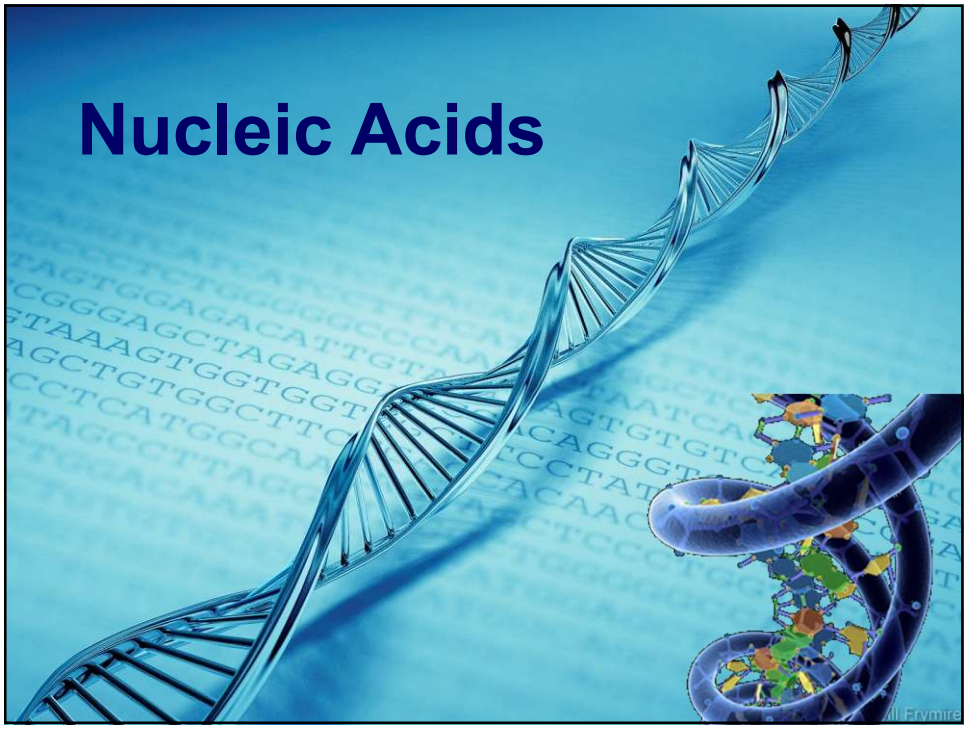



## The Chemical Differences Between DNA and RNA Have Biological Significance

Two fundamental chemical differences distinguish DNA from RNA:

1. DNA contains 2-deoxyribose instead of ribose.
2. DNA contains thymine instead of uracil.

What are the consequences of these differences, and do they hold any significance in common? An argument can be made that, because of these differences, DNA is chemically more stable than RNA. The greater stability of DNA over RNA is consistent with the respective roles these macromolecules have assumed in heredity and information transfer.

Consider first why DNA contains thymine instead of uracil. The key observation is that *cytosine deaminates to form uracil* at a finite rate in vivo (Figure 10.25). Because C in one DNA strand pairs with G in the other strand, whereas U would pair with A, conversion of a C to a U could potentially result in a heritable change of a C:G pair to a U:A pair. Such a change in nucleotide sequence would constitute a *mutation* in the DNA. To prevent this C deamination from leading to permanent changes in nucleotide sequence, a cellular repair mechanism "proofreads" DNA, and when a U arising from C deamination is encountered, it is treated as inappropriate and is replaced by a C. If DNA normally contained U rather than T, this repair system could not readily distinguish U formed by C deamination from U correctly paired with A. However, the U in DNA is "5-methyl-U" or, as it is conventionally known, thymine (Figure 10.26). That is, the 5-methyl group on T labels it as if to say "this U belongs; do not replace it."

The other chemical difference between RNA and DNA is that the ribose 2′-OH group on each nucleotide in RNA is absent in DNA. Consequently, the ubiquitous 3′-O of polynucleotide backbones lacks a vicinal hydroxyl neighbor in DNA. This difference leads to a greater resistance of DNA to alkaline hydrolysis, examined in detail in the following section. To view it another way, RNA is less stable than DNA because its vicinal 2′-OH group makes the 3′-phosphodiester bond susceptible to nucleophilic cleavage (Figure 10.27). For just this reason, it is selectively advantageous for the heritable form of genetic information to be DNA rather than RNA.

