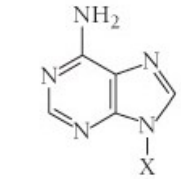
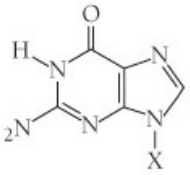
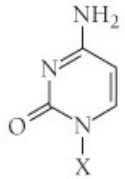
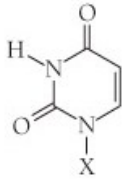
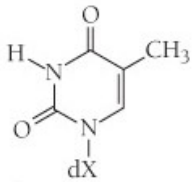
# Nucleosides, Nucleotides, Oligonucleotides, Nucleic Acids

CHEM 420 – Principles of Biochemistry
Instructor – Anthony S. Serianni

Spring 2015
Chapters 5, 7, 28, 29 and 32: Voet/Voet, *Biochemistry*, 2011

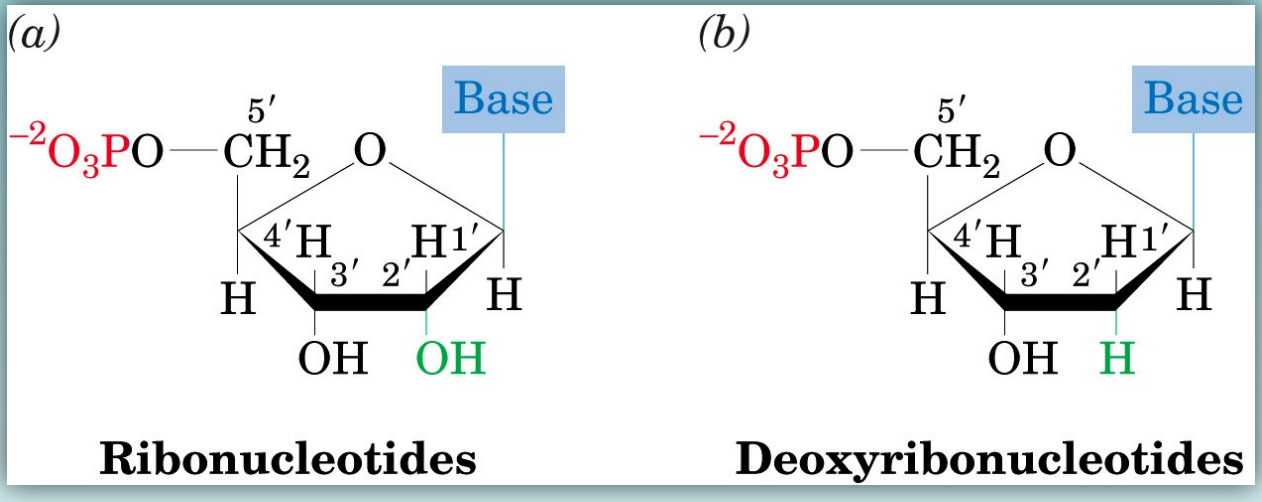
Extra Credit Lectures:
Fall 2015

| Base Formula | Base (X = H) | Nucleoside (X = ribose[a]) | Nucleotide[b] (X = ribose phosphate[a]) |
|---|---|---|---|
|  | Adenine<br>Ade<br>A | Adenosine<br>Ado<br>A | Adenylic acid<br>Adenosine monophosphate<br>AMP |
|  | Guanine<br>Gua<br>G | Guanosine<br>Guo<br>G | Guanylic acid<br>Guanosine monophosphate<br>GMP |
|  | Cytosine<br>Cyt<br>C | Cytidine<br>Cyd<br>C | Cytidylic acid<br>Cytidine monophosphate<br>CMP |
|  | Uracil<br>Ura<br>U | Uridine<br>Urd<br>U | Uridylic acid<br>Uridine monophosphate<br>UMP |
|  | Thymine<br>Thy<br>T | Deoxythymidine<br>dThd<br>dT | Deoxythymidylic acid<br>Deoxythymidine monophosphate<br>dTMP |

[a]The presence of a 2′-deoxyribose unit in place of ribose, as occurs in DNA, is implied by the prefixes "deoxy" or "d." For example, the deoxynucleoside of adenine is deoxyadenosine or dA. However, for thymine-containing residues, which rarely occur in RNA, the prefix is redundant and may be dropped. The presence of a ribose unit may be explicitly implied by the prefixes "ribo" or "r." Thus the ribonucleotide of thymine is ribothymidine or rT.

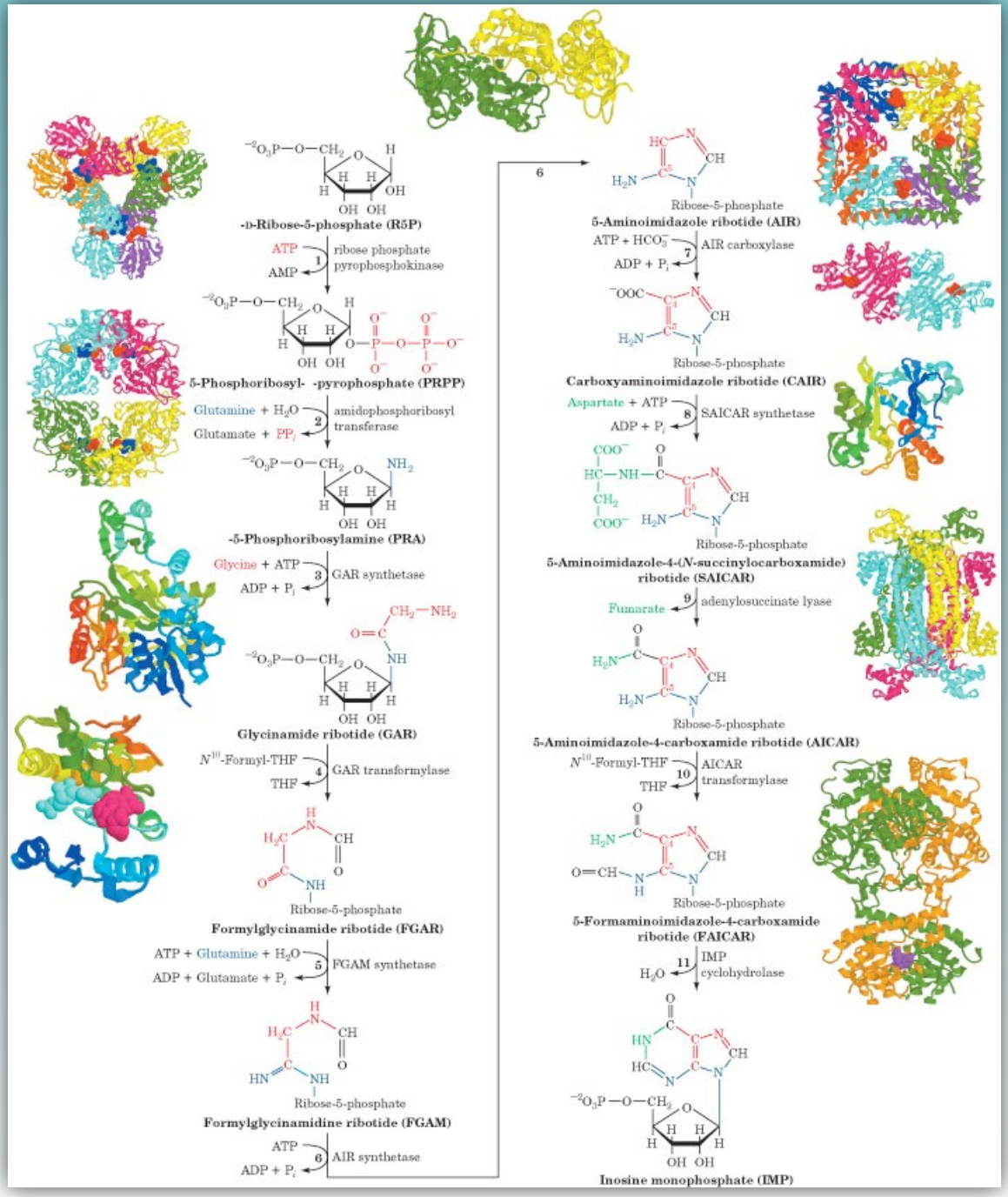
[b]The position of the phosphate group in a nucleotide may be explicitly specified as in, for example, 3 -AMP and 5′-GMP.

# Names and abbreviations of the free nitrogen bases, nucleosides, and nucleotides

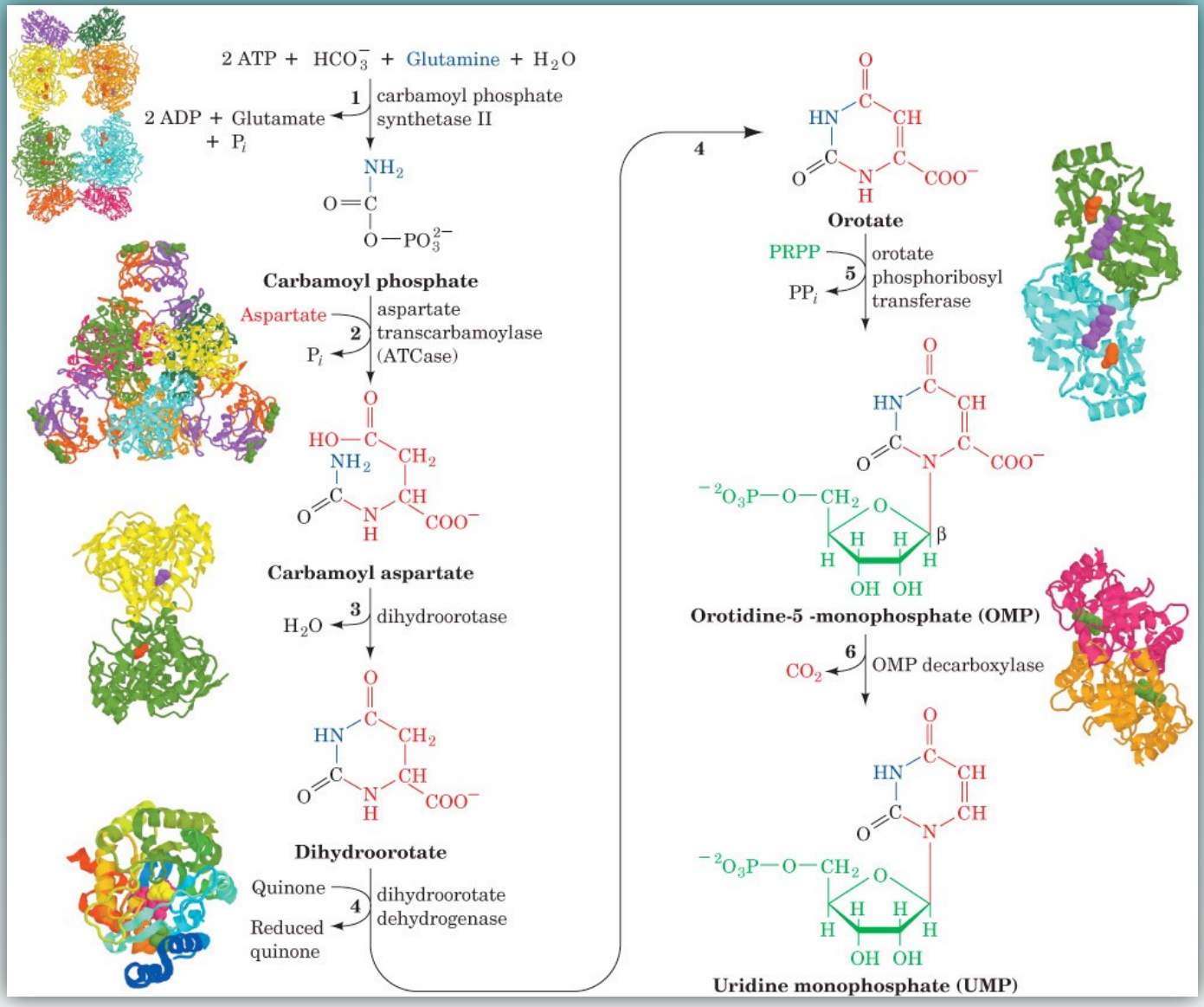



**NMP** **dNMP**

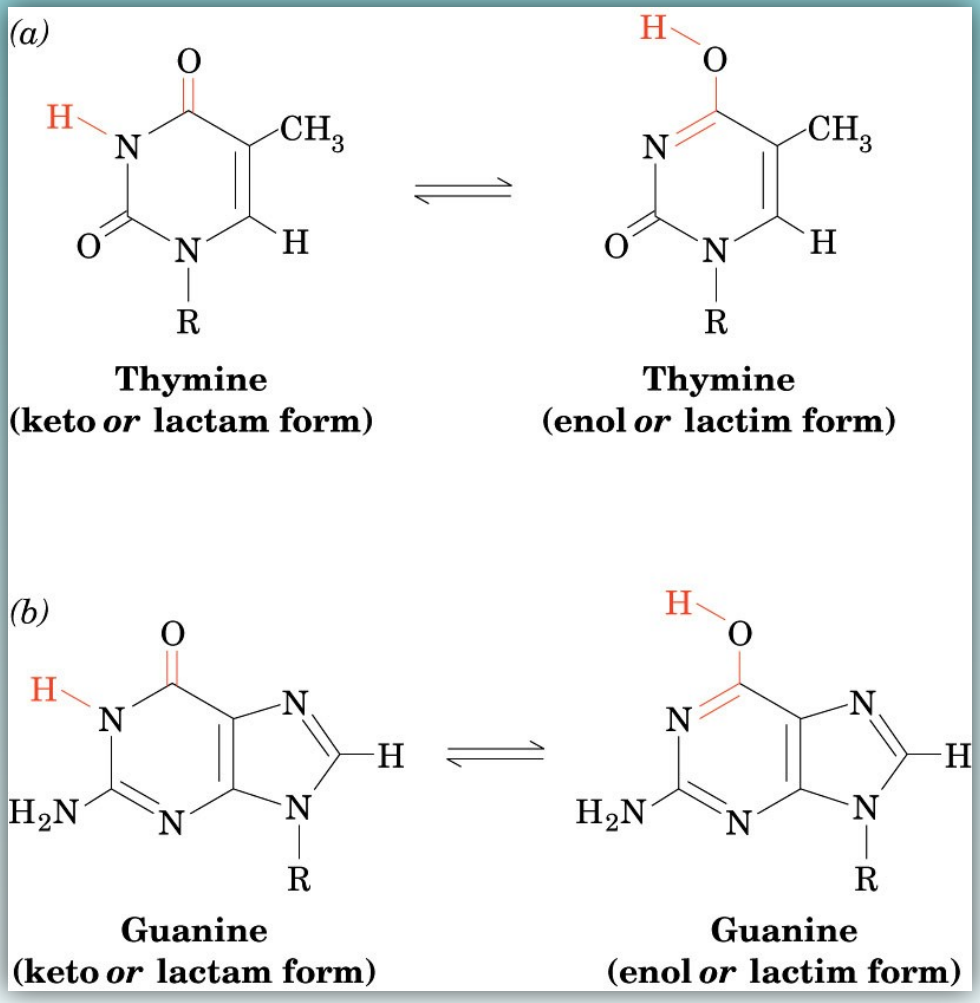
Core chemical structures of (*a*) ribo(mono)nucleotides and (*b*) 2' -deoxyribo(mono)nucleotides




# Metabolic pathway for the *de novo* biosynthesis of IMP in humans

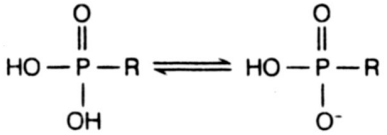
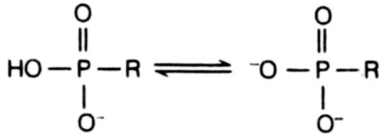



