When mammalian cells are damaged, they release urate, a product of nucleotide metabolism. White blood cells, such as the one shown here, respond to the urate by activating other immune defenses and by increasing their phagocytic activity in order to engulf and eliminate the damaged cell. [David Scharf/Photo Researchers, Inc.]  

Nucleotides are phosphate esters of a pentose (ribose or deoxyribose) in which a purine or pyrimidine base is linked to C1’ of the sugar (Section 3-1). Nucleoside triphosphates are the monomeric units that act as precursors of nucleic acids; nucleotides also perform a wide range of other biochemical functions. For example, we have seen how the cleavage of “high-energy” compounds such as ATP provides the free energy that makes various reactions thermodynamically favorable. We have also seen that nucleotides are components of some of the central cofactors of metabolism, including FAD, NAD⁺, and coenzyme A. The importance of nucleotides in cellular metabolism is indicated by the observation that nearly all cells can synthesize them both de novo (anew) and from the degradation products of nucleic acids. However, unlike carbohydrates, amino acids, and fatty acids, nucleotides do not provide a significant source of metabolic energy.

In this chapter, we consider the nature of the nucleotide biosynthetic pathways. In doing so, we will examine how they are regulated and the consequences of their blockage, both by genetic defects and through the administration of chemotherapeutic agents. We then discuss how nucleotides are degraded. In following the general chemical themes of nucleotide metabolism, we will break our discussion into sections on purines, pyrimidines, and deoxynucleotides (including thymidylate). The structures and nomenclature of the major purines and pyrimidines are given in Table 3-1.
**1 Synthesis of Purine Ribonucleotides**

**KEY CONCEPTS**
- IMP is synthesized through the assembly of a purine base on ribose-5-phosphate.
- Kinases convert IMP-derived AMP and GMP to ATP and GTP.
- Purine nucleotide synthesis is regulated by feedback inhibition and feedforward activation.
- Salvage reactions convert purines to their nucleotide forms.

In 1948, John Buchanan obtained the first clues to the *de novo* synthesis of purine nucleotides by feeding a variety of isotopically labeled compounds to pigeons and chemically determining the positions of the labeled atoms in their excreted uric acid (a purine; at left).

The results of his studies demonstrated that N1 of purines arises from the amino group of aspartate; C2 and C8 originate from formate; N3 and N9 are contributed by the amide group of glutamine; C4, C5, and N7 are derived from glycine (strongly suggesting that this molecule is wholly incorporated into the purine ring); and C6 comes from HCO$_3^-$.

The actual pathway by which the precursors are incorporated into the purine ring was elucidated in subsequent investigations performed largely by Buchanan and G. Robert Greenberg. The initially synthesized purine derivative is inosine monophosphate (IMP; at left), the nucleotide of the base hypoxanthine. IMP is the precursor of both AMP and GMP. Thus, contrary to expectation, purines are initially formed as ribonucleotides rather than as free bases. Additional studies have demonstrated that such widely divergent organisms as *E. coli*, yeast, pigeons, and humans have virtually identical pathways for the biosynthesis of purine nucleotides, thereby further demonstrating the biochemical unity of life.

**A Purine Synthesis Yields Inosine Monophosphate**

IMP is synthesized in a pathway composed of 11 reactions (Fig. 23-1):

1. **Activation of ribose-5-phosphate.** The starting material for purine biosynthesis is α-D-ribose-5-phosphate, a product of the pentose phosphate pathway (Section 15-6). In the first step of purine biosynthesis, ribose phosphate pyrophosphokinase activates the ribose by reacting it with ATP to form 5-phosphoribosyl-α-pyrophosphate (PRPP). This compound is also a precursor in the biosynthesis of pyrimidine nucleotides (Section 23-2A) and the amino acids histidine and tryptophan (Section 21-5B). As is expected for an enzyme at such an important biosynthetic crossroads, the activity of ribose phosphate pyrophosphokinase is precisely regulated.

2. **Acquisition of purine atom N9.** In the first reaction unique to purine biosynthesis, amidophosphoribosyl transferase catalyzes the displacement of PRPP’s pyrophosphate group by glutamine’s amide nitrogen. The reaction occurs with inversion of the α configuration at C1 of PRPP, thereby forming β-5-phosphoribosylamine and establishing the anomeric form of the future nucleotide. The reaction, which is driven to completion by the subsequent hydrolysis of the released PP$_n$, is the pathway’s flux-controlling step.
FIG. 23-1  The metabolic pathway for the de novo biosynthesis of IMP. Here the purine residue is built up on a ribose ring in 11 enzyme-catalyzed reactions. The X-ray structures for all the enzymes are shown to the outside of the corresponding reaction arrow. The peptide chains of monomeric enzymes are colored in rainbow order from N-terminus (blue) to C-terminus (red). The oligomeric enzymes, all of which consist of identical polypeptide chains, are viewed along a rotation axis with their various chains differently colored. Bound ligands are shown in space-filling form with C green, N blue, O red, and P orange. [PDBids: enzyme 1, 1DKU; enzyme 2, 1A00; enzyme 3, 1GSO; enzyme 4, 1CDE; enzyme 5, 1VK3; enzyme 6, 1CLI; enzyme 7, 1D7A (PurE) and 1B6S (PurK); enzyme 8, 1A48; enzyme 9, 1C3U; enzymes 10 and 11, 1G8M.]

Identify the type of reaction that occurs at each step.
3. Acquisition of purine atoms C4, C5, and N7. Glycine’s carboxyl group forms an amide with the amino group of phosphoribosylamine, yielding glycinamide ribotide (GAR). This reaction is reversible, despite its concomitant hydrolysis of ATP to ADP + P<sub>i</sub>. It is the only step of the purine biosynthetic pathway in which more than one purine ring atom is acquired.

4. Acquisition of purine atom C8. GAR’s free -amino group is formylated to yield formylglycinamide ribotide (FGAR). The formyl donor in the reaction is N<sup>10</sup>-formyltetrahydrofolate (N<sup>10</sup>-formyl-THF), a coenzyme that transfers C<sub>1</sub> units (THF cofactors are described in Section 21-4D). The X-ray structure of the enzyme catalyzing the reaction, GAR transformylase, in complex with GAR and the THF analog 5-deazatetrahydrofolate (5dTHF) was determined by Robert Almassy (Fig. 23-2). Note the proximity of the GAR amino group to N10 of 5dTHF. This supports enzymatic studies suggesting that the GAR transformylase reaction proceeds via the nucleophilic attack of the GAR amine group on the formyl carbon of N<sup>10</sup>-formyl-THF to yield a tetrahedral intermediate.

5. Acquisition of purine atom N3. The amide amino group of a second glutamine is transferred to the growing purine ring to form formylglycinamidine ribotide (FGAM). This reaction is driven by the coupled hydrolysis of ATP to ADP + P<sub>i</sub>.

6. Formation of the purine imidazole ring. The purine imidazole ring is closed in an ATP-requiring intramolecular condensation that yields 5-aminoimidazole ribotide (AIR). The aromatization of the imidazole ring is facilitated by the tautomeric shift of the reactant from its imine to its enamine form.

7. Acquisition of C6. Purine C6 is introduced as (CO<sub>2</sub>) in a reaction catalyzed by AIR carboxylase that yields carboxyaminoimidazole ribotide (CAIR). In yeast, plants, and most prokaryotes (including E. coli), AIR carboxylase consists of two proteins called PurE and PurK. Although PurE alone can catalyze the carboxylation reaction, its K<sub>M</sub> for HCO<sub>3</sub> is ~110 mM, so the reaction would require an unphysiologically high HCO<sub>3</sub> concentration (~100 mM) to proceed. PurK decreases the HCO<sub>3</sub> concentration required for the PurE reaction by ~1000-fold but at the expense of ATP hydrolysis.