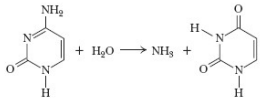



**FIGURE 10.25** Deamination of cytosine forms uracil.

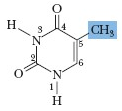



**FIGURE 10.26** The 5-methyl group on thymine labels it as a special kind of uracil.

## RNA Is Susceptible to Hydrolysis by Base, but DNA Is Not

RNA is relatively resistant to the effects of dilute acid, but gentle treatment of DNA with 1 m*M* HCl leads to hydrolysis of purine glycosidic bonds and the loss of purine bases from the DNA. The glycosidic bonds between pyrimidine bases and 2′-deoxyribose are not affected, and in this case, the polynucleotide's sugar–phosphate backbone remains intact. The purine-free polynucleotide product is called **apurinic acid.**

DNA is not susceptible to alkaline hydrolysis. On the other hand, RNA is alkali labile and is readily hydrolyzed by hydroxide ions (Figure 10.27). DNA has no 2′-OH; therefore, DNA is alkali stable.

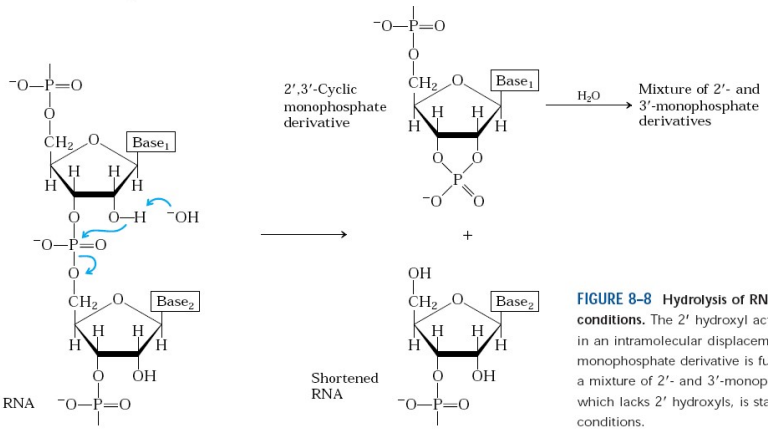



**FIGURE 8–8 Hydrolysis of RNA under alkaline conditions.** The 2′ hydroxyl acts as a nucleophile in an intramolecular displacement. The 2′,3′-cyclic monophosphate derivative is further hydrolyzed to a mixture of 2′- and 3′-monophosphates. DNA, which lacks 2′ hydroxyls, is stable under similar conditions.

## 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 carbonyl and ring nitrogen 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 and Figure 10.7 for guanine. The keto tautomers of uracil, thymine, and guanine vastly predominate at neutral pH. In other words, p$K_a$ values for ring nitrogen atoms 1 and 3 in uracil (Figure 10.6) are greater than 8 (the p$K_a$ value for N-3 is 9.5). In contrast, the enamine form of cytosine predominates at pH 7 and the p$K_a$ value for N-3 in this pyrimidine is 4.5. Similarly, for guanine (Figure 10.7), the p$K_a$ value is 9.4 for N-1 and less than 5 for N-3. These p$K_a$ values specify whether protons are associated with the various ring nitrogens at neutral pH. As such, they are important in determining

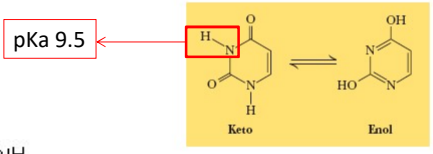
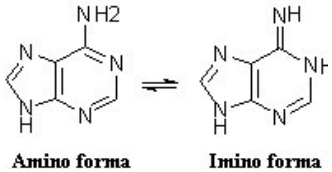