**Metabolic pathway for the *de novo* synthesis of UMP in humans**




Some possible tautomeric forms of the nitrogen bases

**TABLE 4.1 Ionization constants of ribonucleotides expressed as $pK_a$ values**

| | Phosphate | | | Base | |
|---|---|---|---|---|---|
| | Primary Ionization | | Secondary Ionization | | |
| |  | |  | | |
| | $pK_{a1}$ + $H^+$ | | $pK_{a2}$ + $H^+$ | $pK_a$ | Reaction (as Loss of Proton from) |
| 5′ AMP | 0.9 | | 6.1 | 3.8 | N-1 |
| 5′ GMP | 0.7 | | 6.1 | 2.4 | N-7 |
| | | | | 9.4 | N-1 |
| 5′ UMP | 1.0 | | 6.4 | 9.5 | N-3 |
| 5′ CMP | 0.8 | | 6.3 | 4.5 | N-3 |

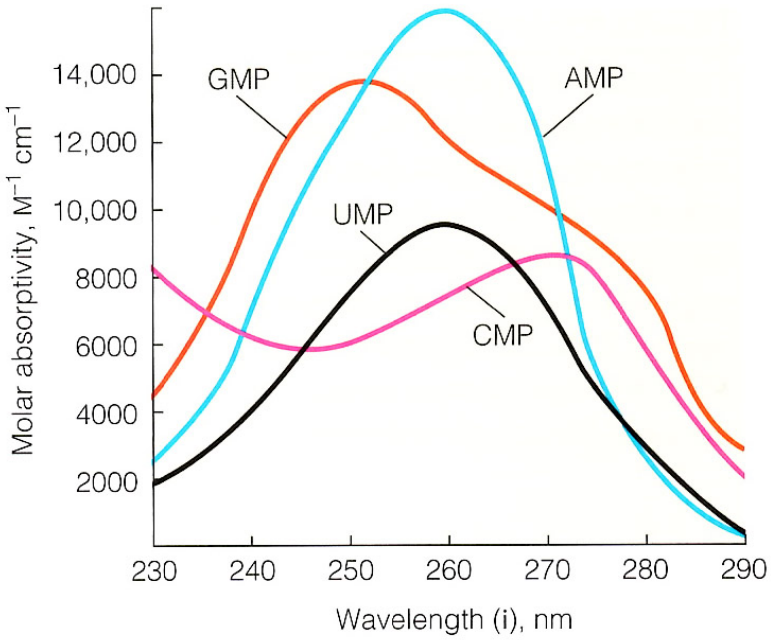
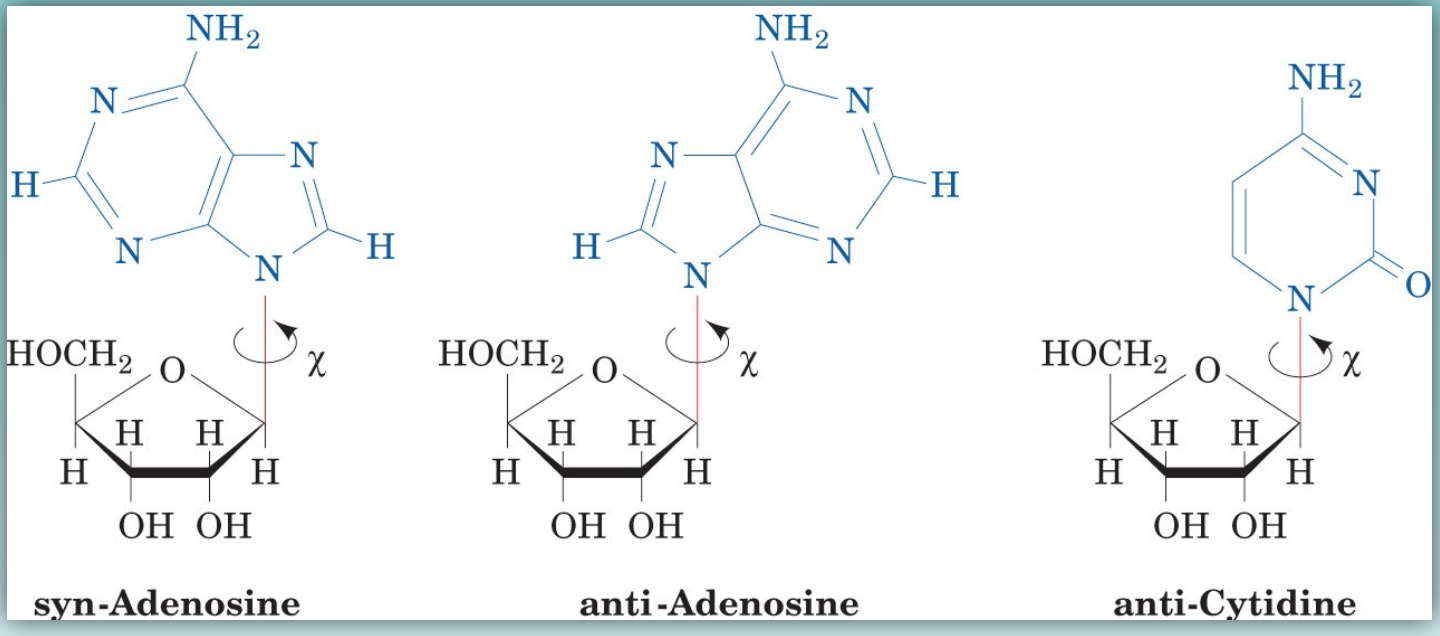



**FIGURE 4.5**

**Ultraviolet absorption spectra of ribonucleotides.** The dimensions of the absorption coefficients are $M^{-1}cm^{-1}$. Thus a $10^{-4}$ solution of UMP would have an absorbance of 0.95 at 260 mm in a 1-cm-thick cuvette. (Absorbance = molar absorptivity × light path in cm × molar concentration; see Tools of Biochemistry 6A).

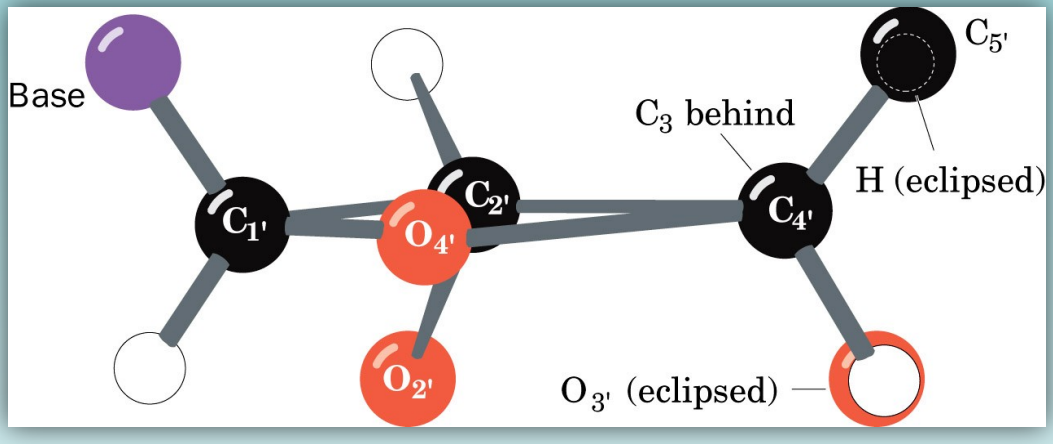


**The UV absorption properties of AMP, GMP, CMP and UMP**

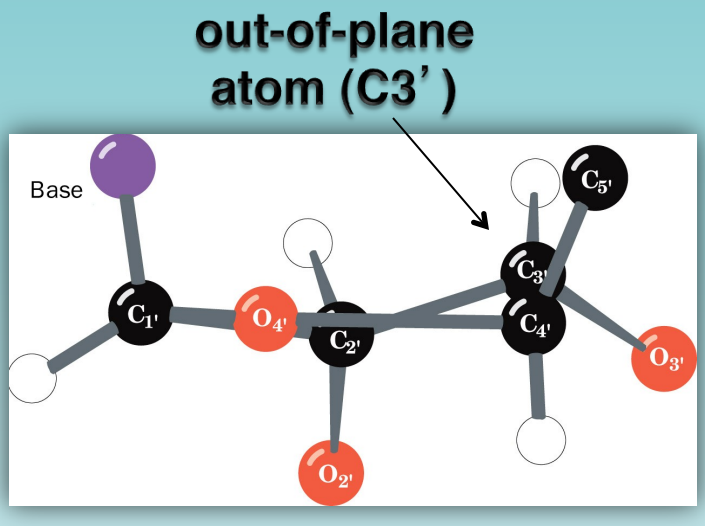



## *N*-Glycoside conformation: *syn* and *anti* conformations

The *syn-anti* equilibrium favors the *anti* form for most nucleosides and mononucleotides in aqueous solution. The *syn-anti* equilibrium is influenced by furanose conformation (*i.e.*, *N*-glycoside and furanose conformation are correlated in nucleosides/tides).




**Furanose ring conformation:** A planar β-D-ribofuranose ring of a ribonucleoside viewed down the C3′-C4′ bond showing the eclipsed substituents. The eclipsing of multiple substituents at C1'-C4' destabilizes the planar form relative to non-planar forms, although the energy difference is relatively small (< 6 kcal/mol).




**Furanose ring pucker**: Steric strain present in the planar form (caused by multiple eclipsed substituents) is partially relieved by ring puckering to form non-planar forms. In the above case, C3′ is the out-of-plane atom (an envelope (E) form denoted $^{3}$E or C3' -*endo*)(C4' -O4' -C1' -C2' are coplanar).

Alternate non-planar forms are twist forms (T) in which three contiguous atoms are coplanar and the remaining two are out-of-plane. Ten E and ten T forms are possible.

E and T forms are energetically more stable than the planar form.

In monomers (nucleosides/nucleotides), the furanose ring exchanges conformationally between $^{3}E$ (C3' -*endo*; north; N) and $^{2}E$ (C2' -*endo*; south; S) conformers. This exchange occurs freely in aqueous solution, and the equilibrium depends on the sugar and base structures.

C3' -*endo* $\rightleftharpoons$ C2' -*endo*

$^{3}E$ $\rightleftharpoons$ $^{2}E$

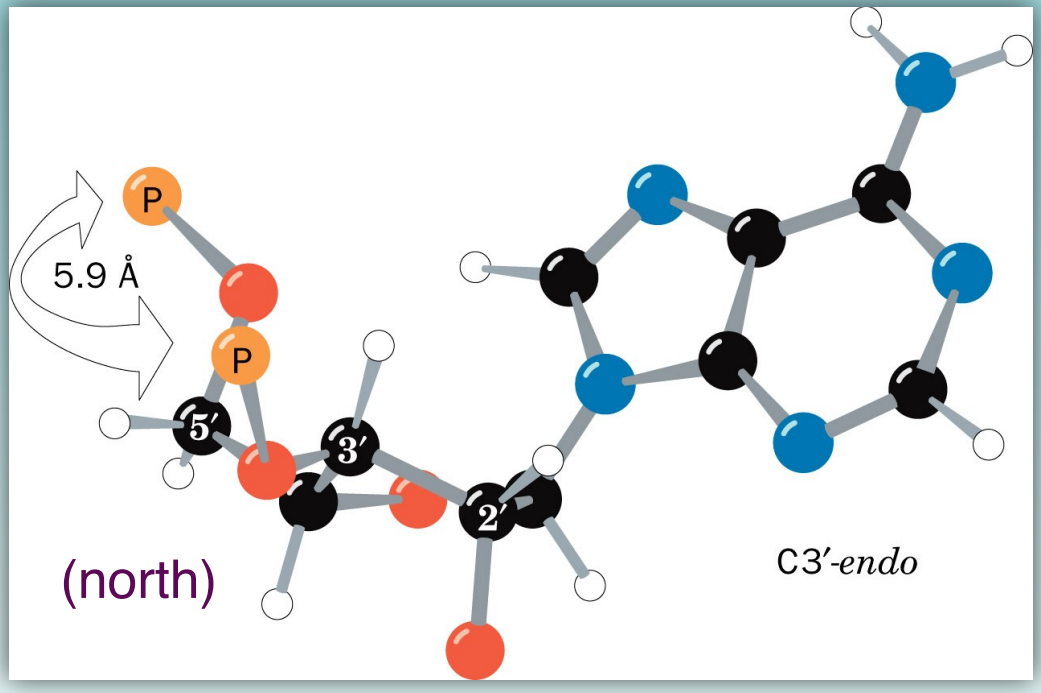
N $\rightleftharpoons$ S

equivalent representations

In the polymers (oligo- and polynucleotides), furanose conformational flexibility is more restricted, especially in duplex (double-stranded) molecules.

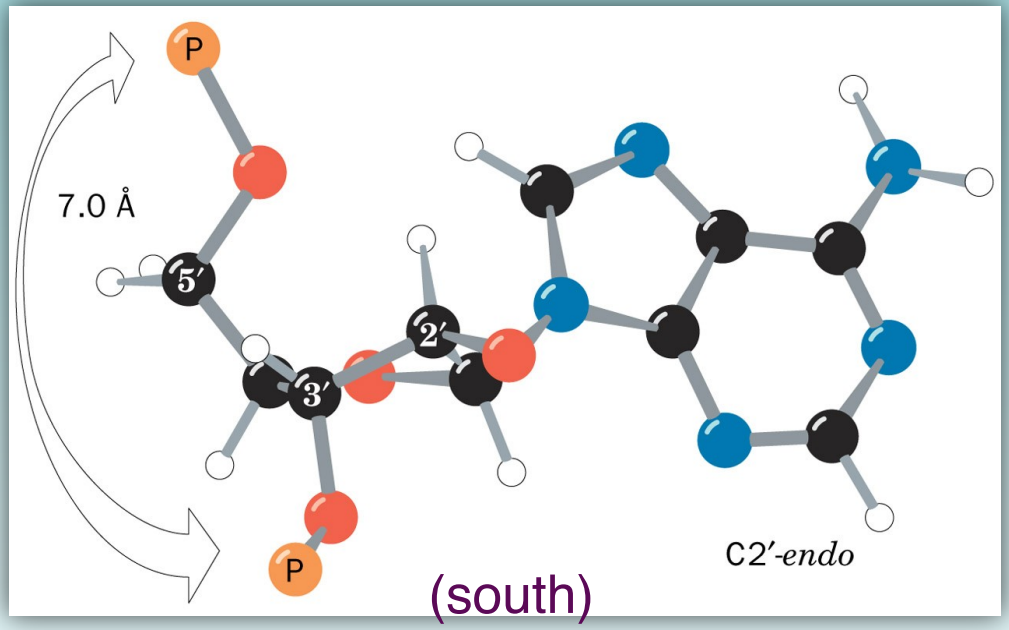
A strict N/S exchange model between $^{3}E$ and $^{2}E$ is a simplification of what really occurs in solution; other modes of conformational exchange may also exist.