8. Acquisition of N1. Purine atom N1 is contributed by aspartate in an amide-forming condensation reaction yielding 5-aminoimidazole-4-(N-succinylcarboxamide) ribotide (SACAIR). This reaction, which is driven by the hydrolysis of ATP, chemically resembles Reaction 3.

9. Elimination of fumarate. SACAIR is cleaved with the release of fumarate, yielding 5-aminoimidazole-4-carboxamide ribotide (AICAR). Reactions 8 and 9 chemically resemble the reactions in the urea cycle in which citrulline is aminated to form arginine (Section 21-3A). In both pathways, aspartate’s amino group is transferred to an acceptor through an ATP-driven coupling reaction followed by the elimination of the aspartate carbon skeleton as fumarate.

10. Acquisition of C2. The final purine ring atom is acquired through formylation by N<sup>10</sup>-formyl-THF, yielding 5-formaminoimidazole-4-carboxamide ribotide (FAICAR). This reaction and Reaction 4 of purine biosynthesis are inhibited indirectly by sulfonamides, structural analogs of the p-aminobenzoic acid constituent of THF (Section 21-4D).

11. Cyclization to form IMP. The final reaction in the purine biosynthetic pathway, ring closure to form IMP, occurs through the elimination of water. In contrast to Reaction 6, the cyclization that forms the imidazole ring, this reaction does not require ATP hydrolysis.
In animals, Reactions 10 and 11 are catalyzed by a bifunctional enzyme, as are Reactions 7 and 8. Reactions 3, 4, and 6 also take place on a single protein. The intermediate products of these multifunctional enzymes are not readily released to the medium but are channeled to the succeeding enzymatic activities of the pathway. As in the reactions catalyzed by the pyruvate dehydrogenase complex (Section 17-2), fatty acid synthase (Section 20-4C), bacterial glutamate synthase (Section 21-7), and tryptophan synthase (Section 21-5B), channeling in the nucleotide synthetic pathways increases the overall rate of these multistep processes and protects intermediates from degradation by other cellular enzymes.

B IMP Is Converted to Adenine and Guanine Ribonucleotides

IMP does not accumulate in the cell but is rapidly converted to AMP and GMP. AMP, which differs from IMP only in the replacement of its 6-keto group by an amino group, is synthesized in a two-reaction pathway (Fig. 23-3, left). In the first reaction, aspartate’s amino group is linked to IMP in a reaction powered by the hydrolysis of GTP to GDP + P_i to yield adenylosuccinate. In the second reaction, adenylosuccinate lyase eliminates fumarate from adenylosuccinate to form AMP. The same enzyme catalyzes Reaction 9 of the IMP pathway (Fig. 23-1). Both reactions add a nitrogen with the elimination of fumarate.

GMP is also synthesized from IMP in a two-reaction pathway (Fig. 23-3, right). In the first reaction, IMP is dehydrogenated via the reduction of NAD^+ to form xanthosine monophosphate (XMP; the ribonucleotide of the base xanthine). XMP is then converted to GMP by the transfer of the glutamine...
amide nitrogen in a reaction driven by the hydrolysis of ATP to AMP + PP_i (and subsequently to 2 P_i). In B and T lymphocytes, which mediate the immune response, IMP dehydrogenase activity is high in order to supply the guanosine the cells need for proliferation. The fungal compound mycophenolic acid (at left) inhibits the enzyme and is used as an immunosuppressant following kidney transplants.

Nucleoside Diphosphates and Triphosphates Are Synthesized by the Phosphorylation of Nucleoside Monophosphates. In order to participate in nucleic acid synthesis, nucleoside monophosphates must first be converted to the corresponding nucleoside triphosphates. First, nucleoside diphosphates are synthesized from the corresponding nucleoside monophosphates by base-specific nucleoside monophosphate kinases. For example, adenylate kinase (Section 14-2C) catalyzes the phosphorylation of AMP to ADP:

\[
AMP + ATP \rightleftharpoons 2 ADP
\]

Similarly, GDP is produced by guanylate kinase:

\[
GMP + ATP \rightleftharpoons GDP + ADP
\]

These nucleoside monophosphate kinases do not discriminate between ribose and deoxyribose in the substrate.

Nucleoside diphosphates are converted to the corresponding triphosphates by nucleoside diphosphate kinase; for instance,

\[
GDP + ATP \rightleftharpoons GTP + ADP
\]

Although the reaction is written with ATP as the phosphoryl donor, this enzyme exhibits no preference for the bases of its substrates or for ribose over deoxyribose. Furthermore, the nucleoside diphosphate kinase reaction, as might be expected from the nearly identical structures of its substrates and products, normally operates close to equilibrium (\(\Delta G \approx 0\)). ADP is, of course, also converted to ATP by a variety of energy-releasing reactions such as those of glycolysis and oxidative phosphorylation. Indeed, it is those reactions that ultimately drive the foregoing kinase reactions.

C Purine Nucleotide Biosynthesis Is Regulated at Several Steps

The pathways synthesizing IMP, ATP, and GTP are individually regulated in most cells so as to control the total amounts of purine nucleotides available for nucleic acid synthesis, as well as the relative amounts of ATP and GTP! This control network is diagrammed in Fig. 23-4.

The IMP pathway is regulated at its first two reactions: those catalyzing the synthesis of PRPP and 5-phosphoribosylamine. Ribose phosphate pyrophosphokinase, the enzyme catalyzing Reaction 1 of the IMP pathway (Fig. 23-1), is inhibited by both ADP and GDP. Amidophosphoribosyl transferase, the enzyme catalyzing the first committed step of the IMP pathway (Reaction 2), is likewise subject to feedback inhibition. In this case, the enzyme binds ATP, ADP, and AMP at one inhibitory site and GTP, GDP, and GMP at another. The rate of IMP production is therefore independently but synergistically controlled by the levels of adenine nucleotides and guanine nucleotides. Moreover, amidophosphoribosyl transferase is allosterically stimulated by PRPP (feedforward activation).

A second level of regulation occurs immediately below the branch point leading from IMP to AMP and GMP. AMP and GMP are each competitive inhibitors of IMP in their own synthesis, which prevents excessive buildup of the pathway products. In addition, the rates of adenine and guanine nucleotide synthesis are coordinated. Recall that GTP powers the synthesis of AMP from IMP, whereas ATP powers the synthesis of GMP from IMP (Fig. 23-3). This reciprocity balances the production of AMP and GMP (which are required in roughly equal amounts in nucleic acid biosynthesis): The rate of synthesis of GMP increases with [ATP], whereas that of AMP increases with [GTP].
Purines Can Be Salvaged

In most cells, the turnover of nucleic acids, particularly some types of RNA, releases adenine, guanine, and hypoxanthine (Section 23-4A). These free purines are reconverted to their corresponding nucleotides through salvage pathways. In contrast to the de novo purine nucleotide synthetic pathway, which is virtually identical in all cells, salvage pathways are diverse in character and distribution. In mammals, purines are mostly salvaged by two different enzymes. Adenine phosphoribosyltransferase (APRT) mediates AMP formation using PRPP:

$$\text{Adenine} + \text{PRPP} \rightleftharpoons \text{AMP} + \text{PP}_i$$

Hypoxanthine–guanine phosphoribosyltransferase (HGPRT) catalyzes the analogous reaction for both hypoxanthine and guanine:

$$\text{Hypoxanthine} + \text{PRPP} \rightleftharpoons \text{IMP} + \text{PP}_i$$
$$\text{Guanine} + \text{PRPP} \rightleftharpoons \text{GMP} + \text{PP}_i$$

Lesch–Nyhan Syndrome Results from HGPRT Deficiency. The symptoms of Lesch–Nyhan syndrome, which is caused by a severe HGPRT deficiency, indicate that purine salvage reactions have functions other than conservation of the energy required for de novo purine biosynthesis. This sex-linked congenital defect (it affects mostly males) results in excessive uric acid production (uric acid is a purine degradation product; Section 23-4A) and neurological abnormalities such as spasticity, mental retardation, and highly aggressive and destructive behavior, including a bizarre compulsion toward self-mutilation. For example, many children with Lesch–Nyhan syndrome have such an irresistible urge to bite their lips and fingers that they must be restrained. If the restraints are removed, communicative patients will plead that the restraints be replaced, even as they attempt to injure themselves.