**FIGURE 10.6** The keto–enol tautomerization of uracil.



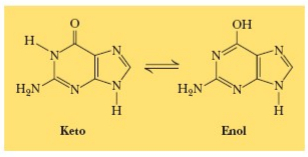




**FIGURE 10.7** The tautomerization of the purine guanine.

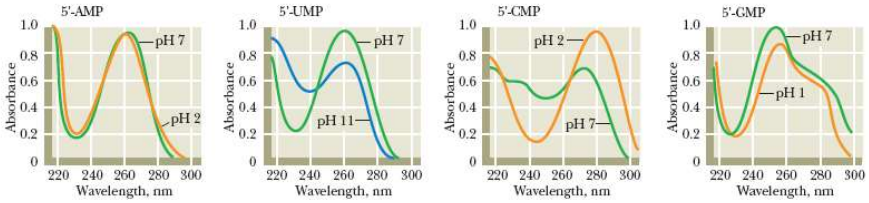
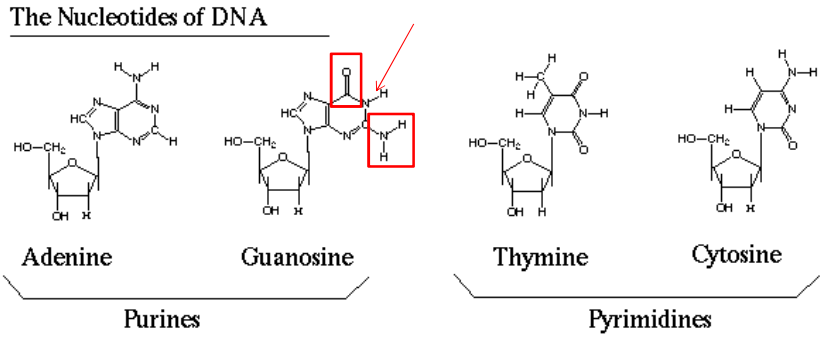



**FIGURE 10.8** The UV absorptioan spectra of the common ribonucleotides.

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 10.17).

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.



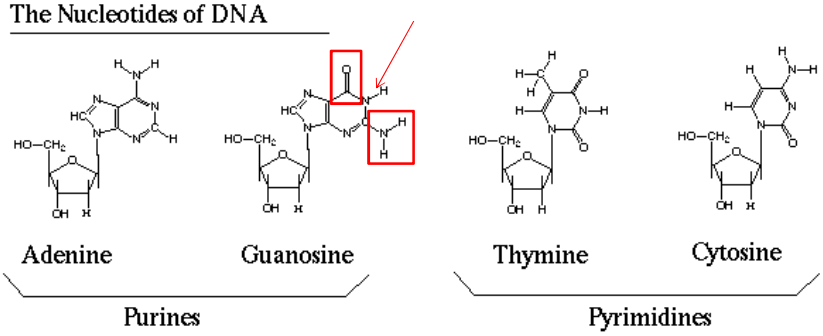

**The purine and pyrimidine bases**

• hydrophobic

• relatively insoluble in water at the near-neutral pH of the cell. At acidic or alkaline pH the bases become charged and their solubility in water increases.

• Hydrophobic stacking interactions in which two or more bases are positioned with the planes of their rings parallel (like a stack of coins) are one of two important modes of interaction between bases in nucleic acids. Base stacking helps to minimize contact of the bases with water, and base-stacking interactions are very important in stabilizing the three-dimensional structure of nucleic Acids.

## The Nucleotides of DNA




**Functional groups of purine and pyrimidine bases**

- **Ring nitrogens**, **carbonyl** groups, and exocyclic **amino** groups.
- Hydrogen bonds involving the amino and carbonyl groups are the second important mode of interaction between bases in nucleic acid molecules.
- Hydrogen bonds between bases permit a complementary association of two strands of nucleic acid.