# Impact of furanose ring conformation on backbone structure in polynucleotides

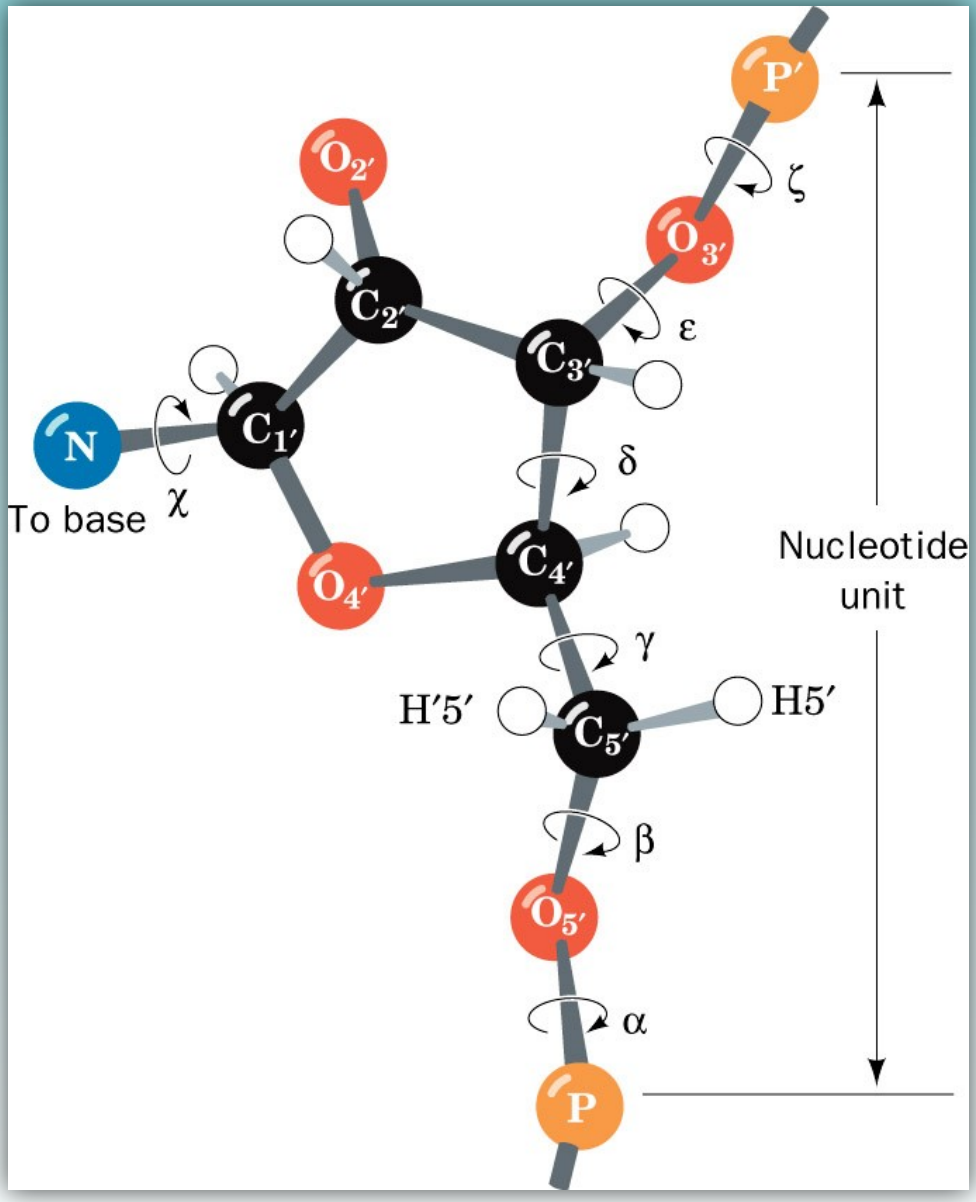



**A P-to-P internuclear distance of 5.9 Å** is correlated with the C3′-*endo* conformation (out-of-plane atom on the same side of the sugar ring as C5′) that is found in A-DNA and A-RNA.

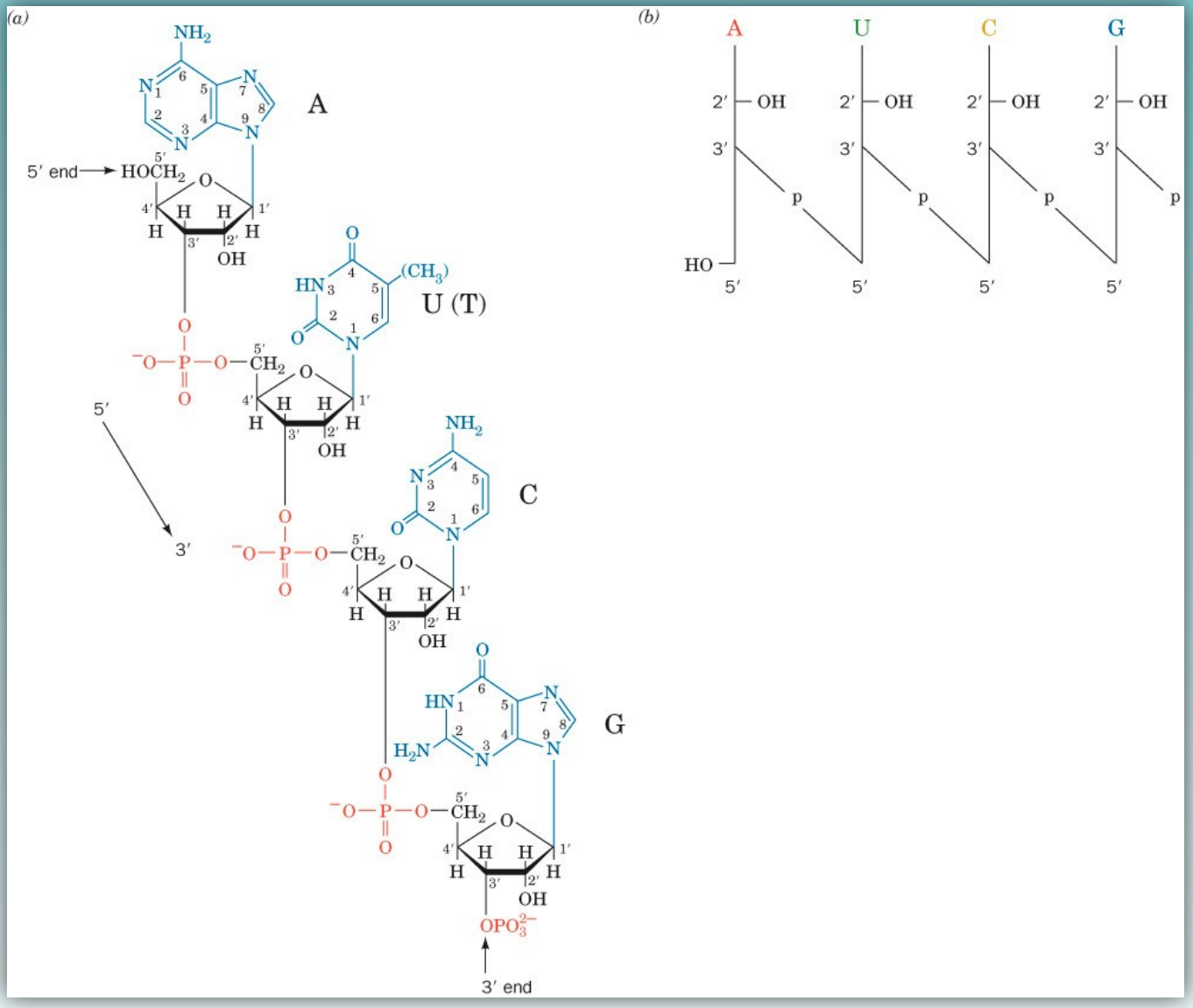
# Impact of furanose ring conformation on backbone structure in polynucleotides




**A P-to-P internuclear distance of 7.0 Å** is correlated with the *C2′-endo* conformation that is found in B-DNA.




Oligonucleotide conformation is determined by the seven indicated torsion angles, $\alpha$-$\zeta$. Note the role of the furanose ring as the "connector" between *N*-glycoside conformation ($\chi$) and backbone conformation.




# Chemical structure of a nucleic acid (RNA)

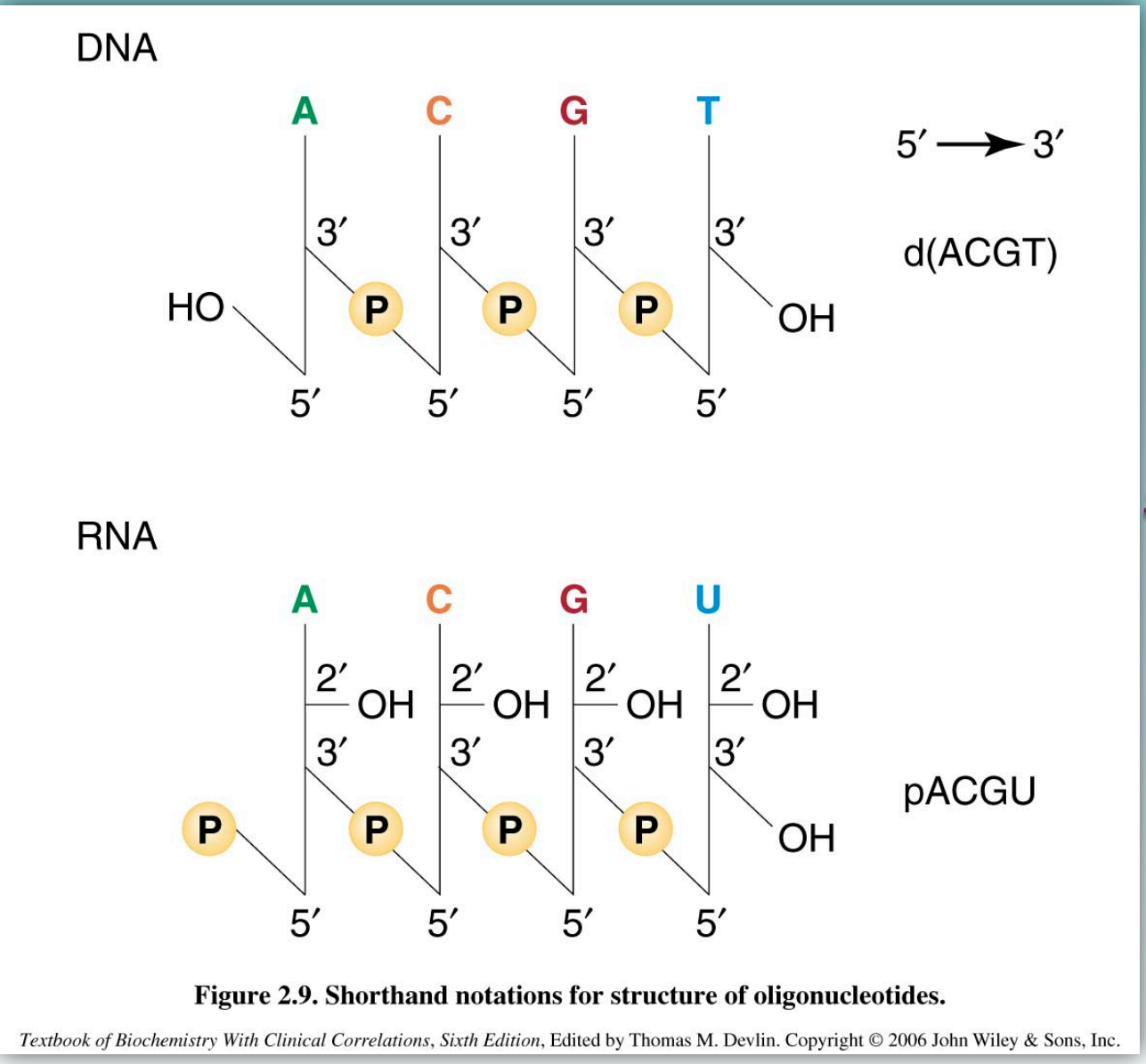
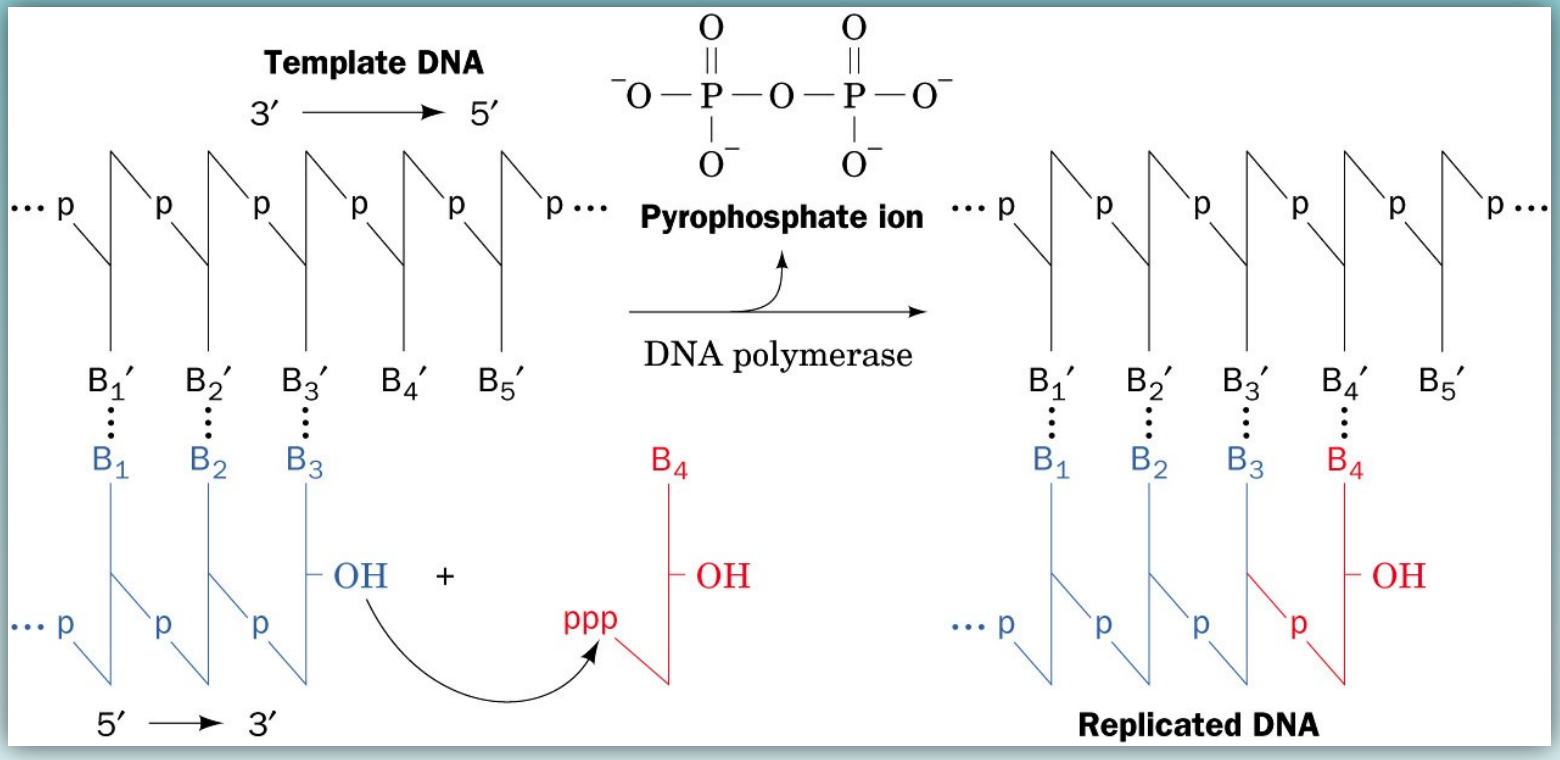