The excessive uric acid production in patients with Lesch–Nyhan syndrome is readily explained. The lack of HGPRT activity leads to an accumulation of the PRPP that would normally be used to salvage hypoxanthine and
guanine. The excess PRPP activates amidophosphoribosyl transferase (which catalyzes Reaction 2 of the IMP biosynthetic pathway), thereby greatly accelerating the synthesis of purine nucleotides and thus the formation of their degradation product, uric acid. Yet the physiological basis of the associated neurological abnormalities remains obscure. That a defect in a single enzyme can cause such profound but well-defined behavioral changes nevertheless has important neurophysiological implications.

2 Synthesis of Pyrimidine Ribonucleotides

**KEY CONCEPTS**

- UMP is synthesized as a pyrimidine base to which ribose-5-phosphate is added.
- CTP and UTP are derived from UMP.
- The early steps of pyrimidine nucleotide synthesis are the pathway’s major control points.

The biosynthesis of pyrimidines is simpler than that of purines. Isotopic labeling experiments have shown that atoms N1, C4, C5, and C6 of the pyrimidine ring are all derived from aspartic acid, C2 arises from , and N3 is contributed by glutamine.

![Pyrimidine Ring Structure](image)

**A UMP Is Synthesized in Six Steps**

UMP, which is also the precursor of CMP, is synthesized in a six-reaction pathway ([Fig. 23-5](#)). In contrast to purine nucleotide synthesis, the pyrimidine ring is coupled to the ribose-5-phosphate moiety after the ring has been synthesized.

1. **Synthesis of carbamoyl phosphate.** The first reaction of pyrimidine biosynthesis is the synthesis of carbamoyl phosphate from HCO₃⁻ and the amide nitrogen of glutamine by the cytosolic enzyme carbamoyl phosphate synthetase II. This reaction consumes two molecules of ATP: One provides a phosphate group and the other energizes the reaction. Carbamoyl phosphate is also synthesized in the urea cycle (Section 21-3A). In that reaction, catalyzed by the mitochondrial enzyme carbamoyl phosphate synthetase I, ammonia is the nitrogen source.

2. **Synthesis of carbamoyl aspartate.** Condensation of carbamoyl phosphate with aspartate to form carbamoyl aspartate is catalyzed by aspartate transcarbamoylase (ATCase). This reaction proceeds without ATP hydrolysis because carbamoyl phosphate is already “activated.” The structure and regulation of *E. coli* ATCase are discussed in Section 12-3.

3. **Ring closure to form dihydroorotate.** The third reaction of the pathway is an intramolecular condensation catalyzed by dihydroorotate to yield dihydroorotate.

4. **Oxidation of dihydroorotate.** Dihydroorotate is irreversibly oxidized to orotate by dihydroorotate dehydrogenase. The eukaryotic enzyme, which contains FMN and nonheme Fe, is located on the outer surface of the inner mitochondrial membrane, where quinones supply its oxidizing power. The other five enzymes of pyrimidine nucleotide biosynthesis are cytosolic in animal cells. Inhibition of dihydroorotate dehydrogenase blocks pyrimidine synthesis in T lymphocytes, thereby attenuating the autoimmune disease rheumatoid arthritis.
5. Acquisition of the ribose phosphate moiety. Orotate reacts with PRPP to yield orotate-5'-monophosphate (OMP) in a reaction catalyzed by orotate phosphoribosyl transferase. The reaction, which is driven by the hydrolysis of the eliminated PP, fixes the anomeric form of pyrimidine nucleotides in the β configuration. Orotate phosphoribosyl transferase also salvages other pyrimidine bases, such as uracil and cytosine, by converting them to their corresponding nucleotides.

6. Decarboxylation to form UMP. The final reaction of the pathway is the decarboxylation of OMP by OMP decarboxylase (ODCase) to form UMP. ODCase enhances the rate ($k_{cat}/K_M$) by a factor of $2 \times 10^{23}$ over that of the uncatalyzed reaction, making it the most catalytically proficient enzyme known. Nevertheless, the reaction requires no cofactors to help stabilize its putative carbanion intermediate. Although the mechanism of the ODCase reaction is not fully understood, the removal of OMP's phosphate group, which is quite distant from the
C6 carboxyl group, decreases the reaction rate by a factor of $7 \times 10^3$, thus providing a striking example of how binding energy can be applied to catalysis (preferential transition state binding).

In bacteria, the six enzymes of UMP biosynthesis occur as independent proteins. In animals, however, as Mary Ellen Jones demonstrated, the first three enzymatic activities of the pathway—carbamoyl phosphate synthetase II, ATCase, and dihydroorotase—occur on a single 210-kD polypeptide chain. The pyrimidine biosynthetic pathway is a target for antiparasitic drugs. For example, the parasite *Toxoplasma gondii* (Fig. 23-6), which infects most mammals, causes toxoplasmosis, a disease whose complications include blindness, neurological dysfunction, and death in immunocompromised individuals (e.g., those with AIDS). Most parasites have evolved to take advantage of nutrients supplied by their hosts, but *T. gondii* is unable to meet its needs exclusively through nucleotide salvage pathways and retains the ability to synthesize uracil *de novo*. Drugs that target the parasite's carbamoyl phosphate synthetase II (an enzyme whose structure and kinetics distinguish it from its mammalian counterpart) could therefore prevent *T. gondii* growth. Moreover, there is evidence that *T. gondii* strains that have been engineered to lack carbamoyl phosphate synthetase II are avirulent and could be useful as vaccines in humans and livestock.

**B UMP Is Converted to UTP and CTP**

The synthesis of UTP from UMP is analogous to the synthesis of purine nucleoside triphosphates (Section 23-1B). The process occurs by the sequential actions of a nucleoside monophosphate kinase and nucleoside diphosphate kinase:

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

CTP is formed by the amination of UTP by CTP synthetase (Fig. 23-7). In animals, the amino group is donated by glutamine, whereas in bacteria it is supplied directly by ammonia.

**C Pyrimidine Nucleotide Biosynthesis Is Regulated at ATCase or Carbamoyl Phosphate Synthetase II**

In bacteria, the pyrimidine biosynthetic pathway is primarily regulated at Reaction 2, the ATCase reaction (Fig. 23-8a). In *E. coli*, control is exerted through the allosteric stimulation of ATCase by ATP and its inhibition by CTP (Section 12-3). In many bacteria, however, UTP is the major ATCase inhibitor.