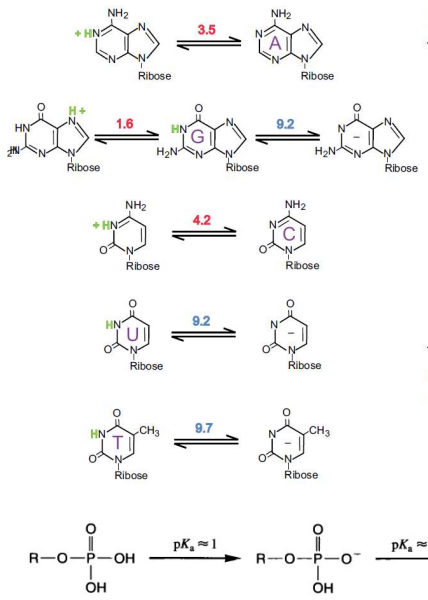




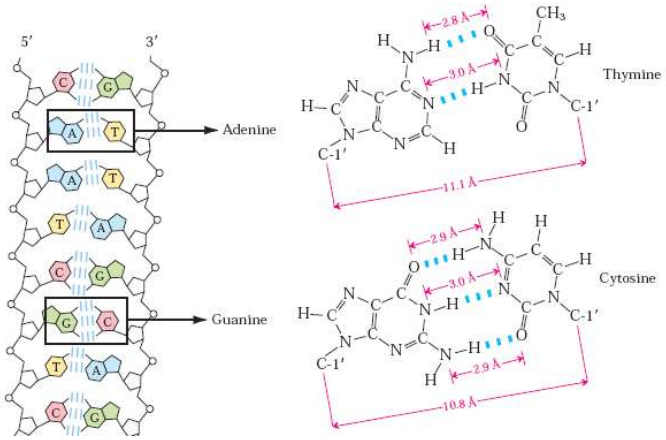


**Table 2-2.** Ionization and UV Absorbance Properties of Nucleoside Monophosphates.

| Nucleotide | $pK_a$ of ring $-NH_2$ | UV absorbance[a] $\lambda_{max}$ (nm) | UV absorbance[a] Molar absorbance ($10^3$ $M^{-1}$ $cm^{-1}$) |
|---|---|---|---|
| AMP | 3.8 | 257 | 15.0 |
| CMP | 4.5 | 280 | 13.2 |
| GMP | 2.4 | 256 | 12.2 |
| UMP | | 262 | 10.0 |
| dAMP | 4.4 | 258 | 14.3 |
| dCMP | 4.6 | 280 | 13.5 |
| dGMP | 2.9 | 255 | 11.8 |
| dTMP | | 267 | 10.2 |
| cAMP | | 256 | 14.5 |
| cGMP | | 256.5 | 11.4 |

[a] Measured at pH 1 to 2.

Data from C.K. Mathews (1999) In *Encyclopedia of Molecular Biology* (T.E. Creighton, ed.) Wiley-Interscience, New York, p. 1676.




## 11.3 Can the Secondary Structure of DNA Be Denatured and Renatured?

### Thermal Denaturation of DNA Can Be Observed by Changes in UV Absorbance

When duplex DNA molecules are subjected to conditions of pH, temperature, or ionic strength that disrupt base-pairing interactions, the strands are no longer held together. That is, the double helix is **denatured,** and the strands separate as individual random coils. If temperature is the denaturing agent, the double helix is said to *melt.* The course of this dissociation can be followed spectrophotometrically because the relative absorbance of the DNA solution at 260 nm increases as much as 40% as the bases unstack. This absorbance increase, or **hyperchromic shift,** is due to the fact that the aromatic