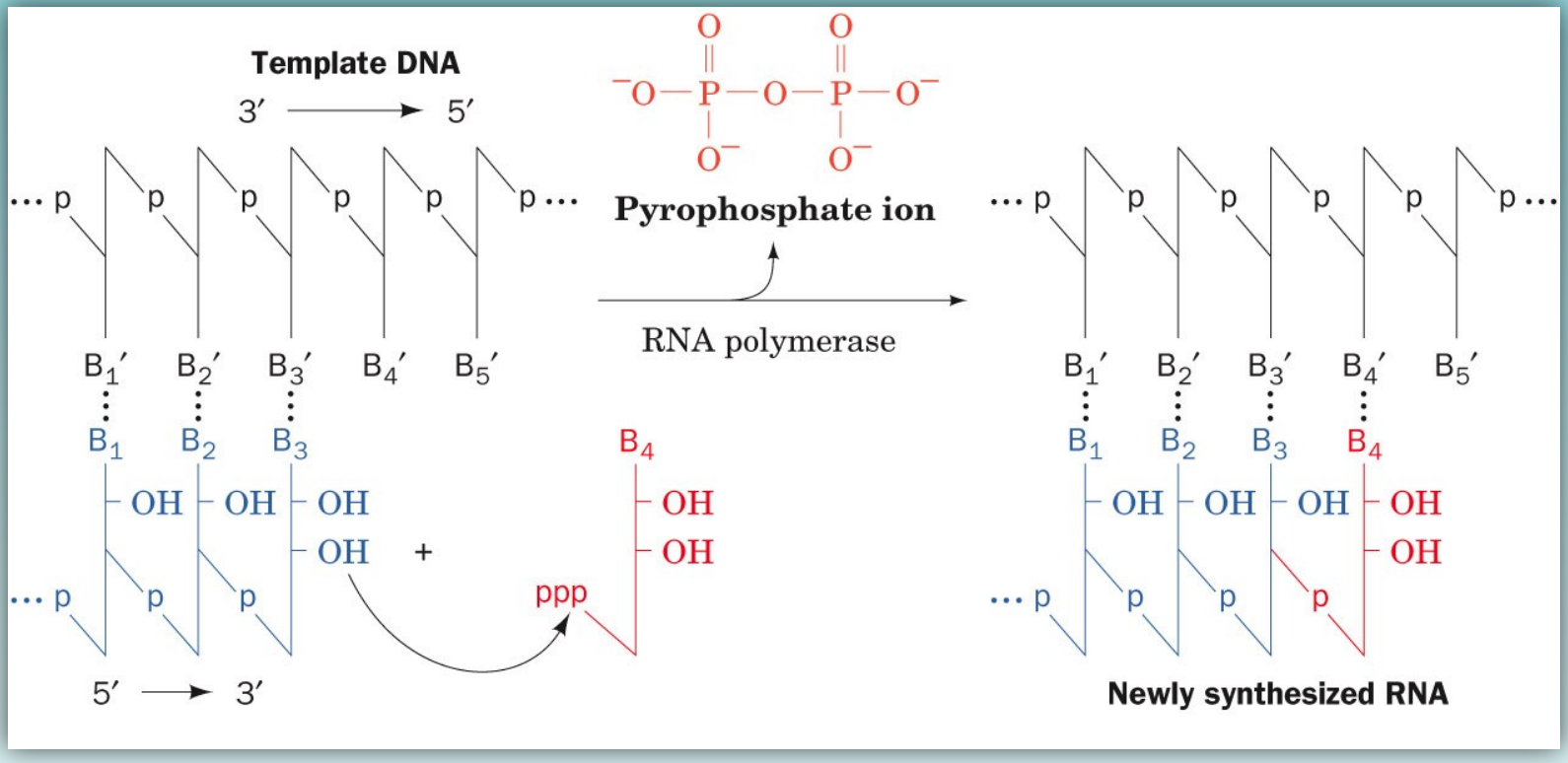

**Figure 2.9. Shorthand notations for structure of oligonucleotides.**



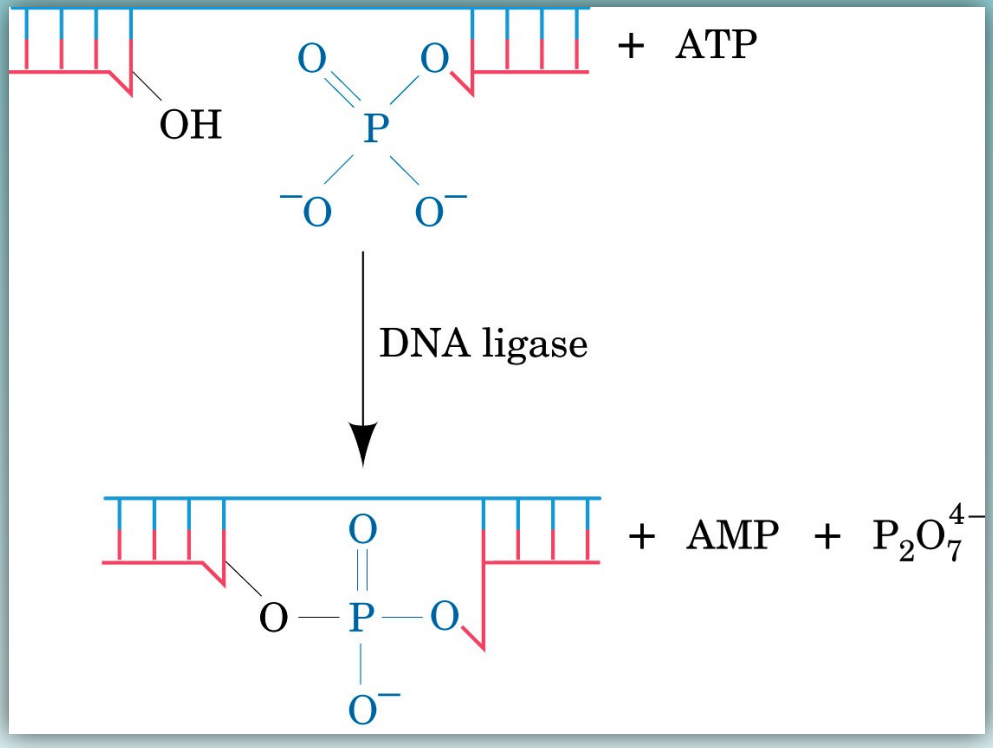
Shorthand notations for oligonucleotide structures. Sequences are always drawn left to right from the 5'-end to the 3'-end.




**General mode of action of DNA polymerases**. Note the cleavage of NTPs in an $\alpha,\beta$-fashion to generate $PP_i$, which is subsequently hydrolyzed by the pyrophosphatases to drive each insertion reaction to completion.




**General mode of action of RNA polymerases.** Note the cleavage of NTPs in an $\alpha,\beta$-fashion to generate $PP_i$, which is subsequently hydrolyzed by the pyrophosphatases to drive each insertion reaction to completion.

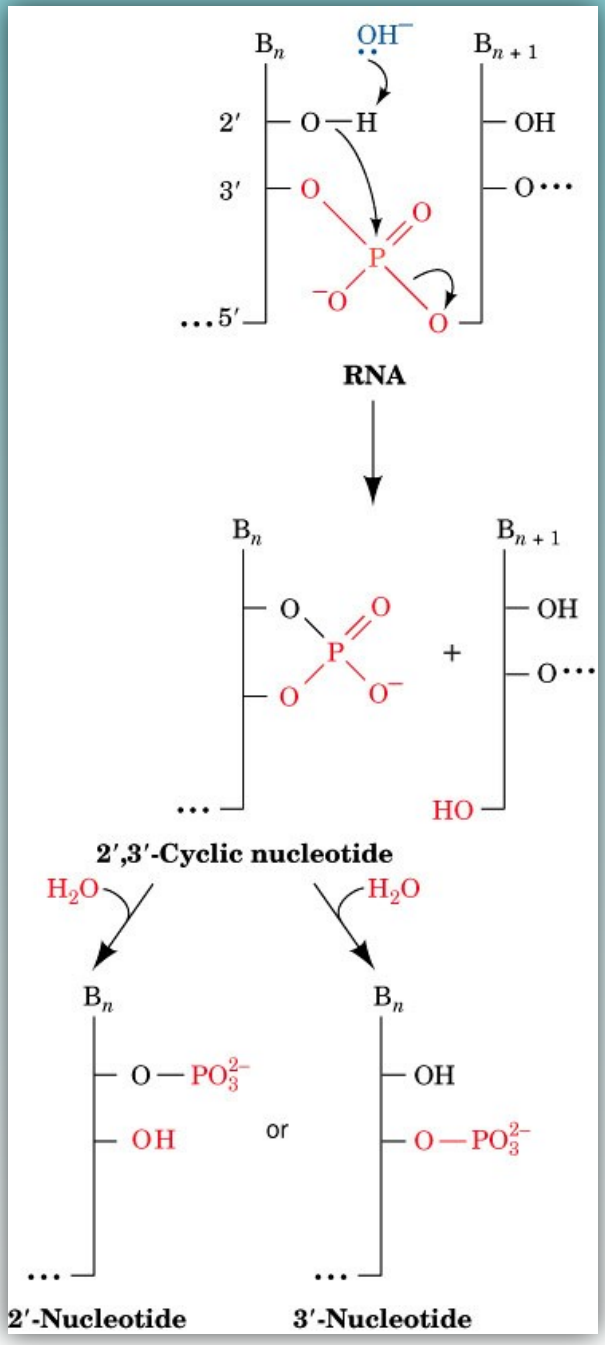



**Mode of action of DNA ligase.** Formation of a phosphodiester bond at the expense of two phosphoanhydride bonds

## A. Hydrolysis of DNA and RNA: phosphodiester

1. Chemical:
    a. acid-catalyzed: DNA and RNA
    b. base-catalyzed: RNA
2. Enzymic:
    a. nucleases

## B. Hydrolysis of DNA and RNA: *N*-glycoside




**Mechanism of base-catalyzed hydrolysis of RNA.** The 2’,3’-cyclic phosphate is produced as an intermediate. This mechanism explains the much greater rate of base-catalyzed hydrolysis of RNA compared to DNA.

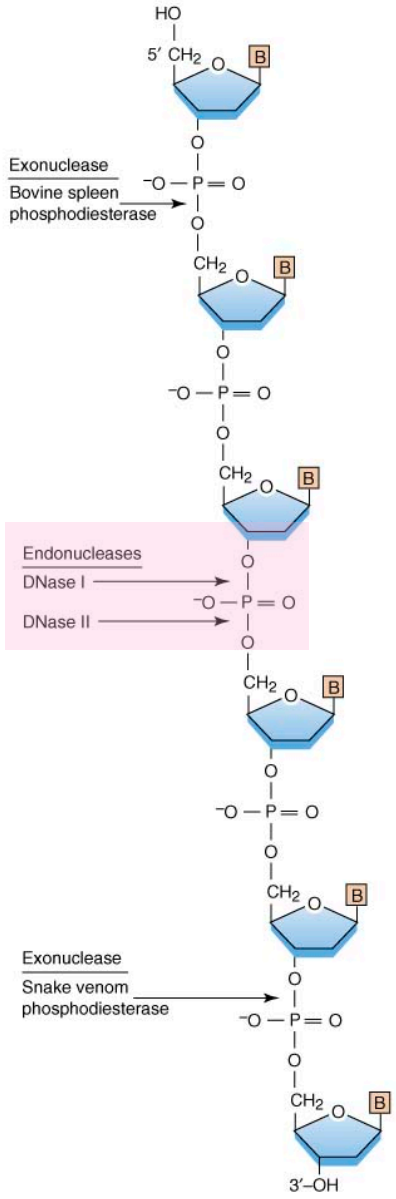


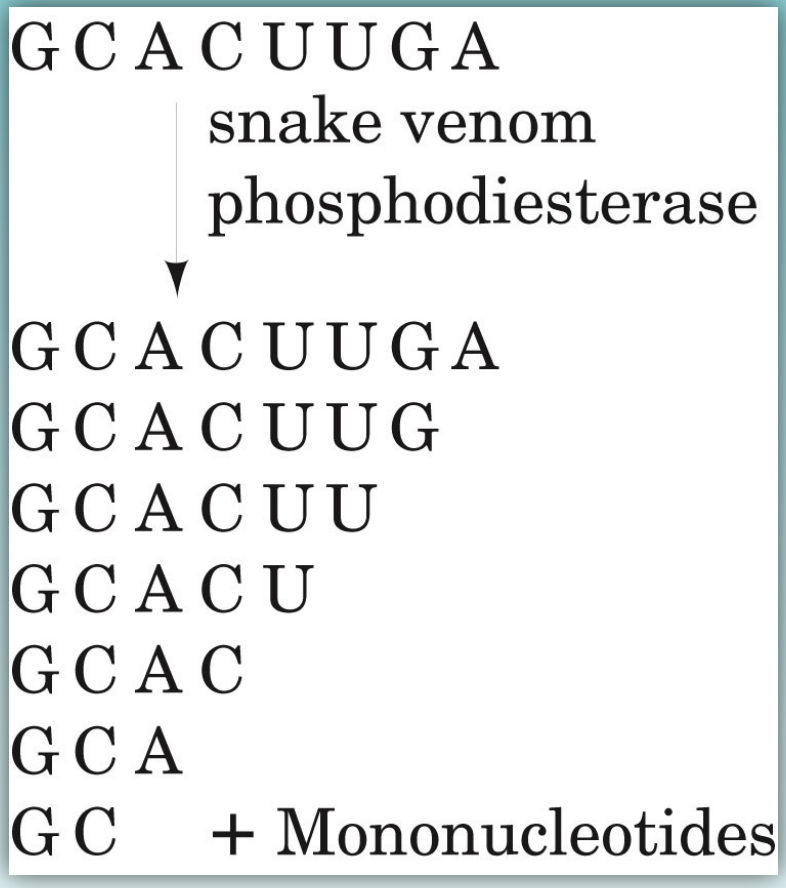

**Figure 2.12. Specificity of nucleases.**



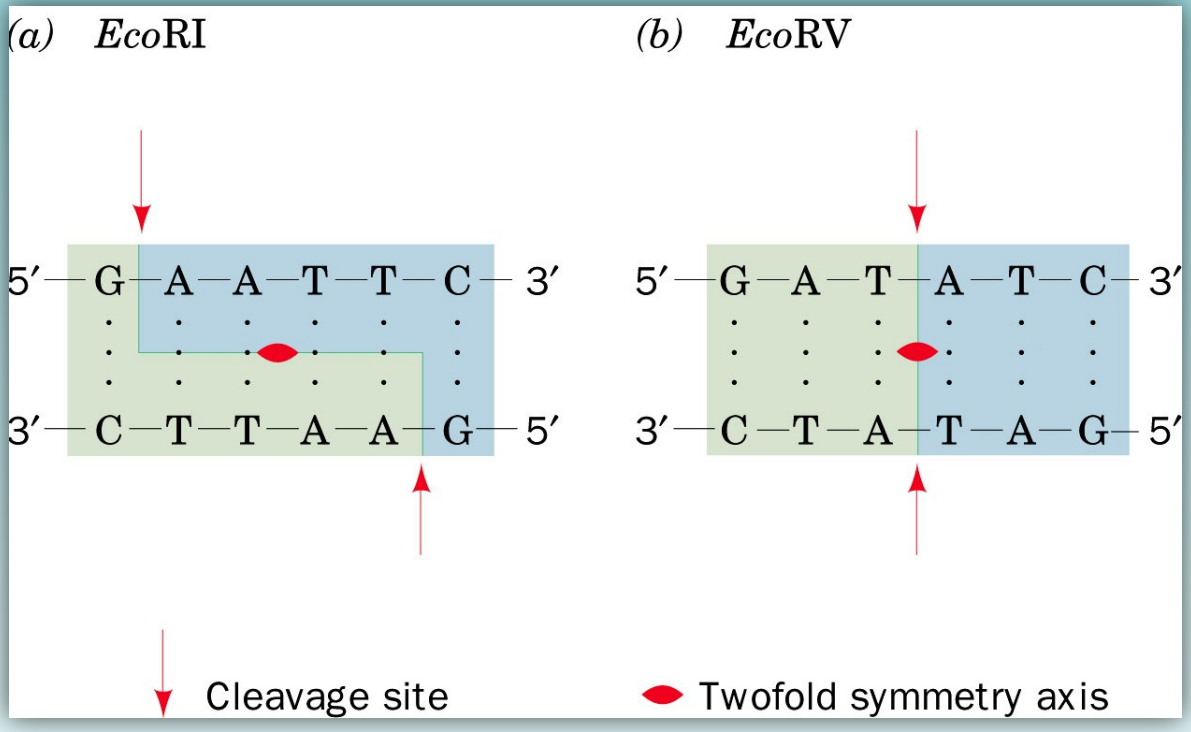
**Type a**: DNase I and snake venom phosphodiesterase