In animals, ATCase is not a regulatory enzyme. Rather, pyrimidine biosynthesis is controlled by the activity of carbamoyl phosphate synthetase II, which is inhibited by UDP and UTP and activated by ATP and PRPP (Fig. 23-8b). A second level of control in the mammalian pathway occurs at OMP decarboxylase, for which UMP and to a lesser extent CMP are competitive in-
hibitors. In all organisms, the rate of OMP production varies with the availability of its precursor, PRPP. Recall that the PRPP level depends on the activity of ribose phosphate pyrophosphokinase (Fig. 23-1, Reaction 1), which is inhibited by ADP and GDP (Section 23-1C).

Orotic Aciduria Results from an Inherited Enzyme Deficiency. Orotic aciduria, an inherited human disease, is characterized by the urinary excretion of large amounts of orotic acid, retarded growth, and severe anemia. It results from a deficiency in the bifunctional enzyme catalyzing Reactions 5 and 6 of pyrimidine nucleotide biosynthesis. Consideration of the biochemistry of this situation led to its effective treatment: the administration of uridine and/or cytidine. The UMP formed through the phosphorylation of the nucleosides, besides replacing that normally synthesized, inhibits carbamoyl phosphate synthetase II so as to attenuate the rate of orotic acid synthesis. No other genetic deficiency in pyrimidine nucleotide biosynthesis is known in humans, presumably because such defects are lethal in utero.

3 Formation of Deoxyribonucleotides

**KEY CONCEPTS**

- Ribonucleotide reductase uses a free radical mechanism to convert ribonucleotides to deoxyribonucleotides.
- Thymidylate synthase transfers a methyl group to dUMP to form thymine.

DNA differs chemically from RNA in two major respects: (1) Its nucleotides contain 2’-deoxyribose residues rather than ribose residues, and (2) it contains
the base thymine (5-methyluracil) rather than uracil. In this section, we consider the biosynthesis of these DNA components.

A Ribonucleotide Reductase Converts Ribonucleotides to Deoxyribonucleotides

Deoxyribonucleotides are synthesized from their corresponding ribonucleotides by the reduction of their C2\(^{-}\) position rather than by their de novo synthesis from deoxyribose-containing precursors.

Enzymes that catalyze the formation of deoxyribonucleotides by the reduction of the corresponding ribonucleotides are named ribonucleotide reductases (RNRs). There are three classes of RNRs, which differ in their prosthetic groups, although they all replace the 2\(^{-}\)-OH group of ribose with H via a free-radical mechanism. We will discuss the mechanism of the so-

**FIG. 23-9** Class I ribonucleotide reductase from *E. coli.*
(a) Schematic diagram of the quaternary structure. The enzyme consists of two identical pairs of subunits, R1\(_2\) and R2\(_2\). Each R2 subunit contains a binuclear Fe(III) complex that generates a phenoxy radical at Tyr 122. The R1 subunits each contain three different allosteric effector sites and five catalytically important Cys residues. The enzyme’s two active sites occur at the interface between the R1 and R2 subunits. (b) A ribbon diagram of R2\(_2\) viewed perpendicularly to its twofold axis with the subunits drawn in blue and yellow. The Fe(III) ions are represented by orange spheres, and the radical-harboring Tyr 122 side chains are shown in space-filling representation with their C and O atoms green and red. (c) The binuclear Fe(III) complex of R2. Each Fe(III) ion is octahedrally coordinated by a His N atom and five O atoms, including those of the O\(^{2-}\) ion and the Glu carboxyl group that bridges the two Fe(III) ions. [Part b based on an X-ray structure by Hans Eklund, Swedish University of Agricultural Sciences, Uppsala, Sweden. PDBid 1RIB.] See Interactive Exercise 33.
called Class I RNRs, which contain an Fe or Mn prosthetic group and which occur in most eukaryotes and aerobic prokaryotes.

Class I RNRs reduce ribonucleoside diphosphates (NDPs) to the corresponding deoxyribonucleoside diphosphates (dNDPs). *E. coli* ribonucleotide reductase, as Peter Reichard demonstrated, is mainly present *in vitro* as a heterotetramer that can be decomposed to two catalytically inactive homodimers, R12 and R22 (Fig. 23-9a; opposite). Each R1 subunit contains a substrate-binding site that includes several redox-active thiol groups. The R1 subunits also contain three effector-binding sites that control the enzyme’s catalytic activity as well as its substrate specificity (see below).

The X-ray structure of R22 (Fig. 23-9b), determined by Hans Eklund, reveals that the subunits are bundles of eight unusually long helices. Each subunit contains a novel binuclear Fe(III) prosthetic group whose Fe(III) ions are liganded by a variety of groups including an O2− ion (Fig. 23-9c). The Fe(III) complex interacts with Tyr 122 to form an unusual tyrosyl free radical. Recall that tyrosine radicals also take part in the reactions catalyzed by cytochrome *c* oxidase (Section 18-2F) and plant Photosystem II (Section 19-2C).

JoAnne Stubbe has proposed the following catalytic mechanism for *E. coli* ribonucleotide reductase (Fig. 23-10):

1. Ribonucleotide reductase’s free radical (X•) abstracts an H atom from C3′ of the substrate in the reaction’s rate-determining step.
2 and 3. Acid-catalyzed cleavage of the C2′–OH bond releases H2O to yield a radical–cation intermediate. The C3′-OH group’s unshared

![Enzymatic mechanism of ribonucleotide reductase](image)
electron pair stabilizes the C2’ cation. This accounts for the radical’s catalytic role.

4. The radical–cation intermediate is reduced by the enzyme’s redox-active sulfhydryl pair to yield a 3’-deoxyxynucleotide radical and a protein disulfide group (this group must eventually be reduced to regenerate the enzyme’s activity).

5. The 3’ radical abstracts an H atom from the protein to yield the product deoxynucleoside diphosphate and restore the enzyme to its radical state.

The Tyr 122 radical in R2 is buried 10 Å beneath the surface of the protein, too far for the enzyme’s catalytic site to abstract an electron directly from the substrate. Evidently, the protein mediates electron transfer from this tyrosyl radical to another group that is closer to the substrate, probably the thyl radical (—S—) form of Cys 439 in R1 (represented as X· in Fig. 23-10). Two other R1 Cys residues probably form the redox-active sulfhydryl pair that directly reduces the substrate. The resulting disulfide bond is reduced via disulfide interchange with yet two other Cys residues, which are positioned to accept electrons from external reducing agents to regenerate the active enzyme. Thus, each R1 subunit contains at least five Cys residues that chemically participate in nucleotide reduction.

The Inability of Oxidized Ribonucleotide Reductase to Bind Substrate Serves an Essential Protective Function. Comparison of the X-ray structures of reduced R1 (in which the redox-active Cys 225 and Cys 462 residues are in their SH forms) and oxidized R1 (in which Cys 225 and Cys 462 are disulfide-linked) reveals that Cys 462 in reduced R1 has rotated away from its position in oxidized R1 to become buried in a hydrophobic pocket, whereas Cys 225 moves into the region formerly occupied by Cys 462. The distance between the formerly disulfide-linked S atoms thereby increases from 2.0 Å to 5.7 Å. These movements are accompanied by small shifts of the surrounding polypeptide chain. R1 Cys 225 in oxidized ribonucleotide reductase prevents the binding of substrate through steric interference of its S atom with the substrate NDP’s O2’ atom.

The inability of oxidized ribonucleotide reductase to bind substrate has functional significance. In the absence of substrate, the enzyme’s free radical is stored in the interior of the R2 subunit, close to its dinuclear iron center. When substrate is bound, the radical is presumably transferred to it via a series of protein side chains in both R2 and R1. If the substrate is unable to properly react after accepting this free radical, as would be the case if the enzyme were in its oxidized state, the free radical could potentially destroy both the substrate and the enzyme. Thus, an important role of the enzyme is to control the release of the radical’s powerful oxidizing capability. It does so in part by preventing the binding of substrate while the enzyme is in its oxidized form.

