deoxynucleoside triphosphates dATP, dTTP, dGTP, and dCTP. The particular DNA polymerase used is one called *Taq polymerase* that is stable and active at the temperature at which the third step of the cycle is carried out (72°C).

Taq polymerase was first found in a bacterium (*Thermus aquaticus*) that lives in hot springs in Yellowstone National Park. Bacteria of this type are called thermophiles because they thrive in warm environments.

The products of the first cycle are two DNAs, each of which is composed of a longer and a shorter strand. These products are subjected to a second three-step cycle [Figure 28.14(*e*)–(*f*)] to give four DNAs. Two of these four contain a "strand" which is nothing more than the target region flanked by primers. In the third cycle, these two ultrashort "strands" produce two DNAs of the kind shown in Figure 28.14(*g*). This product contains only the target region plus the primers and is the one that increases disproportionately in subsequent cycles.

Since its introduction in 1985, PCR has been applied to practically every type of study that requires samples of DNA. These include screening for genetic traits such as sickle cell anemia, Huntington's disease, and cystic fibrosis. PCR can detect HIV infection when the virus is present in such small concentrations that no AIDS symptoms have as yet appeared. In forensic science, analysis of PCR-amplified DNA from tiny amounts of blood or semen have helped convict the guilty and free the innocent. Anthropologists increasingly use information from DNA analysis to trace the origins of racial and ethnic groups but sometimes find it difficult, for cultural reasons, to convince individuals to volunteer blood samples. Thanks to PCR, a strand of hair is now sufficient.

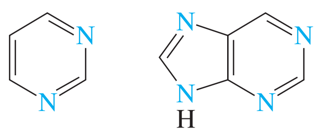
Within a few weeks of being brought into a case, scientists at the U.S. Centers for Disease Control (CDC) used PCR to help identify the infectious agent responsible for an outbreak of an especially dangerous hemorrhagic fever that struck the U.S. southwest in 1993. By annealing with synthetic oligonucleotide primers having sequences complementary to known hantaviruses, portions of the viral DNA obtained from those infected with the disease could be sucessfully amplified. Not only did this provide material for analysis, it also suggested that the new viral DNA had stretches where its sequence was the same as already known hantaviruses. Thus, the "Four Corners virus" was found to be a new strain of hantavirus and diagnostic procedures were developed specific for it.

Although not an official name, "Four Corners" succinctly describes where the virus was first discovered. It is the region where Arizona, New Mexico, Colorado, and Utah meet.

More recently, PCR proved to be a valuable detection and analytical tool during the terrorist-inspired anthrax outbreak in the fall of 2001.

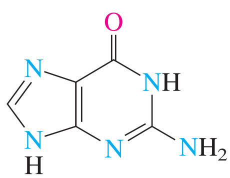
28.17 SUMMARY

Section 28.1 Many biologically important compounds are related to the heterocyclic aromatic compounds pyrimidine and purine.



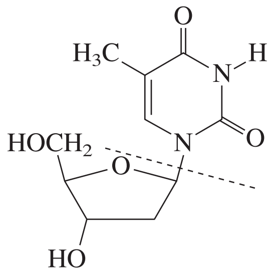
Pyrimidine Purine

The structure of guanine illustrates an important feature of substituted pyrimidines and purines. Oxygen substitution on the ring favors the keto form rather than the enol. Amino substitution does not.



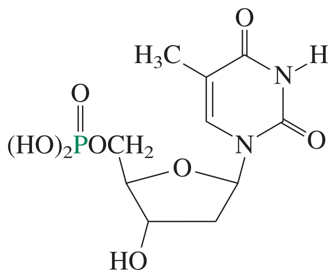
Guanine

Section 28.2 **Nucleosides** are carbohydrate derivatives of pyrimidine and purine bases. The most important nucleosides are derived from D-ribose and 2-deoxy-D-ribose.



Thymidine

Section 28.3 **Nucleotides** are phosphoric acid esters of nucleosides.

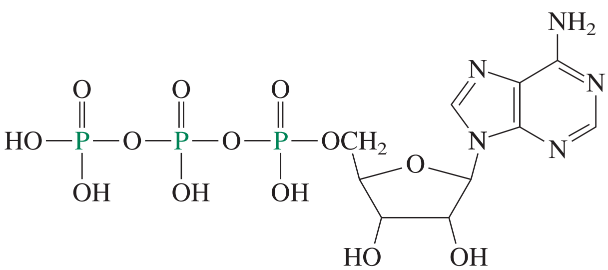


Thymidine 5′-monophosphate

In the example shown, the 5′-OH group is phosphorylated. Nucleotides are also possible in which some other OH group bears the phosphate ester function. Cyclic phosphates are common and important as biochemical messengers.

Section 28.4 **Bioenergetics** is concerned with the thermodynamics of biological processes. Particular attention is paid to $\Delta G^{\circ\prime}$, the standard free-energy change of reactions at pH = 7. When the sign of $\Delta G^{\circ\prime}$ is +, the reaction is **endergonic;** when the sign of $\Delta G^{\circ\prime}$ is −, the reaction is **exergonic.**

Section 28.5 **Adenosine triphosphate (ATP)** is a key compound in biological energy storage and delivery.