**Type b**: spleen phosphodiesterase and DNase II

**Exonucleases** Spleen phosphodiesterase is a 5' -exonuclease; snake venom phosphodiesterase is a 3' -exonuclease.




Sequence determination of an oligonucleotide by partial digestion with snake venom phosphodiesterase (a type-a 3' -exonuclease)




**Mode of recognition and action of two restriction endonucleases:** Recognition of palindromic sequences

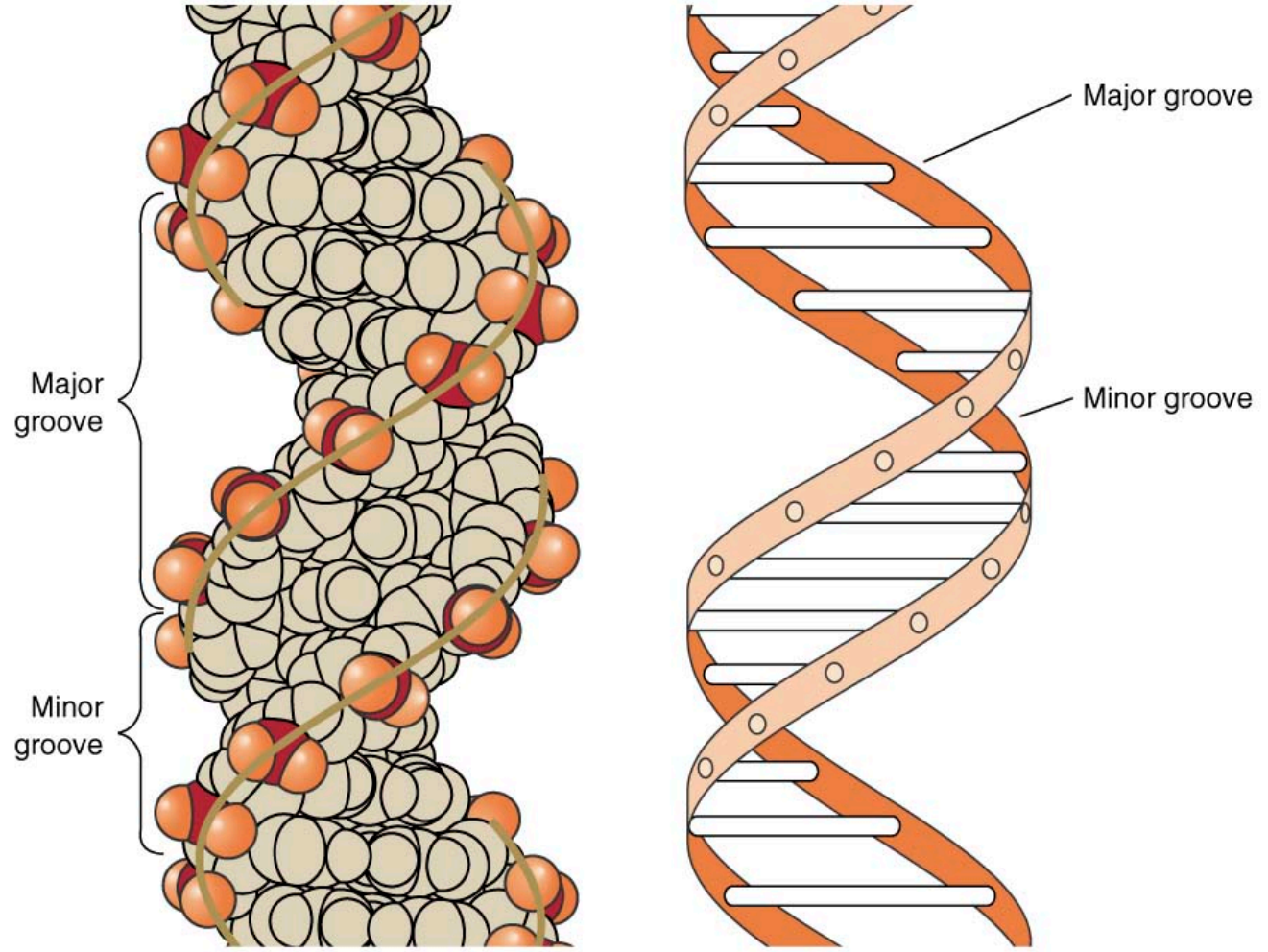



**Figure 2.15. The Watson–Crick model of DNA**. Redrawn from Rich, A. J. *Biomol. Struct. Dyn.* 1:1, 1983.

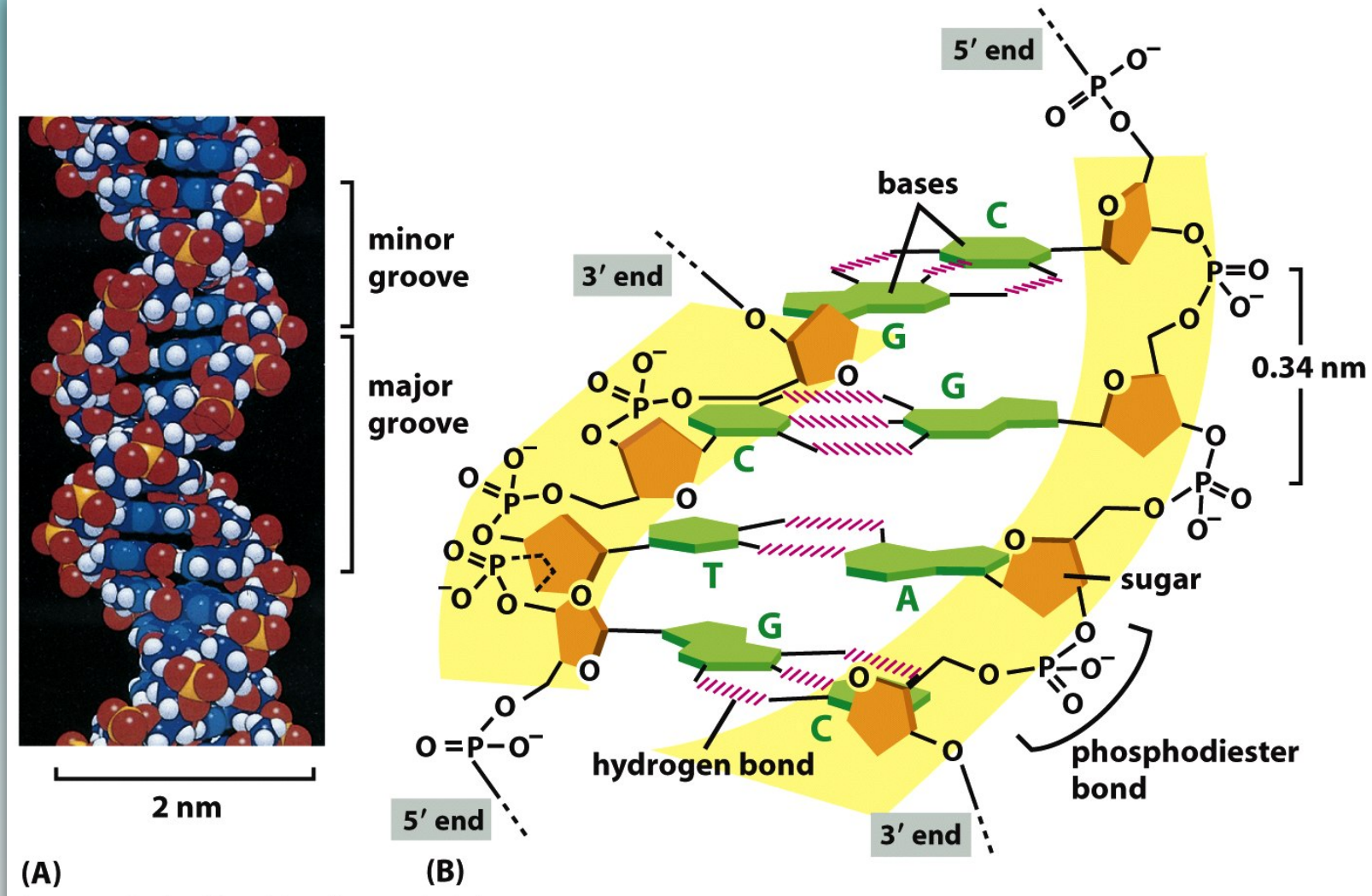
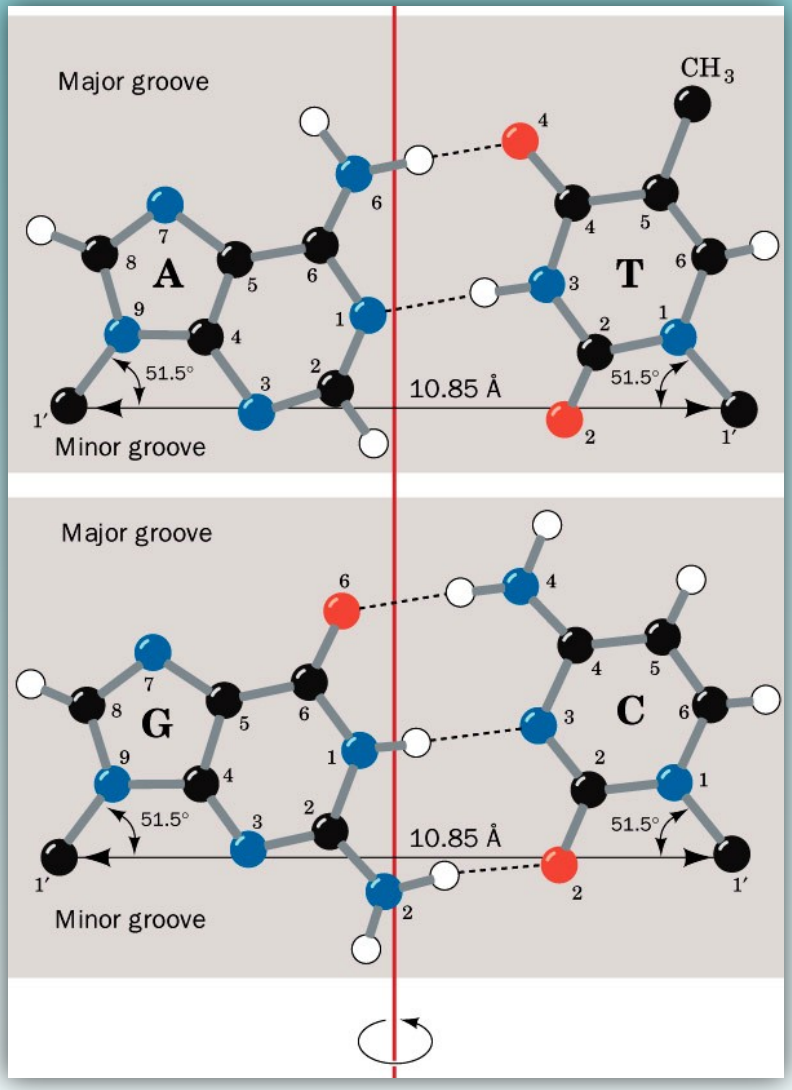



Figure 4-5 Molecular Biology of the Cell 5/e (© Garland Science 2008)




**Watson-Crick base pairs**

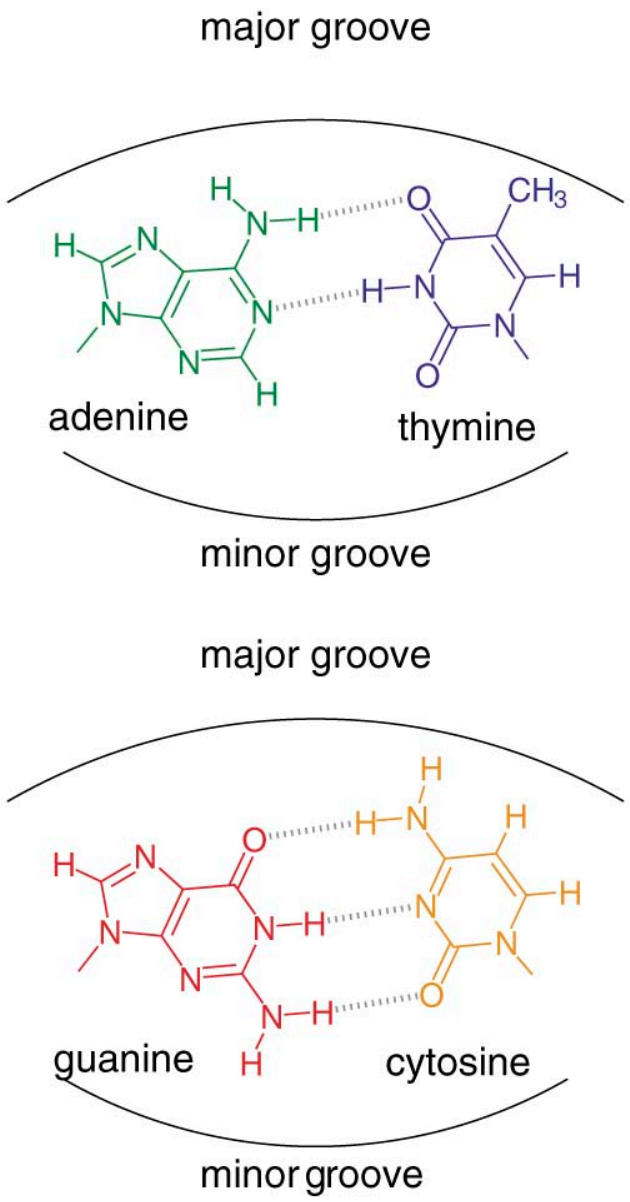



**Figure 2.16. Watson–Crick base pairs.**



Note that different edges of the A-T and C-G base pairs are accessible in the minor and major grooves of DNA. This difference dictates the types of interactions available for binding of DNA to other molecules such as proteins.

# Molecular recognition "handles": Base pairs in a DNA duplex

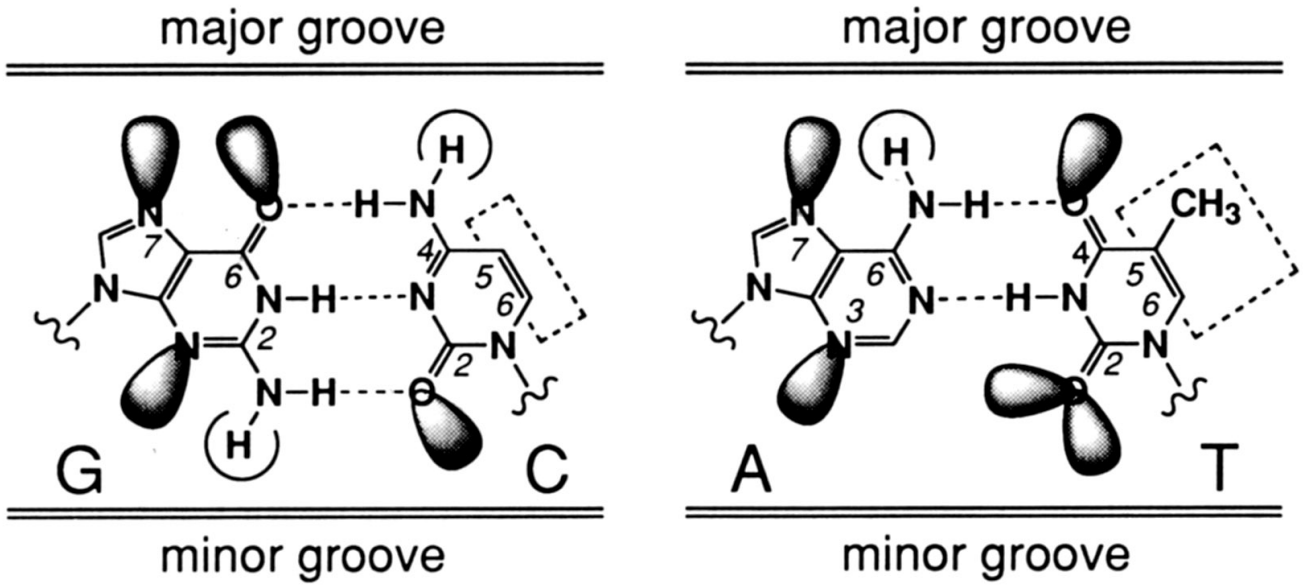



**Figure 11-2.** Potential contact functionally present on base pairs in DNA. Shaded lobes, hydrogen bond acceptors; circles, hydrogen bond donors; dashed rectangles, nonpolar surfaces.