Thioredoxin Reduces Ribonucleotide Reductase. The final step in the ribonucleotide reductase catalytic cycle is reduction of the enzyme’s newly formed disulfide bond to re-form its redox-active sulfhydryl pair. One of the enzyme’s physiological reducing agents is thioredoxin, a ubiquitous monomeric protein with a pair of neighboring Cys residues (and which also participates in regulating the Calvin cycle; Section 19-3C). Thioredoxin reduces oxidized ribonucleotide reductase via disulfide interchange.
The X-ray structure of thioredoxin (Fig. 23-11) reveals that its redox-active disulfide group is located on a molecular protrusion, making the protein the only known example of a “male” enzyme.

Oxidized thioredoxin is, in turn, reduced in a reaction mediated by thioredoxin reductase, which contains redox-active thiol groups and an FAD prosthetic group. This enzyme, a homolog of glutathione reductase (Box 15-4) and lipoamide dehydrogenase (Section 17-2B), catalyzes a similar reaction, the NADPH-mediated reduction of a substrate disulfide bond. NADPH therefore serves as the terminal reducing agent in the ribonucleotide reductase–catalyzed reduction of NDPs to dNDPs (Fig. 23-12).

**Ribonucleotide Reductase Is Regulated by a Complex Feedback Network.**

The synthesis of the four dNTPs in the amounts required for DNA synthesis is accomplished through feedback control. Maintaining the proper intracellular ratios of dNTPs is essential for normal growth. Indeed, a deficiency of any dNTP is lethal, whereas an excess is mutagenic because the probability that a given dNTP will be erroneously incorporated into a growing DNA strand increases with its concentration relative to those of the other dNTPs.

The activities of both *E. coli* and mammalian ribonucleotide reductases are remarkably responsive to the levels of nucleotides in the cell. For example, ATP induces the reduction of CDP and UDP; dTTP induces the reduction of GDP and inhibits the reduction of CDP and UDP; and dATP inhibits the reduction of all NDPs. Barry Cooperman has shown that the catalytic activity of mouse ribonucleotide reductase varies with its state of oligomerization, which in turn is governed by the binding of nucleotide effectors to three independent allosteric sites on R1 (Fig. 23-9a): (1) the specificity site, which binds ATP, dATP, dGTP, and dTTP; (2) the activity site, which binds ATP and dATP; and (3) the hexamerization site, which binds only ATP. Cooperman’s model for the allosteric regulation of Class I ribonucleotide reductase...
ductase, which quantitatively accounts for the enzyme's regulatory properties, has the following features (Fig. 23-13):

1. The binding of ATP, dATP, dGTP, or dTTP to the specificity site induces the catalytically inactive R1 monomers to form a catalytically active dimer, R1₂.

2. The binding of dATP or ATP to the activity site causes the dimers to form tetramers, R1₄a, that slowly but reversibly change conformation to a catalytically inactive state, R1₄b.

3. The binding of ATP to the hexamerization site induces the tetramers to further aggregate to form catalytically active hexamers, R1₆, the enzyme's major active form.

The concentration of ATP in a cell is such that, in vivo, R1 is almost entirely in its tetrameric or hexameric forms. As a consequence, ATP couples the overall rate of DNA synthesis to the cell's energy state.

dNTPs Are Produced by Phosphorylation of dNDPs. The final step in the production of all dNTPs is the phosphorylation of the corresponding dNDPs:

\[
dNTP + ATP \rightleftharpoons dNTP + ADP
\]

This reaction is catalyzed by nucleoside diphosphate kinase, the same enzyme that phosphorylates NDPs (Section 23-1B). As before, the reaction is written with ATP as the phosphoryl donor, although any NTP or dNTP can function in that capacity.

B dUMP Is Methylated to Form Thymine

The dTTP substrate for DNA synthesis is derived from dUTP, which is hydrolyzed to dUMP by dUTP diphosphohydrolase (dUTPase):

\[
dUTP + H_2O \rightarrow dUMP + PP_i
\]

The dUMP is then methylated to generate dTMP, and the dTMP is phosphorylated to form dTTP. The apparent reason for the energetically wasteful process of dephosphorylating dUTP and repolishing dTMP is that cells must minimize their concentration of dUTP in order to prevent incorporation of uracil into their DNA (the enzyme system that synthesizes DNA from dNTPs does not efficiently discriminate between dUTP and dTTP; Section 25-2A).

Human dUTPase is a homotrimer of 141-residue subunits. Its X-ray structure, determined by John Tainer, reveals the basis for the enzyme's exquisite specificity for dUTP. Each subunit binds dUTP in a snug-fitting cavity that sterically excludes thymine's C5 methyl group via the side chains of conserved
residues (Fig. 23-14a). The enzyme differentiates uracil from the similarly shaped cytosine via a set of hydrogen bonds that in part mimic adenine's base pairing interactions (Fig. 23-14b). The 2'-OH group of ribose is likewise sterically excluded by the side chain of a conserved Tyr.

**Thymidylate Synthase Transfers a Methyl Group to dUMP.** Thymidylate (dTMP) is synthesized from dUMP by thymidylate synthase with $N^5,N^{10}$-methyleneetrahydrofolate ($N^5,N^{10}$-methylene-THF) as the methyl donor:

\[
\text{dUMP} + N^5,N^{10}\text{-Methylenetetrahydrofolate} \rightarrow \text{dTMP} + \text{Dihydrofolate}
\]

\[
R = \underset{\text{O}}{\text{C}}\overset{\text{N}}{\text{H}}\overset{\text{N}}{\text{H}}\overset{\text{N}}{\text{H}}\overset{\text{N}}{\text{H}}\overset{-\text{COO}^-}{\text{O}}; \quad n = 1-6
\]

**FIG. 23-14  X-Ray structure of human dUTPase.** (a) The active site region of dUTPase in complex with dUTP. The protein is represented by its molecular surface colored according to its electrostatic potential (negative, red; positive, blue; and near neutral, white). The dUTP is shown in ball-and-stick form with its N, O, and P atoms blue, red, and yellow. Mg$^{2+}$ ions that have been modeled into the structure are represented by green spheres. Note the complementary fit of the uracil ring into its binding pocket, particularly the close contacts that discriminate against a methyl group on C5 of the pyrimidine ring and a 2'-OH group on the ribose ring. (b) The binding site of dUMP, showing the hydrogen bonding system responsible for the enzyme's specific binding of a uracil ring. The dUMP and the polypeptide backbone binding it are shown in ball-and-stick form with atoms colored as in Part a; hydrogen bonds are indicated by white dotted lines; and a conserved water molecule is represented by a pink sphere. The side chain of a conserved Tyr is tightly packed against the ribose ring so as to discriminate against the presence of a 2'-OH group. [Courtesy of John Tainer and Clifford Mol, The Scripps Research Institute, La Jolla, California.]
Note that the transferred methylene group (in which the carbon has the oxidation state of formaldehyde) is reduced to a methyl group (which has the oxidation state of methanol) at the expense of the oxidation of the THF cofactor to dihydrofolate (DHF).

Thymidylate synthase, a highly conserved 65-kD homodimeric protein, follows a mechanistic scheme proposed by Daniel Santi (Fig. 23-15):

1. An enzyme nucleophile, identified as the thiolate group of Cys 146, attacks C6 of dUMP to form a covalent adduct.

2. C5 of the resulting enolate ion attacks the CH2 group of the iminium cation in equilibrium with $N^5,N^{10}$-methylene-THF to form an enzyme–dUMP–THF ternary covalent complex.