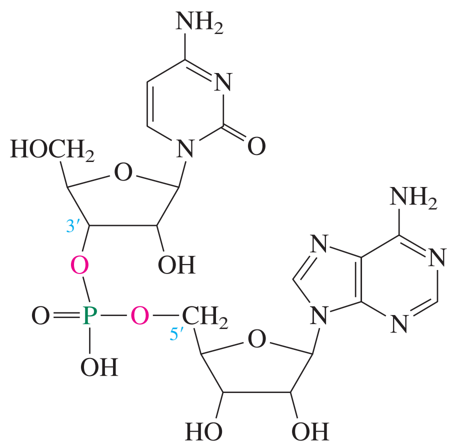
Adenosine triphosphate (ATP)

The hydrolysis of ATP to ADP and HPO_4^{2-} is exergonic.

$$\text{ATP} + H_2O \longrightarrow \text{ADP} + HPO_4^{2-} \qquad \Delta G^{\circ\prime} = -31 \text{ kJ } (-7.4 \text{ kcal})$$

Many formally endergonic biochemical processes become exergonic when they are coupled mechanistically to the hydrolysis of ATP.

Section 28.6 Many important compounds contain two or more nucleotides joined together by a **phosphodiester** linkage. The best known are those in which the phosphodiester joins the 5′-oxygen of one nucleotide to the 3′-oxygen of the other.



Oligonucleotides contain about 50 or fewer nucleotides held together by phosphodiester links; **polynucleotides** can contain thousands of nucleotides.

Section 28.7 **Nucleic acids** are polynucleotides present in cells. The carbohydrate component is D-ribose in ribonucleic acid (RNA) and 2-deoxy-D-ribose in deoxyribonucleic acid (DNA).

Section 28.8 The most common form of DNA is B-DNA, which exists as a right-handed double helix. The carbohydrate–phosphate backbone lies on the outside, the purine and pyrimidine bases on the inside. The double helix is stabilized by complementary hydrogen bonding (base pairing) between adenine (A) and thymine (T), and guanine (G) and cytosine (C).

Section 28.9 Within the cell nucleus, double-helical DNA adopts a **supercoiled** tertiary structure in which short sections are wound around proteins called **histones.** This reduces the effective length of the DNA and maintains it in an ordered arrangement.

Section 28.10 During DNA replication the two strands of the double helix begin to unwind, exposing the pyrimidine and purine bases in the interior. Nucleotides with complementary bases hydrogen bond to the original strands and are joined together by phosphodiester linkages with the aid of DNA polymerase. Each new strand grows in its 5′→3′ direction.

Section 28.11 Three RNAs are involved in gene expression. In the **transcription** phase, a strand of **messenger RNA (mRNA)** is synthesized from a DNA template. The four bases A, G, C, and U, taken three at a time, generate 64 possible combinations called **codons.** These 64 codons comprise the **genetic code** and code for the 20 amino acids found in proteins plus start and stop signals. The mRNA sequence is **translated** into a prescribed protein sequence at the ribosomes. There, small polynucleotides called

transfer RNA (tRNA), each of which contains an **anticodon** complementary to an mRNA codon carries the correct amino acid for incorporation into the growing protein. **Ribosomal RNA (rRNA)** is the main constituent of ribosomes and appears to catalyze protein biosynthesis.

Section 28.12 The start codon for protein biosynthesis is AUG, which is the same as the codon for methionine. Thus, all proteins initially have methionine as their N-terminal amino acid, but lose it subsequent to their formation. The reaction responsible for extending the protein chain is nucleophilic acyl substitution.

Section 28.13 HIV, which causes AIDS, is a retrovirus. Its genetic material is RNA instead of DNA. HIV contains an enzyme called reverse transcriptase that allows its RNA to serve as a template for DNA synthesis in the host cell.

Section 28.14 The nucleotide sequence of DNA can be determined by a technique in which a short section of single-stranded DNA is allowed to produce its complement in the presence of dideoxy analogs of ATP, TTP, GTP, and CTP. DNA formation terminates when a dideoxy analog is incorporated into the growing polynucleotide chain. A mixture of polynucleotides differing from one another by an incremental nucleoside is produced and analyzed by electrophoresis. From the observed sequence of the complementary chain, the sequence of the original DNA is deduced.

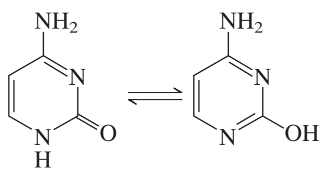
Section 28.15 A rough draft of the sequence of nucleotides that make up the human genome has been completed. There is every reason to believe that the increased knowledge of human biology it offers will dramatically affect the practice of medicine.

Section 28.16 In **DNA profiling** the noncoding regions are cut into smaller fragments using enzymes that recognize specific sequences, and these smaller bits of DNA are then separated by electrophoresis. The observed pattern of DNA fragments is believed to be highly specific for the source of the DNA. Using the **polymerase chain reaction (PCR),** millions of copies of minute amounts of DNA can be produced in a relatively short time.

PROBLEMS

28.14 *5-Fluorouracil* is one component of a mixture of three drugs used in breast-cancer chemotherapy. What is its structure?

28.15 (a) Which isomer, the keto or enol form of cytosine, is the stronger acid?



keto enol

(b) What is the relationship between the conjugate base of the keto form and the conjugate base of the enol form?

28.16 Birds excrete nitrogen as *uric acid*. Uric acid is a purine having the molecular formula $C_5H_4N_4O_3$; it has no C—H bonds. Write a structural formula for uric acid.