# Molecular recognition: H-bonding interactions between DNA bases and protein sidechains

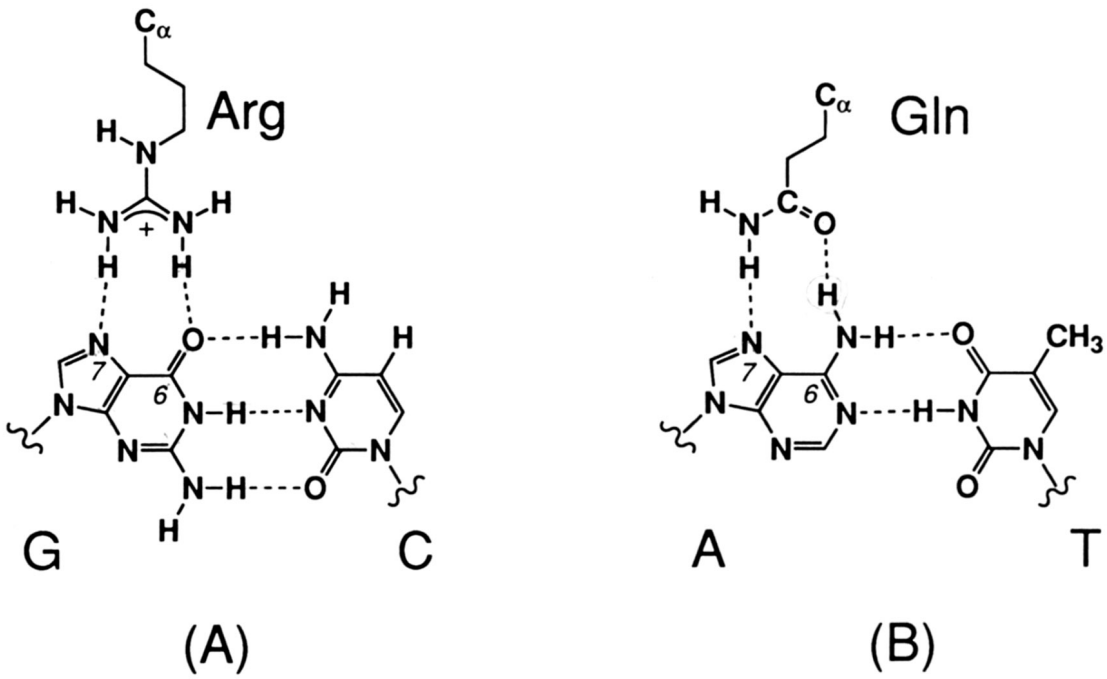



**Figure 11-3.** Bidentate hydrogen bonding interactions between (A) guanine and arginine and (B) adenine and glutamine.

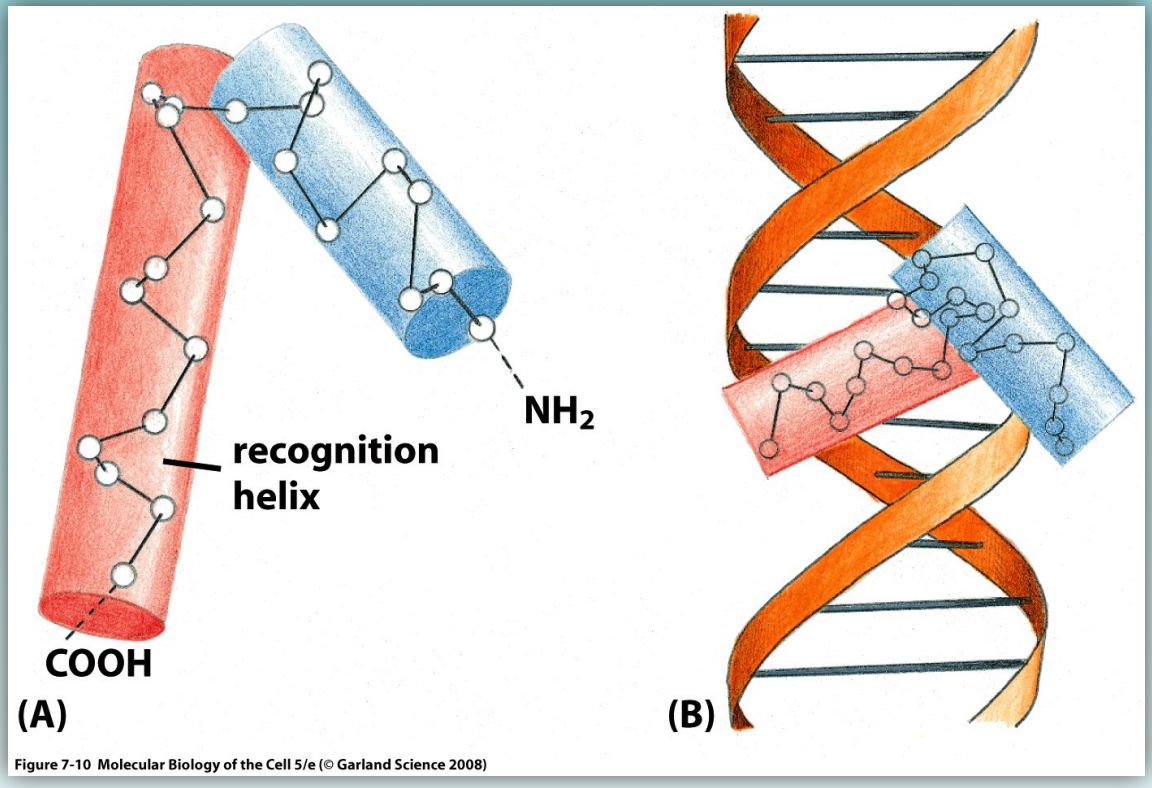


Figure 7-10 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The helix-turn-helix motif in proteins is involved in DNA binding to the major groove.

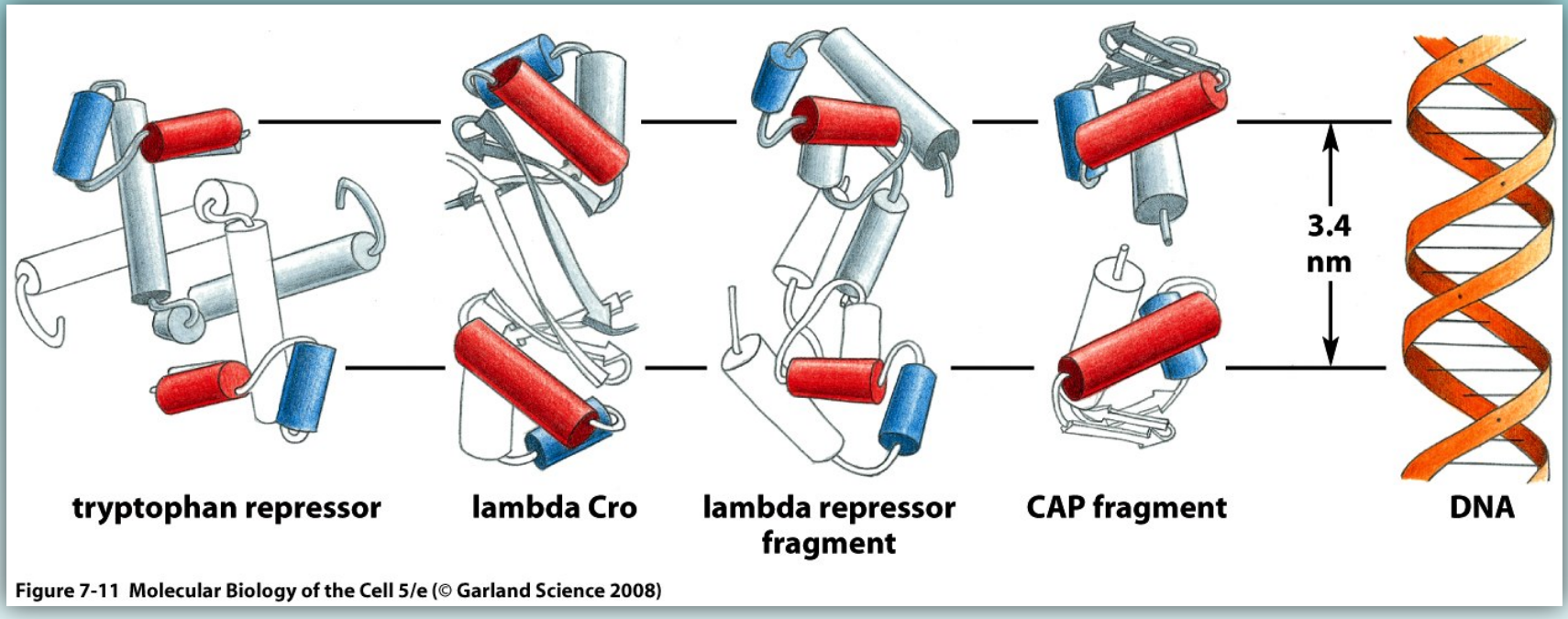



Figure 7-11 Molecular Biology of the Cell 5/e (© Garland Science 2008)

# Some helix-turn-helix DNA-binding proteins

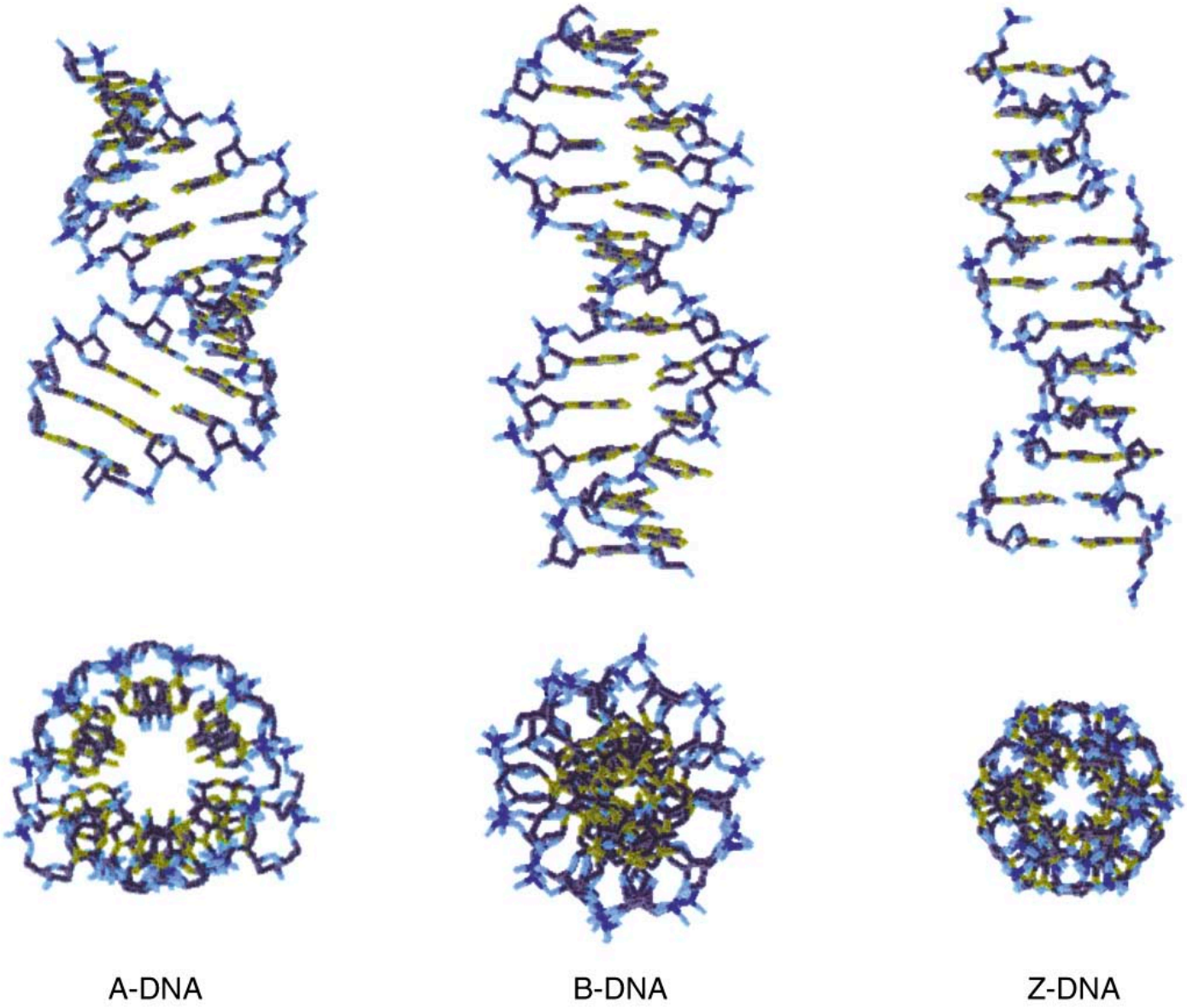
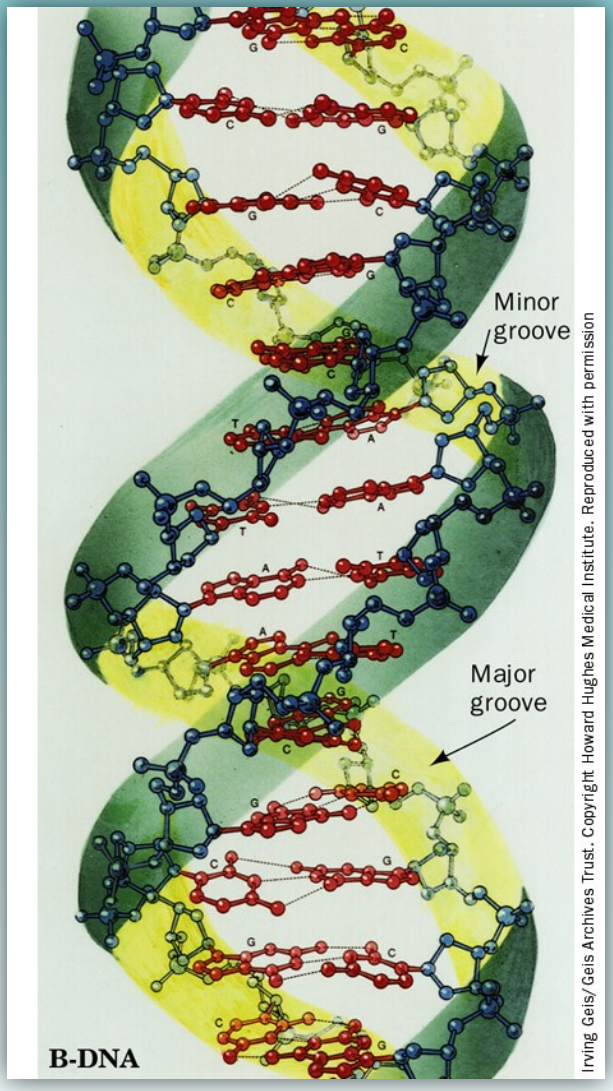
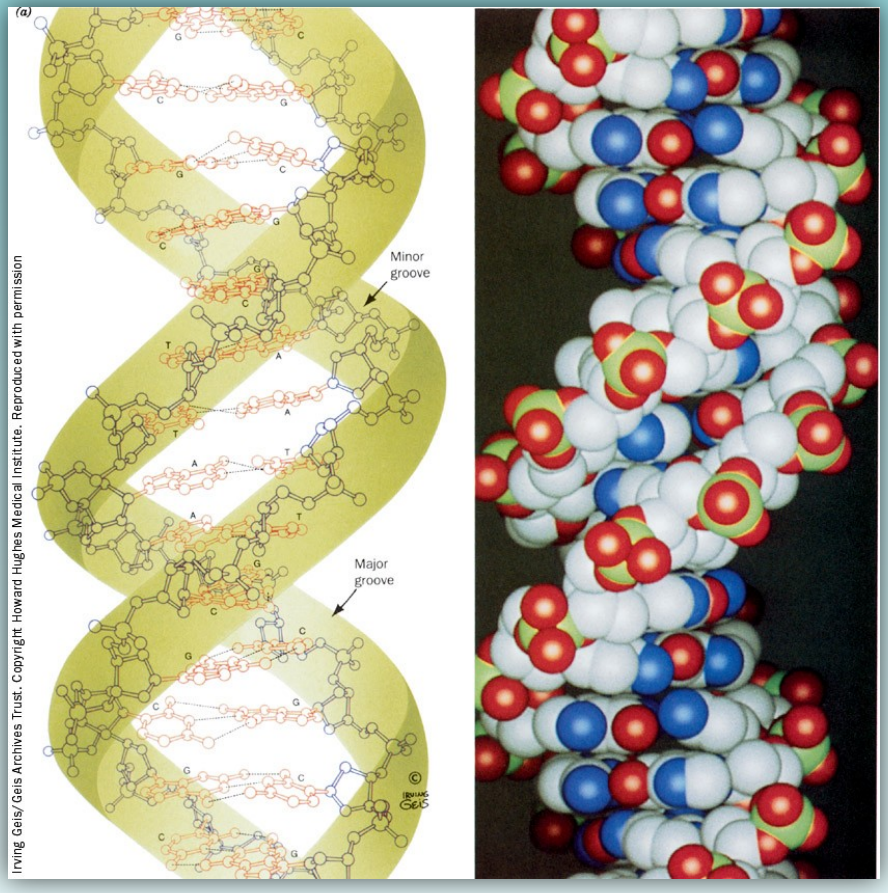