3. An enzyme base abstracts the acidic proton at the C5 position of the enzyme-bound dUMP, forming an exocyclic methylene group and eliminating the THF cofactor. The abstracted proton subsequently exchanges with solvent.
The redox change occurs via the migration of the C6-H atom of THF as a hydride ion to the exocyclic methylene group, converting it to a methyl group and yielding DHF. This reduction promotes the displacement of the Cys thiolate group from the intermediate to release product, dTMP, and re-form the active enzyme.

**Tetrahydrofolate Is Regenerated in Two Reactions.** The thymidylate synthase reaction is biochemically unique in that it oxidizes THF to DHF; no other enzymatic reaction employing a THF cofactor alters this coenzyme’s net oxidation state. The DHF product of the thymidylate synthase reaction is recycled back to $N^5,N^{10}$-methylene-THF through two sequential reactions (Fig. 23-16):

1. DHF is reduced to THF by NADPH as catalyzed by dihydrofolate reductase (DHFR; Fig. 23-17). Although in most organisms DHFR is a monomeric, monofunctional enzyme, in protozoa and some plants

---

**FIG. 23-16** Regeneration of $N^5,N^{10}$-methylene tetrahydrofolate. The DHF product of the thymidylate synthase reaction is converted back to $N^5,N^{10}$-methylene-THF by the sequential actions of (1) dihydrofolate reductase and (2) serine hydroxymethyltransferase. The sites of action of some inhibitors are indicated by red octagons. Thymidylate synthase is inhibited by FdUMP, whereas dihydrofolate reductase is inhibited by the antifolates methotrexate, aminopterin, and trimethoprim (Box 23-1).

**FIG. 23-17** X-Ray structure of human dihydrofolate reductase in complex with folic acid. The polypeptide is colored in rainbow order from its N-terminus (blue) to its C-terminus (red). The folic acid is drawn in space-filling form with C green, N blue, and O red. [Based on an X-ray structure by Joseph Kraut, University of California at San Diego. PDBid 1DHF]
DHFR and thymidylate synthase occur on the same polypeptide chain to form a bifunctional enzyme that has been shown to channel DHF from its thymidylate synthase to its DHFR active sites.

2. Serine hydroxymethyltransferase (Section 21-4A) transfers the hydroxymethyl group of serine to THF yielding \(N^5,N^{10}\)-methylene-THF and glycine.

Inhibition of thymidylate synthase or DHFR blocks dTMP synthesis and is therefore the basis of cancer chemotherapies (Box 23-1).

4 Nucleotide Degradation

**KEY CONCEPTS**

- Purines are broken down to uric acid.
- Uric acid may be further catabolized for excretion.
- Pyrimidines are converted to CoA derivatives for catabolism.

Most foodstuffs, being of cellular origin, contain nucleic acids. Dietary nucleic acids survive the acidic medium of the stomach; they are degraded to their component nucleotides, mainly in the intestine, by pancreatic nucleases and intestinal phosphodiesterases. The ionic nucleotides, which cannot pass through cell membranes, are then hydrolyzed to nucleosides by a variety of group-specific nucleotidases and nonspecific phosphatases. Nucleosides may be directly absorbed by the intestinal mucosa or further degraded to free bases and ribose or ribose-1-phosphate through the action of nucleosidases and nucleoside phosphorylases:

\[
\text{Nucleoside} + H_2O \xrightarrow{\text{nucleosidase}} \text{base} + \text{ribose}
\]

\[
\text{Nucleoside} + P_i \xrightarrow{\text{nucleoside phosphorylase}} \text{base} + \text{ribose-1-P}
\]

Radioactive labeling experiments have demonstrated that only a small fraction of the bases of ingested nucleic acids are incorporated into tissue nucleic acids. Evidently, the de novo pathways of nucleotide biosynthesis largely satisfy an organism’s need for nucleotides. Consequently, ingested bases are mostly degraded and excreted. Cellular nucleic acids are also subject to degradation as part of the continual turnover of nearly all cellular components. In this section, we outline these catabolic pathways and discuss the consequences of several of their inherited defects. A summary of nucleotide metabolism is shown in Fig. 23-18.
Inhibition of Thymidylate Synthesis in Cancer Therapy

5-Fluorodeoxyuridylate (FdUMP) is an irreversible inhibitor of thymidylate synthase. This substance, like dUMP, binds to the enzyme (an F atom is not much larger than an H atom) and undergoes the first two steps of the normal enzymatic reaction (Fig. 23-15). In Step 3, however, the enzyme cannot abstract the F atom as F\(^{−}\) (F is the most electronegative element) so that the enzyme is frozen in an enzyme–FdUMP–THF ternary covalent complex.

The X-ray structure of the covalent thymidylate synthase–FdUMP–THF ternary complex has been determined.

are DHF analogs that competitively although nearly irreversibly bind to DHFR with an ∼1000-fold greater affinity than does DHF. These antifolates (substances that interfere with the action of folate cofactors) are effective anticancer agents, particularly against childhood leukemias. In fact, a successful chemotherapeutic strategy is to treat a cancer victim with a lethal dose of methotrexate and some hours later "rescue" the patient (but hopefully not the cancer) by administering massive doses of 5-formyl-THF and/or thymidine. Trimethoprim binds much more tightly to bacterial DHFRs than to those of mammals and is therefore a clinically useful antibacterial agent. A variety of compounds that inhibit protozoan DHFR are used to treat (and prevent) malaria and other parasitic infections.

[Based on an X-ray structure by William Montfort, University of Arizona. PDBid 1TSN.]
A Purine Catabolism Yields Uric Acid

The major pathways of purine nucleotide and deoxynucleotide catabolism in animals are diagrammed in Fig. 23-19. The pathways in other organisms differ somewhat, but all the pathways lead to uric acid. Of course, the pathway intermediates may be directed to purine nucleotide synthesis via salvage reactions. In addition, ribose-1-phosphate, a product of the reaction catalyzed by purine nucleoside phosphorylase (PNP), is a precursor of PRPP.

Adenosine and deoxyadenosine are not degraded by mammalian PNP. Rather, adenine nucleosides and nucleotides are deaminated by adenosine deaminase (ADA) and AMP deaminase to their corresponding inosine derivatives, which can then be further degraded.

ADA is an eight-stranded α/β barrel (Fig. 23-20) with its active site in a pocket at the C-terminal end of the β barrel, as in nearly all known

**FIG. 23-19** The major pathways of purine catabolism in animals. The various purine nucleotides are all degraded to uric acid. Compare the activity of PNP to that of glycogen phosphorylase (Section 16-1A).

**FIG. 23-20** X-Ray structure of murine adenosine deaminase. The polypeptide is drawn in ribbon form colored according to its secondary structure (helices cyan, β strands magenta, and loops pink) and viewed approximately down the axis of the enzyme’s α/β barrel from the N-terminal ends of its β strands. The transition state analog 6-hydroxy-1,6-dihydropurine ribonucleoside (HDPR) is shown in stick form with its C, N, and O atoms green, blue, and red. The enzyme-bound Zn²⁺ ion, which is coordinated by HDPR’s 6-hydroxyl group, is represented by a silver sphere. [Based on an X-ray structure by Florante Quiocio, Baylor College of Medicine. PDBid 1ADA] See Interactive Exercise 35.
α/β barrel enzymes. A catalytically essential zinc ion is bound in the deepest part of the active site pocket. Mutations that affect the active site of ADA selectively kill lymphocytes, causing severe combined immunodeficiency disease (SCID). Without special protective measures, the disease is invariably fatal in infancy because of overwhelming infection.