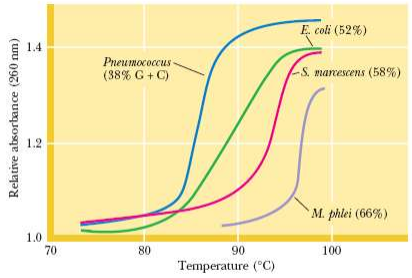
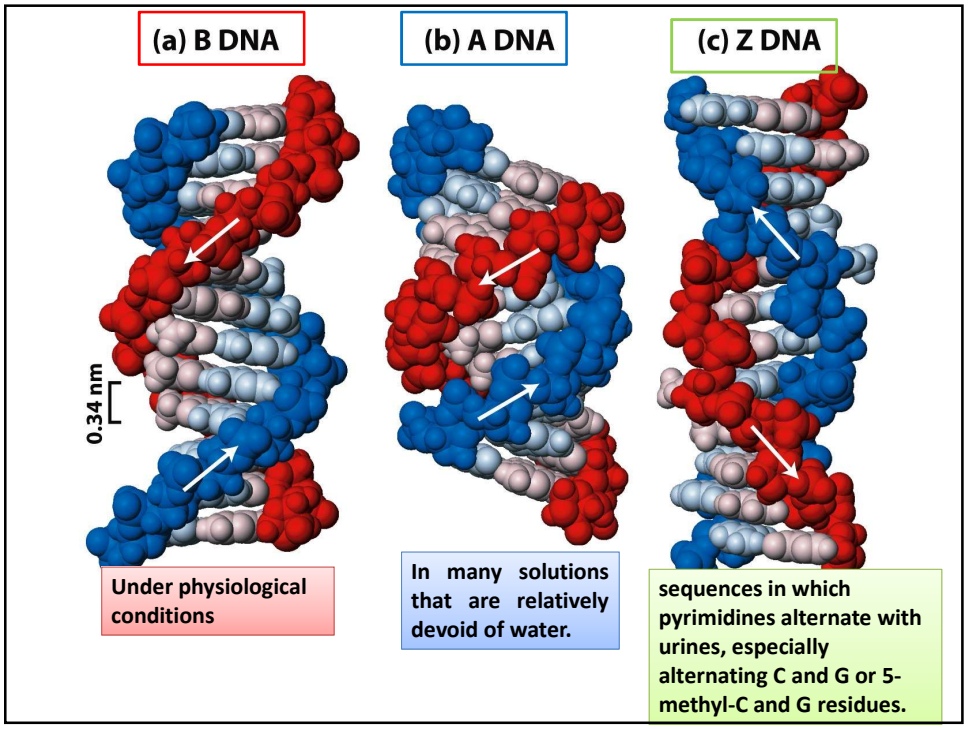



**FIGURE 11.20** Heat denaturation of DNA from various sources, so-called melting curves. (From Marmur, J., 1959. Heterogenity in deoxyribonucleic acids. *Nature* **183**:1427–1429.)

bases in DNA interact via their $\pi$-electron clouds when stacked together in the double helix. Because the UV absorbance of the bases is a consequence of $\pi$-electron transitions, and because the potential for these transitions is diminished when the bases stack, the bases in duplex DNA absorb less 260-nm radiation than expected for their numbers. Unstacking alleviates this suppression of UV absorbance. The rise in absorbance coincides with strand separation, and the midpoint of the absorbance increase is termed the **melting temperature,** $T_m$ (Figure 11.20). DNAs differ in their $T_m$ values because they differ in relative G + C content. The higher the G + C content of a DNA, the higher its melting temperature because G:C pairs have higher base stacking energies than A:T pairs. Also, $T_m$ is dependent on the ionic strength of the solution; the lower the ionic strength, the lower the melting temperature. Because cations suppress the electrostatic repulsion between the negatively charged phosphate groups in the complementary strands of the double helix, the double-stranded form of DNA is more stable in dilute salt solutions. DNA in pure water melts even at room temperature.

## pH Extremes or Strong H-Bonding Solutes also Denature DNA Duplexes

At pH values greater than 10, the bases of DNA become deprotonated, which destroys their base-pairing potential, thus denaturing the DNA duplex. Extensive protonation of the bases below pH 2.3 also disrupts base pairing. Alkali is the preferred denaturant because, unlike acid, it does not hydrolyze the glycosidic bonds linking purine bases to the sugar–phosphate backbone. Small solutes that readily form H bonds can also denature duplex DNA at temperatures below $T_m$. If present in sufficiently high concentrations, such small solutes will form H bonds with the bases, thereby disrupting H-bonding interactions between the base pairs. Examples include formamide and urea.