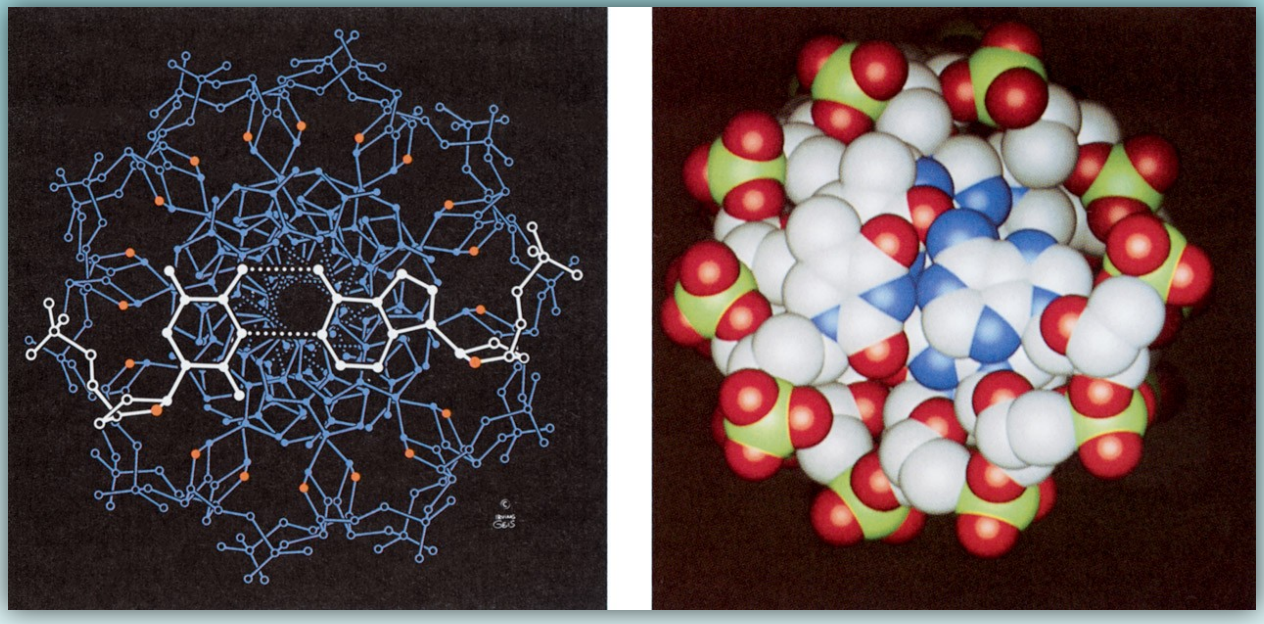

**Figure 2.23. The varied geometries of double-helical DNA.**



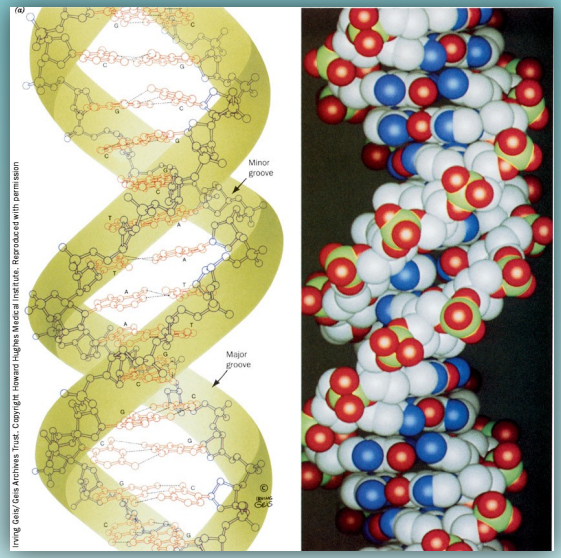

Three-dimensional structure of B-DNA



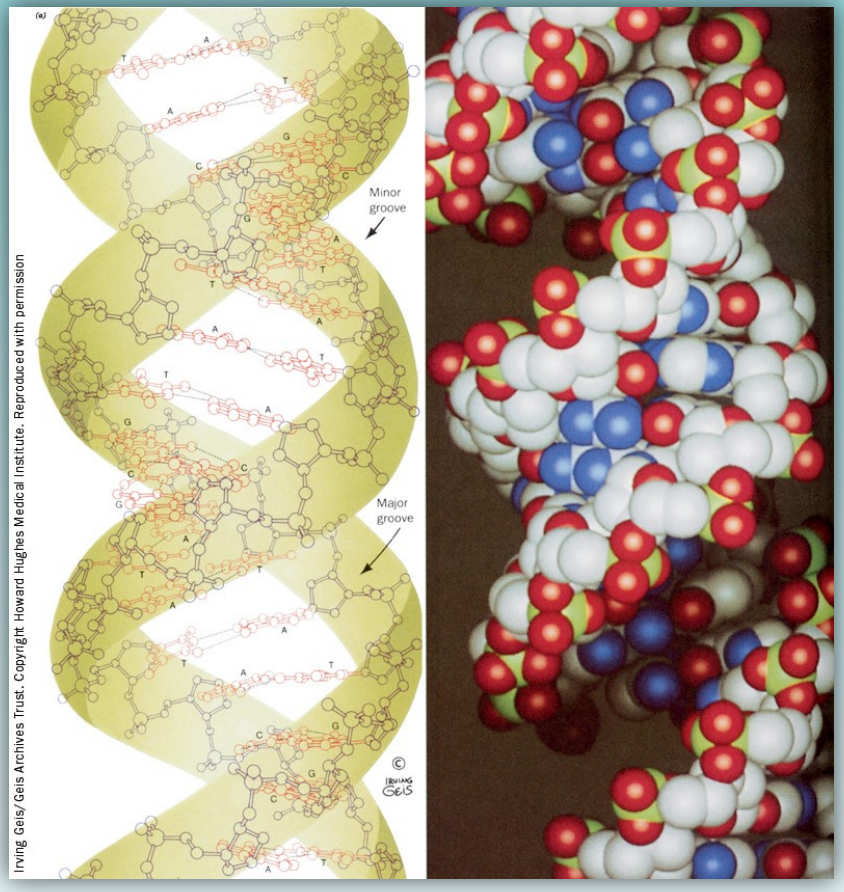

**B-DNA structure**: Ball-and-stick and space-filling models viewed perpendicular to the helical axis



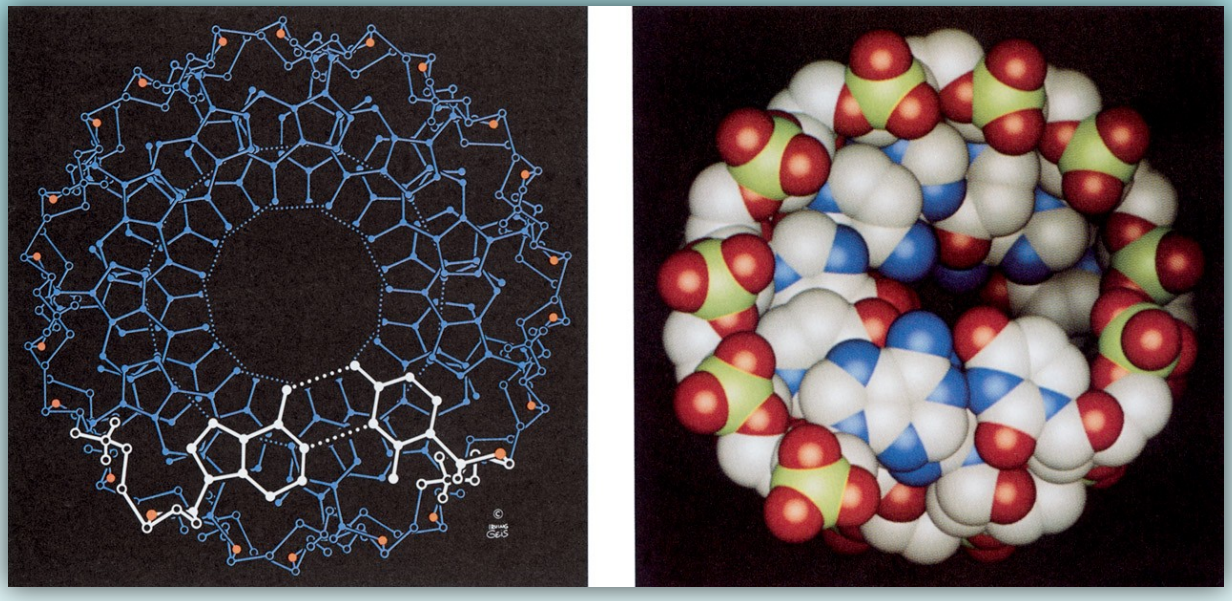
**B-DNA structure**: Ball-and-stick and space-filling models viewed parallel to the helical axis



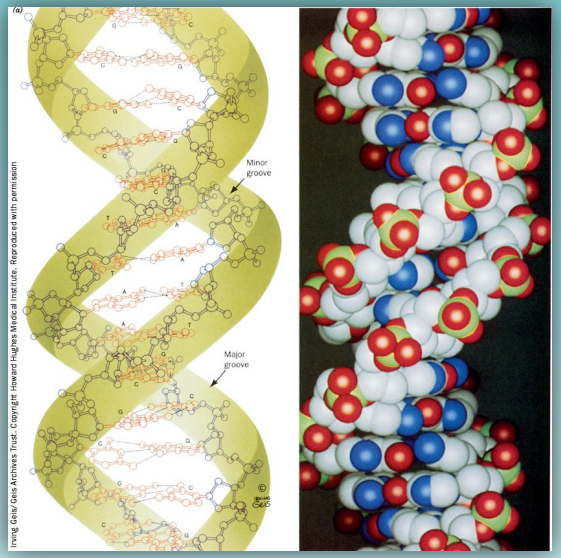

B-DNA



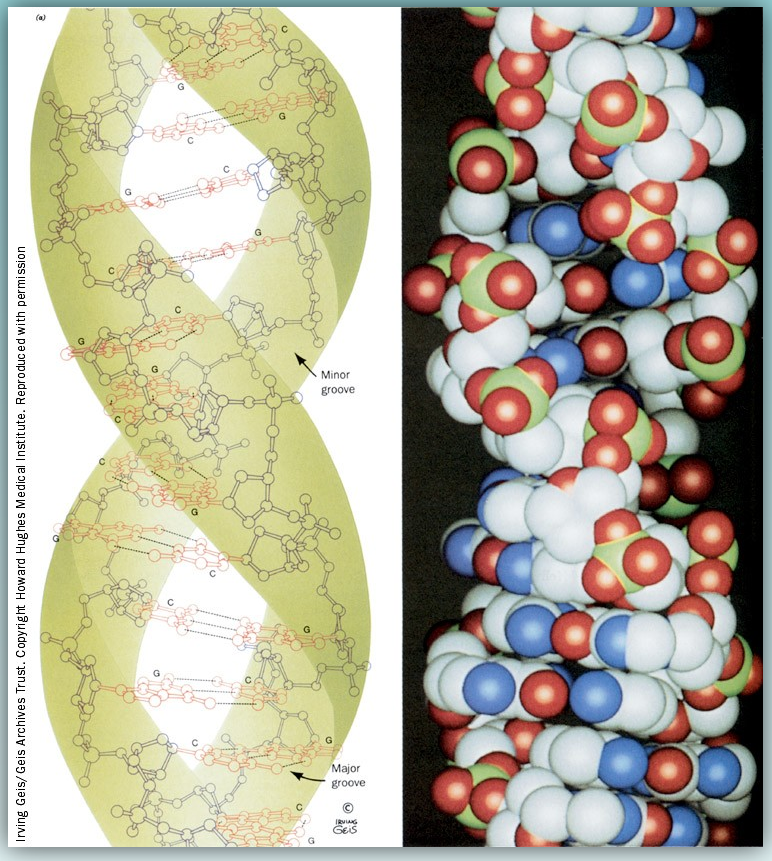

**A-DNA structure**: Ball-and-stick and space-filling models viewed perpendicular to the helical axis



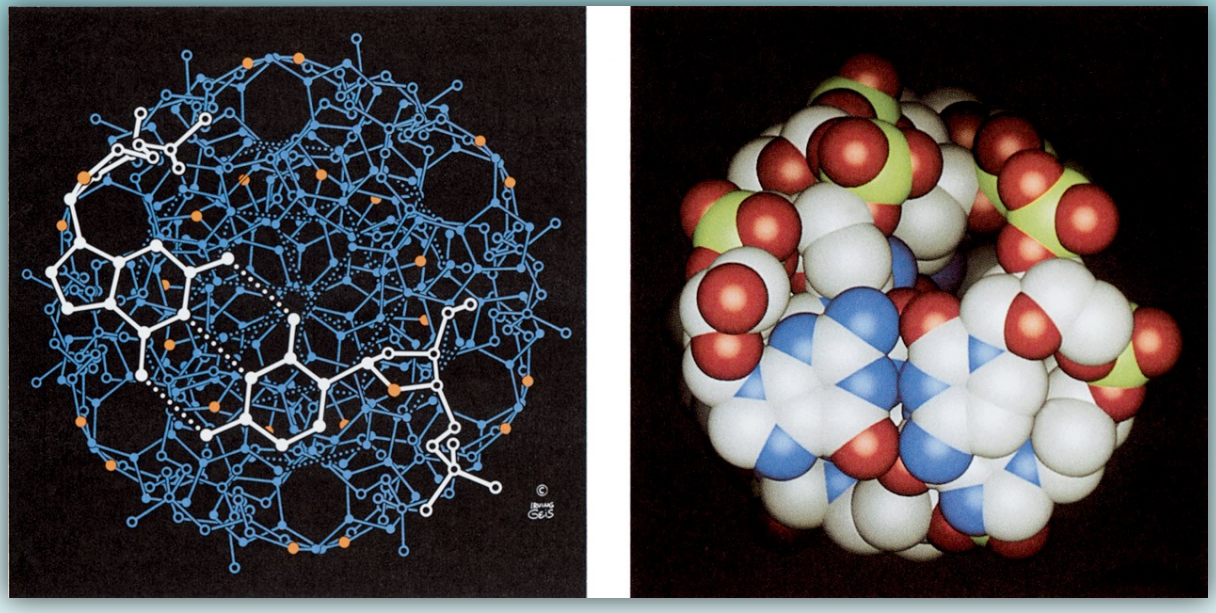
**A-DNA structure**: Ball-and-stick and space-filling models viewed parallel to the helical axis