Biochemical considerations provide a plausible explanation of SCID’s etiology (causes). In the absence of active ADA, deoxyadenosine is phosphorylated to yield levels of dATP that are 50-fold greater than normal. This high concentration of dATP inhibits ribonucleotide reductase (Section 23-3A), thereby preventing the synthesis of the other dNTPs, choking off DNA synthesis and thus cell proliferation. The tissue-specific effect of ADA deficiency on the immune system can be explained by the observation that lymphoid tissue is particularly active in deoxyadenosine phosphorylation. ADA deficiency was one of the first genetic diseases to be successfully treated by gene therapy (Section 3-5D).

The Purine Nucleotide Cycle Generates Fumarate. The deamination of AMP to IMP, when combined with the synthesis of AMP from IMP (Fig. 23-3, left), has the net effect of deaminating aspartate to yield fumarate (Fig. 23-21). John Lowenstein demonstrated that this purine nucleotide cycle has an important metabolic role in skeletal muscle. An increase in muscle activity requires an increase in the activity of the citric acid cycle. This process usually occurs through the generation of additional citric acid cycle intermediates (Section 17-5B). Muscles, however, lack most of the enzymes that catalyze these anaplerotic (filling up) reactions in other tissues. Instead, muscle replenishes its citric acid cycle intermediates with fumarate generated in the purine nucleotide cycle.

The importance of the purine nucleotide cycle in muscle metabolism is indicated by the observation that the activities of the three enzymes involved are all severalfold higher in muscle than in other tissues. In fact, individuals with an inherited deficiency in muscle AMP deaminase (myoadenylate deaminase deficiency) are easily fatigued and usually suffer from cramps after exercise.

Xanthine Oxidase Is a Mini-Electron-Transport System. Xanthine oxidase (XO) converts hypoxanthine (the base of IMP) to xanthine, and xanthine to uric acid (Fig. 23-19, bottom). The reaction product is an enol (which has a pK of 5.4; hence the name uric acid). The enol tautomerizes to the more stable keto form:
In mammals, xanthine oxidase occurs almost exclusively in the liver and the small intestinal mucosa. It is a dimeric protein of identical 130-kD subunits, each of which contains an entire “zoo” of electron-transfer agents: an FAD, a Mo complex that cycles between its Mo(VI) and Mo(IV) oxidation states, and two different Fe–S clusters. The final electron acceptor is O\textsubscript{2}, which is converted to H\textsubscript{2}O\textsubscript{2}, a potentially harmful oxidizing agent (Section 18-4B) that is subsequently converted to H\textsubscript{2}O and O\textsubscript{2} by catalase.

**B Some Animals Degrade Uric Acid**

In humans and other primates, the final product of purine degradation is uric acid, which is excreted in the urine. The same is true in birds, terrestrial reptiles, and many insects, but those organisms, which do not excrete urea, also catabolize their excess amino acid nitrogen to uric acid via purine biosynthesis. This complicated system of nitrogen excretion has a straightforward function: It conserves water. Uric acid is only sparingly soluble in water, so its excretion as a paste of uric acid crystals is accompanied by very little water. In contrast, the excretion of an equivalent amount of the much more water-soluble urea osmotically sequesters a significant amount of water.

In all other organisms, uric acid is further processed before excretion (Fig. 23-22). Mammals other than primates oxidize it to their excretory product, allantoin, in a reaction catalyzed by the Cu-containing enzyme urate oxidase. A further degradation product, allantoic acid, is excreted by teleost (bony) fish. Cartilaginous fish and amphibia further degrade allantoic acid to urea prior to excretion. Finally, marine invertebrates decompose urea to NH\textsubscript{4}+.

**Gout Is Caused by an Excess of Uric Acid.** Gout is a disease characterized by elevated levels of uric acid in body fluids. Its most common manifestation is excruciatingly painful arthritic joint inflammation of sudden onset, most often of the big toe (Fig. 23-23), caused by deposition of nearly insoluble crystals of sodium urate. Sodium urate and/or uric acid may also precipitate in the kidneys and ureters as stones, resulting in renal damage and urinary tract obstruction.

Gout, which affects ~3 per 1000 persons, predominantly males, has been traditionally, although inaccurately, associated with overindulgent eating and drinking. The probable origin of this association is that in previous centuries, when wine was often contaminated with lead during its manufacture and storage, heavy drinking resulted in chronic lead poisoning that, among other things, decreases the kidney’s ability to excrete uric acid.

The most prevalent cause of gout is impaired uric acid excretion (although usually for reasons other than lead poisoning). Gout may also result from a number of metabolic insufficiencies, most of which are not well characterized.

**FIG. 23-22** Degradation of uric acid to ammonia. The process is arrested at different stages in the indicated species, and the resulting nitrogen-containing product is excreted.

**FIG. 23-23** The Gout, a cartoon by James Gillray (1799). [Courtesy of Yale University Medical Historical Library.]
Gertrude Elion and Purine Derivatives

A related compound, azathioprine, is converted to 6-mercaptopurine intracellularly. It turned out to be effective not as an anticancer drug but as an inhibitor of the immune response. This drug helped solve the problem of rejection in organ transplants and was used for the first successful human kidney transplant in 1961.

Studies aimed at improving the effectiveness of purine derivatives by preventing their degradation by xanthine oxidase led to the discovery of allopurinol (Section 23-4B), which is still used to treat gout and some parasitic diseases. Elion also helped develop the antibacterial agent trimethoprim (Box 23-1) and the widely used antiviral drug acyclovir (Zovir).

After Hitchings’ retirement, Elion became head of the Department of Experimental Therapy in 1967. Although she formally retired in 1983, Elion’s line of research continued to bear fruit with the development of azidothymidine (AZT; Box 12-3), a nucleoside analog that was the sole drug that was effective for treating AIDS until 1991 and is still in use.

Elion, together with Hitchings and James Black (who discovered cimetidine (Tagamet), the first drug to inhibit stomach acid secretion, and propranolol (Inderol), which is widely used in the treatment of high blood pressure), was awarded the Nobel Prize for Physiology or Medicine in 1988, a rare accomplishment for a scientist in the pharmaceutical industry. It was her research, which was based on an understanding of nucleotide metabolism, rather than her ability to simply synthesize novel compounds, that merited recognition.

One well-understood cause is HGPRT deficiency (Lesch–Nyhan syndrome in severe cases), which leads to excessive uric acid production through PRPP accumulation (Section 23-1D).

Gout can be treated by administering the xanthine oxidase inhibitor allopurinol (at right), a hypoxanthine analog with interchanged N7 and C8 positions. Xanthine oxidase hydroxylates allopurinol, as it does hypoxanthine, yielding alloxanthine (at right), which remains tightly bound to the reduced form of the enzyme, thereby inactivating it. Allopurinol consequently alleviates the symptoms of gout by decreasing the rate of uric acid production while increasing the levels of the more soluble hypoxanthine and xanthine. Although allopurinol controls the gouty symptoms of Lesch–Nyhan syndrome, it has no effect on its neurological symptoms. Allopurinol, along with several other notable purine derivatives, was developed by the chemist Gertrude Elion (Box 23-2).
Pyrimidines Are Broken Down to Malonyl-CoA and Methylmalonyl-CoA

Animal cells degrade pyrimidine nucleotides to their component bases (Fig. 23-24, top). The reactions, like those of purine nucleotides, occur through dephosphorylation, deamination, and glycosidic bond cleavages. The resulting uracil and thymine are then broken down in the liver through reduction (Fig. 23-24, middle) rather than by oxidation as occurs in purine catabolism. The end products of pyrimidine catabolism, \(\text{\textbeta}-\text{Alanine} \) (\(\text{\textbeta}-\text{Aminoisobutyrate}\)) and \(\text{\textbeta}-\text{Ureidopropionate} \) (\(\text{\textbeta}-\text{Ureidoisobutyrate}\)), are amino acids and are metabolized as such. They are converted, through transamination and activation reactions, to malonyl-CoA and methylmalonyl-CoA (Fig. 23-24, bottom left). Malonyl-CoA is a precursor of fatty acid synthesis (Fig. 20-26), and methylmalonyl-CoA is converted to the citric acid cycle intermediate succinyl-CoA (Fig. 20-16). Thus, to a limited extent, catabolism of pyrimidine nucleotides contributes to the energy metabolism of the cell.

