in the process and naming all of the enzymes involved.
12. (Integrates with Chapter 15.) *E. coli* aspartate transcarbamoylase (ATCase) displays classic allosteric behavior. This $\alpha_6\beta_6$ enzyme is

activated by ATP and feedback-inhibited by CTP. In analogy with the behavior of glycogen phosphorylase shown in Figure 15.15, illustrate the allosteric v versus [aspartate] curves for ATCase (a) in the absence of effectors, (b) in the presence of CTP, and (c) in the presence of ATP.

*13. (Integrates with Chapter 15.) Unlike its allosteric counterpart glycogen phosphorylase (an α_2 enzyme), *E. coli* ATCase has a heteromeric ($\alpha_6\beta_6$) organization. The α-subunits bind aspartate and are considered catalytic subunits, whereas the β-subunits bind CTP or ATP and are considered regulatory subunits. How would you describe the subunit organization of ATCase from a functional point of view?

14. (Integrates with Chapter 20.) Starting from HCO_3^-, glutamine, aspartate, and ribose-5-P, how many ATP equivalents are consumed in the synthesis of dTTP in a eukaryotic cell, assuming dihydroorotate oxidation is coupled to oxidative phosphorylation? How does this result compare with the ATP costs of purine nucleotide biosynthesis calculated in problem 2?

15. (Integrates with Chapter 17.) Write a *balanced* equation for the synthesis of dTMP from UMP and N^5,N^{10}-methylene-THF. Thymidylate synthase has four active-site arginine residues (Arg^{25}, $Arg^{178'}$, $Arg^{179'}$, and Arg^{218}) involved in substrate binding. Postulate a role for the side chains of these Arg residues.

Preparing for the MCAT Exam

16. Examine Figure 26.6 and predict the relative rates of the regulated reactions in the purine biosynthetic pathway from ribose-5-P to GMP and AMP under conditions in which GMP levels are very high.

17. Decide from Figures 18.2, 18.16, 25.31, 26.27, and the Deeper Look box on page 855 which carbon atom(s) in glucose would be most likely to end up as the 5-CH_3 carbon in dTMP.

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Further Reading

Purine Metabolism

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