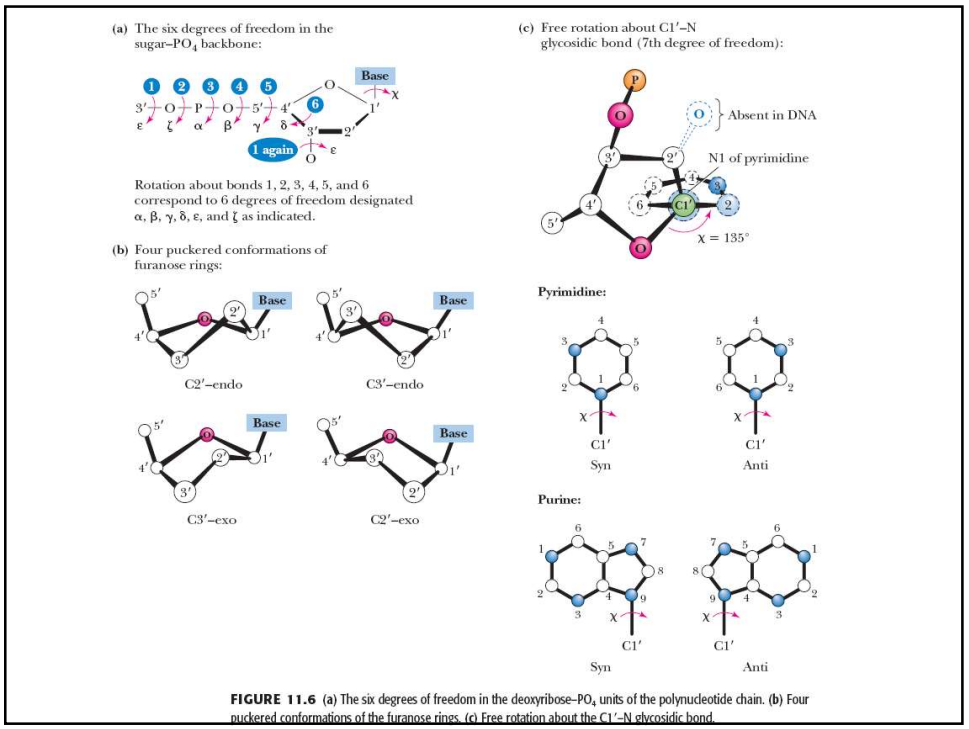
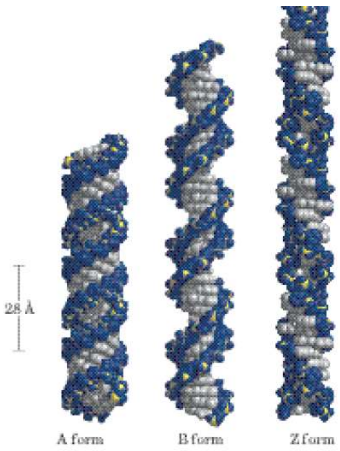




**FIGURE 11.6** (a) The six degrees of freedom in the deoxyribose–$PO_4$ units of the polynucleotide chain. (b) Four puckered conformations of the furanose rings. (c) Free rotation about the C1′–N glycosidic bond.




| | *A form* | *B form* | *Z form* |
|---|---|---|---|
| Helical sense | Right handed | Right handed | Left handed |
| Diameter | ~26 Å | ~20 Å | ~18 Å |
| Base pairs per helical turn | 11 | 10.5 | 12 |
| Helix rise per base pair | 2.6 Å | 3.4 Å | 3.7 Å |
| Base tilt normal to the helix axis | 20° | 6° | 7° |
| Sugar pucker conformation | C-3′ endo | C-2′ endo | C-2′ endo for pyrimidines; C-3′ endo for purines |
| Glycosyl bond conformation | Anti | Anti | Anti for pyrimidines; syn for purines |

**FIGURE 8–19** **Comparison of A, B, and Z forms of DNA.** Each structure shown here has 36 base pairs. The bases are shown in gray, the phosphate atoms in yellow, and the riboses and phosphate oxygens in blue. Blue is the color used to represent DNA strands in later chapters. The table summarizes some properties of the three forms of DNA.