**CHECKPOINT**
- What compounds are produced by the degradation of purines and pyrimidines?
- Describe the reactions catalyzed by nucleoside phosphorylase, adenosine deaminase, and xanthine oxidase.
- What is the function of the purine nucleotide cycle?
- What are the physiological implications of excreting waste nitrogen in the form or urate, urea, or ammonia?
- Describe how purine catabolism is related to SCID, muscle function, and gout.
Summary

1 Synthesis of Purine Ribonucleotides
- The purine nucleotide IMP is synthesized in 11 steps from ribose-5-phosphate, aspartate, fumarate, glutamine, glycine, and HCO$_3^-$.
- Purine nucleotide synthesis is regulated at its first and second steps.
- IMP is the precursor of AMP and GMP, which are phosphorylated to produce the corresponding di- and triphosphates.

2 Synthesis of Pyrimidine Ribonucleotides
- The pyrimidine nucleotide UMP is synthesized from 5-phosphoribosyl pyrophosphate, aspartate, glutamine, and in six reactions. UMP is converted to UTP and CTP by phosphorylation and amination.
- Pyrimidine nucleotide synthesis is regulated in bacteria at the ATCase step and in animals at the step catalyzed by carbamoyl phosphate synthetase II.

3 Formation of Deoxyribonucleotides
- Deoxyribonucleoside diphosphates are synthesized from the corresponding NDPs by the action of ribonucleotide reductase, which contains a binuclear Fe(III) prosthetic group, a tyrosyl radical, and several redox-active sulfhydryl groups. Enzyme activity is regenerated through disulfide interchange with thioredoxin.
- Ribonucleotide reductase is regulated by allosteric effectors, which ensure that deoxynucleotides are synthesized in the amounts required for DNA synthesis.
- dTMP is synthesized from dUMP by thymidylate synthase. The dihydrofolate produced in the reaction is converted back to tetrahydrofolate by dihydrofolate reductase (DHFR).

4 Nucleotide Degradation
- Purine nucleotides are degraded by nucleosidases and purine nucleoside phosphorylase (PNP). Adenine nucleotides are deaminated by adenosine deaminase and AMP deaminase. The synthesis and degradation of AMP in the purine nucleotide cycle yield the citric acid cycle intermediate fumarate in muscles. Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and of xanthine to uric acid.
- In primates, birds, reptiles, and insects, the final product of purine degradation is uric acid, which is excreted. Other organisms degrade urate further.
- Pyrimidines are broken down to intermediates of fatty acid metabolism.

Key Terms
- PRPP
- feedforward activation
- salvage pathway
- mechanism-based inhibitor
- purine nucleotide cycle

Problems
1. Which reaction of the IMP $\rightarrow$ AMP pathway resembles a reaction of the urea cycle?
2. From which purine is caffeine derived?

![Caffeine](image)

3. List all the enzymes of nucleotide biosynthesis that use glutamine as an amino group donor.
4. Certain glutamine analogs irreversibly inactivate enzymes that bind glutamine. Identify the nucleotide biosynthetic intermediates that accumulate in the presence of those compounds.
5. Calculate the cost, in ATP equivalents, of synthesizing de novo (a) IMP, (b) AMP, and (c) CTP. Assume all substrates (e.g., ribose-5-phosphate and glutamine) and cofactors are available.
6. Rats are given cytidine that is $^{14}$C-labeled at both its base and ribose components. Their DNA is then extracted and degraded with nucleases. Describe the labeling pattern of the recovered deoxyctydylate residues if deoxycytidylate production in the cell followed a pathway in which (a) intact CDP is reduced to dCDP, and (b) CDP is broken down to cytosine and ribose before reduction.
7. Why does it make metabolic sense for UTP to inhibit carbamoyl phosphate synthetase II, whereas ATP activates the enzyme?
8. Why does it make metabolic sense for ADP and GDP to inhibit ribose phosphate pyrophosphokinase?
9. Explain why hydroxyurea, which destroys tyrosyl radicals, is useful as an antitumor agent.
10. Why is dATP toxic to mammalian cells?
11. Mouse embryonic stem cells divide extremely rapidly, about once every 5 hours. These cells require large amounts of threonine in their medium. Explain how threonine catabolism (Fig. 21-14) helps meet the cells’ energy needs and their high rate of DNA synthesis.

12. The purine and pyrimidine rings are built from the amino acids aspartate, glutamine, and glycine. Why is serine required for the synthesis of deoxyribonucleotides?

13. Why do individuals who are undergoing chemotherapy with FdUMP or methotrexate often temporarily go bald?

14. Normal cells die in a nutrient medium containing thymidine and methotrexate, whereas mutant cells defective in thymidylate synthase survive and grow. Explain.

15. Explain why methotrexate inhibits the synthesis of histidine and methionine.

16. Some microorganisms lack DHFR activity, but their thymidylate synthase has an FAD cofactor. What is the function of the FAD?

17. Is trimethoprim a mechanism-based inhibitor of bacterial dihydrofolate reductase?

18. Is allopurinol a mechanism-based inhibitor of xanthine oxidase?

19. Why is it important that muscle cells have low levels of glutamate dehydrogenase?

20. Why does von Gierke’s glycogen storage disease (Box 16-2) cause symptoms of gout?

21. Describe how the fumarate produced by the purine nucleotide cycle could be catabolized to CO₂.

22. Calculate the ATP yield of converting the carbons of thymine to CO₂.

23. Individuals with a complete deficiency of dihydroorotidine dehydrogenase exhibit a variety of symptoms. What compounds are likely to be present at high levels in the urine of these patients?

24. Individuals with a partial deficiency of dihydroorotidine dehydrogenase experience severe toxic effects when given high doses of the anticancer drug 5-fluorouracil. Explain.

25. In animals, one pathway for NAD⁺ synthesis begins with nicotinamide. Draw the structures generated by the reactions shown.

\[
\text{Nicotinamide} \\
\text{PRPP} \xrightarrow{\text{nicotinamide phosphoribosyl transferase}} \text{PP} + \text{nicotinamide + PRPP} \\
\text{ATP} \xrightarrow{\text{pyrophosphorylase}} \text{NAD}^+ + \text{PP}_i
\]

26. In the synthesis of FAD, riboflavin is phosphorylated by a kinase to form FMN (Fig. 18-10). The FMN then reacts with ATP. How many “high-energy” bonds are broken in this process?

MORE TO EXPLORE One long-standing shortcoming of the RNA world hypothesis (Section 3-2C) is the lack of a plausible explanation for the origin of ribonucleotides. Although a molecule such as UMP has a modular structure (base + ribose + phosphate), why is it unlikely that these components spontaneously assembled to form the first nucleotides? Why is it more likely that such compound arose from the combination of two molecules that were half-base, half-ribose?

References