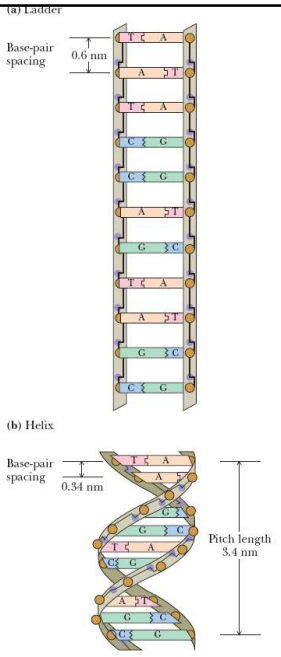



**FIGURE 11.7** **(a)** Double-stranded DNA as an imaginary ladderlike structure. **(b)** A simple right-handed twist converts the ladder to a helix.

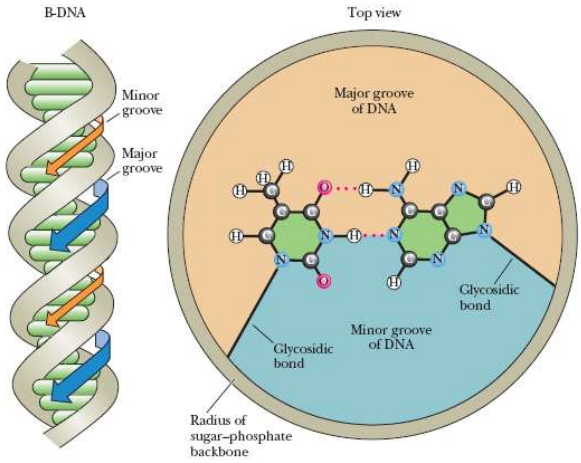
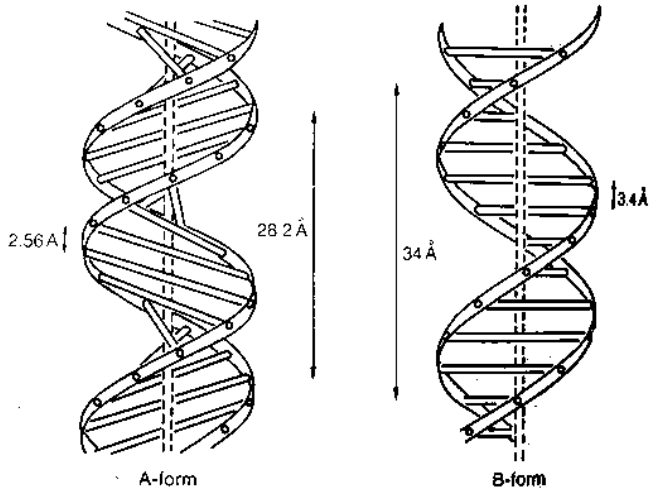
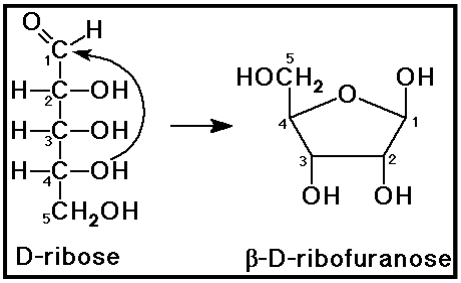



**FIGURE 11.9** The major and minor grooves of B-DNA.

FIGURE 4.8. Schemes of the A and B forms of DNA.



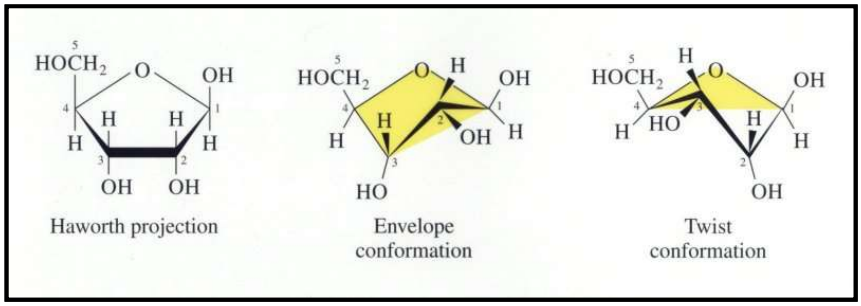



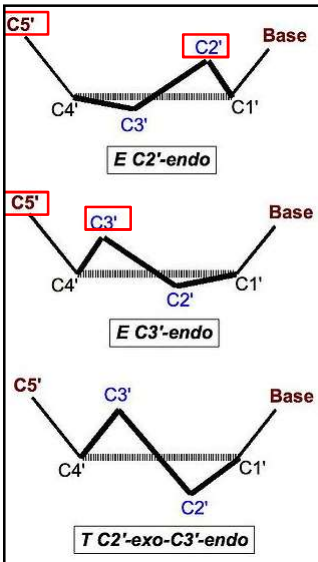

**In solution**, the straight chain (aldehyde) and ring (β-D-furanose) forms of **free** ribose are in equilibrium.

**In RNA**, exists solely as β-D-ribofuranose.

**In DNA**, exists solely as 2'-β-D-deoxyribofuranose.

**Envelope: only a single atom is displaced**
**Twists: Two atoms is displaced**

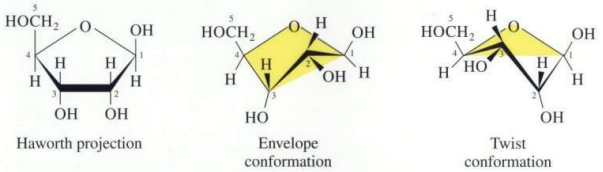





The lowest energy conformations are 2'-*endo* and 3'-*endo*.

**B-DNA**, the form described by Watson and Crick, adopts 2'-*endo*

whereas **RNA** and the **A form of DNA** adopt the 3'-*endo* twist.



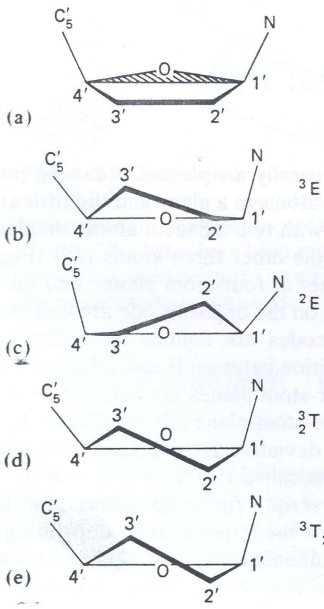
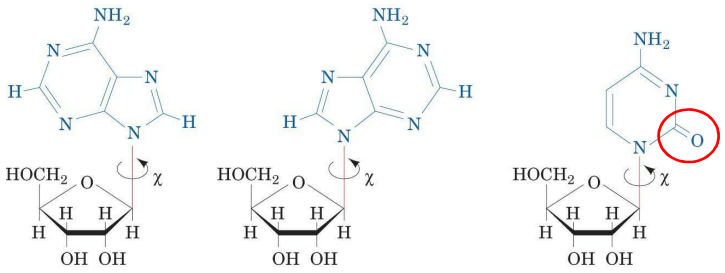




**Figure 2-7.** Definition of sugar puckering modes. (a) Starting position with flat five-membered sugar, a situation never observed. Place $C_{1'}-O_{4'}-C_{4'}$ is shown hatched. (b-e) View with this plane perpendicular to the paper. (b) Envelope $C_{3'}$–*endo*, $^3$E. (c) Envelope $C_{2'}$–*endo*. $^2$E. (d) Symmetrical twist or half-chair $C_{2'}$–*exo*–$C_{3'}$–*endo*. $^3_2$T. (e) Unsymmetrical twist with major $C_{3'}$–*endo* and minor $C_{2'}$–*exo* pucker, $^3$T$_2$.

Because of steric constraints, **purines** in purine nucleotides are restricted to two stable conformations with respect to deoxyribose, called *syn* and *anti*.

**Pyrimidines** are generally restricted to the *anti* conformation because of steric interference between the sugar and the carbonyl oxygen at C-2 of the pyrimidine.



**syn-Adenosine** **anti-Adenosine** **anti-Cytidine**