B-DNA




**Z-DNA structure**: Ball-and-stick and space-filling models viewed perpendicular to the helical axis



**Z-DNA structure**: Ball-and-stick and space-filling models viewed parallel to the helical axis

| | A-DNA | B-DNA | Z-DNA |
|---|---|---|---|
| Helical sense | Right-handed | Right-handed | Left-handed |
| Diameter | ~26 Å | ~20 Å | ~18 Å |
| Base pairs per helical turn | 11.6 | 10 | 12 (6 dimers) |
| Helical twist per base pair | 31° | 36° | 9° for pyrimidine–purine steps; 51° for purine–pyrimidine steps |
| Helix pitch (rise per turn) | 34 Å | 34 Å | 44 Å |
| Helix rise per base pair | 2.9 Å | 3.4 Å | 7.4 Å per dimer |
| Base tilt normal to the helix axis | 20° | 6° | 7° |
| Major groove | Narrow and deep | Wide and deep | Flat |
| Minor groove | Wide and shallow | Narrow and deep | Narrow and deep |
| Sugar pucker | C3′-*endo* | C2′-*endo* | C2′-*endo* for pyrimidines; C3 -*endo* for purines |
| Glycosidic bond | Anti | Anti | Anti for pyrimidines; syn for purines |

*Source:* Mainly Arnott, S., *in* Neidle, S. (Ed.), *Oxford Handbook of Nucleic Acid Structure, p.* 35, Oxford University Press (1999).

**Structural features of ideal A-, B-, and Z-DNA**

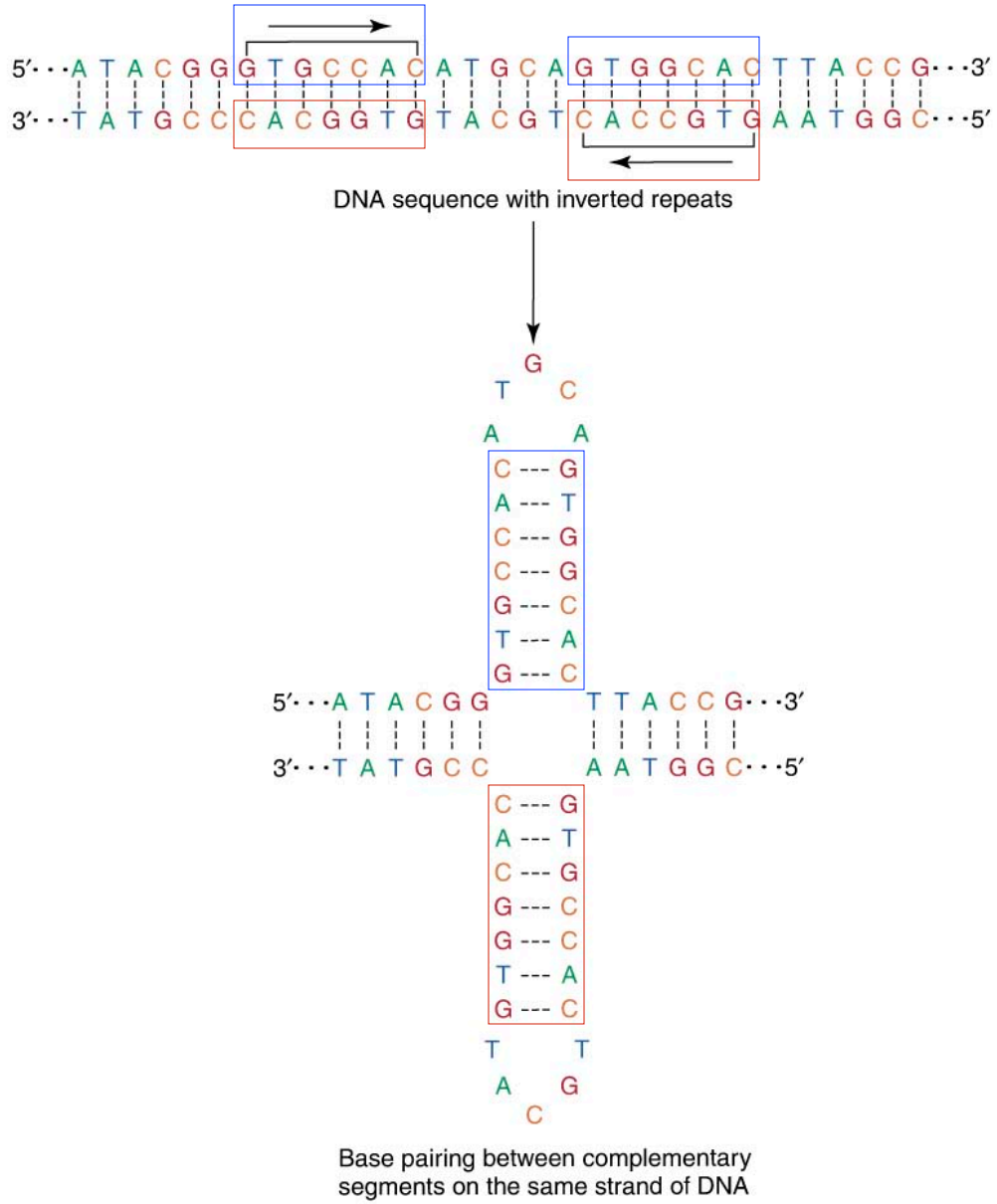
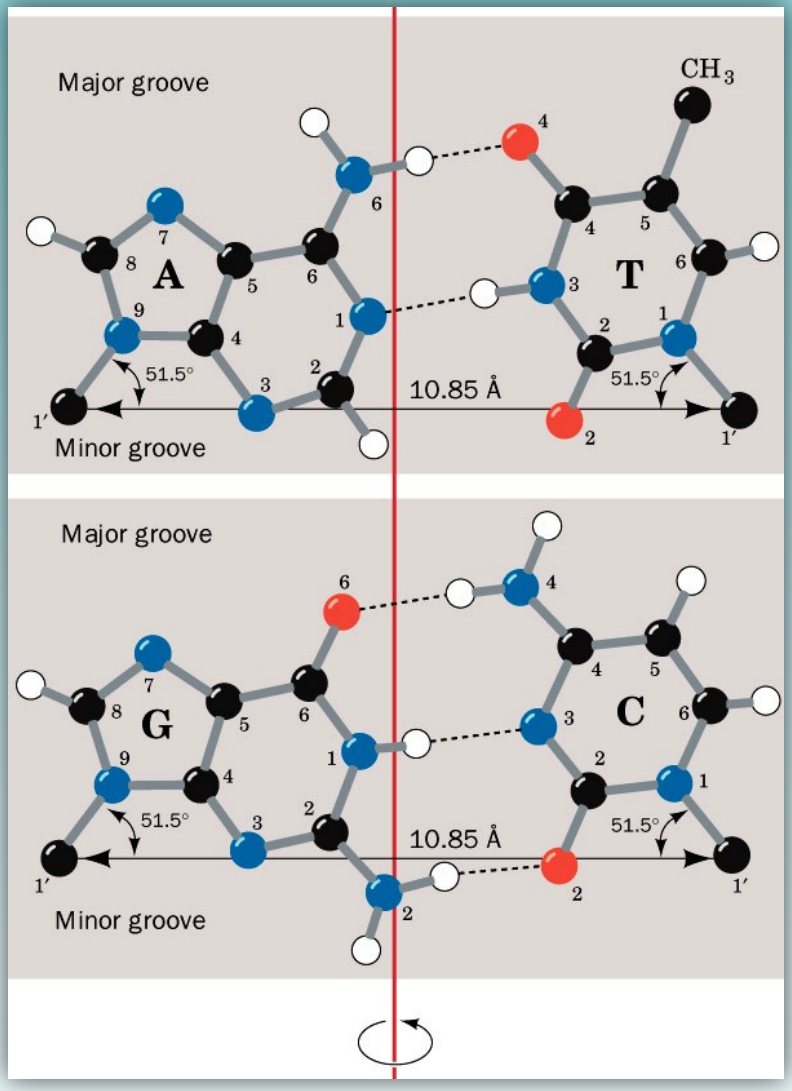



**Figure 2.27. Formation of cruciform structures in DNA.**




**Watson-Crick base pairs**

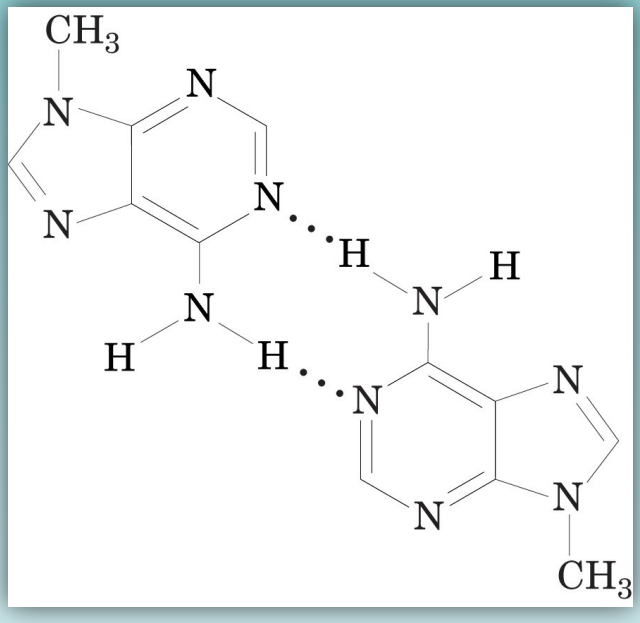
| Base Pair | $K\ (M^{-1})^a$ |
|---|---|
| ***Self-association*** | |
| A · A | 3.1 |
| U · U | 6.1 |
| C · C | 28 |
| G · G | $10^3$–$10^4$ |
| ***Watson–Crick Base Pairs*** | |
| A · U | 100 |
| G · C | $10^4$–$10^5$ |

[a]Data measured in deuterochloroform at 25°C.

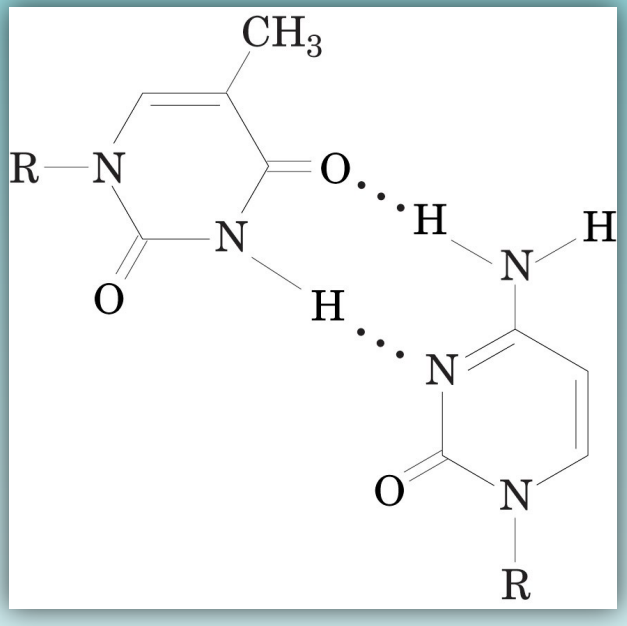
*Source:* Kyogoku, Y., Lord, R.C., and Rich, A., *Biochim. Biophys. Acta* **179,** 10 (1969).

electronic complementarity

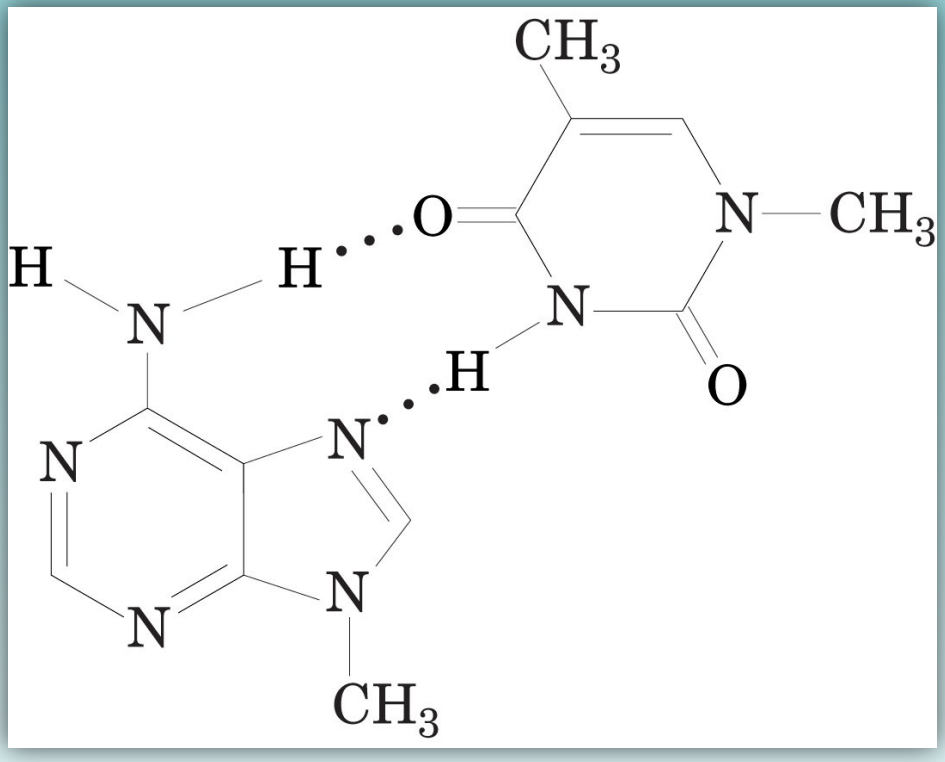
**Association constants for base pair formation**




**Non-Watson-Crick base pairs**: Pairing of adenine residues in the crystal structure of 9-methyladenine




**Non-Watson-Crick base pairs**: Hypothetical pairing between cytosine and thymine residues




**Non-Watson-Crick base pairs**: **Hoogsteen** pairing between adenine and thymine residues in the crystal structure of 9-methyladenine·1-methylthymine

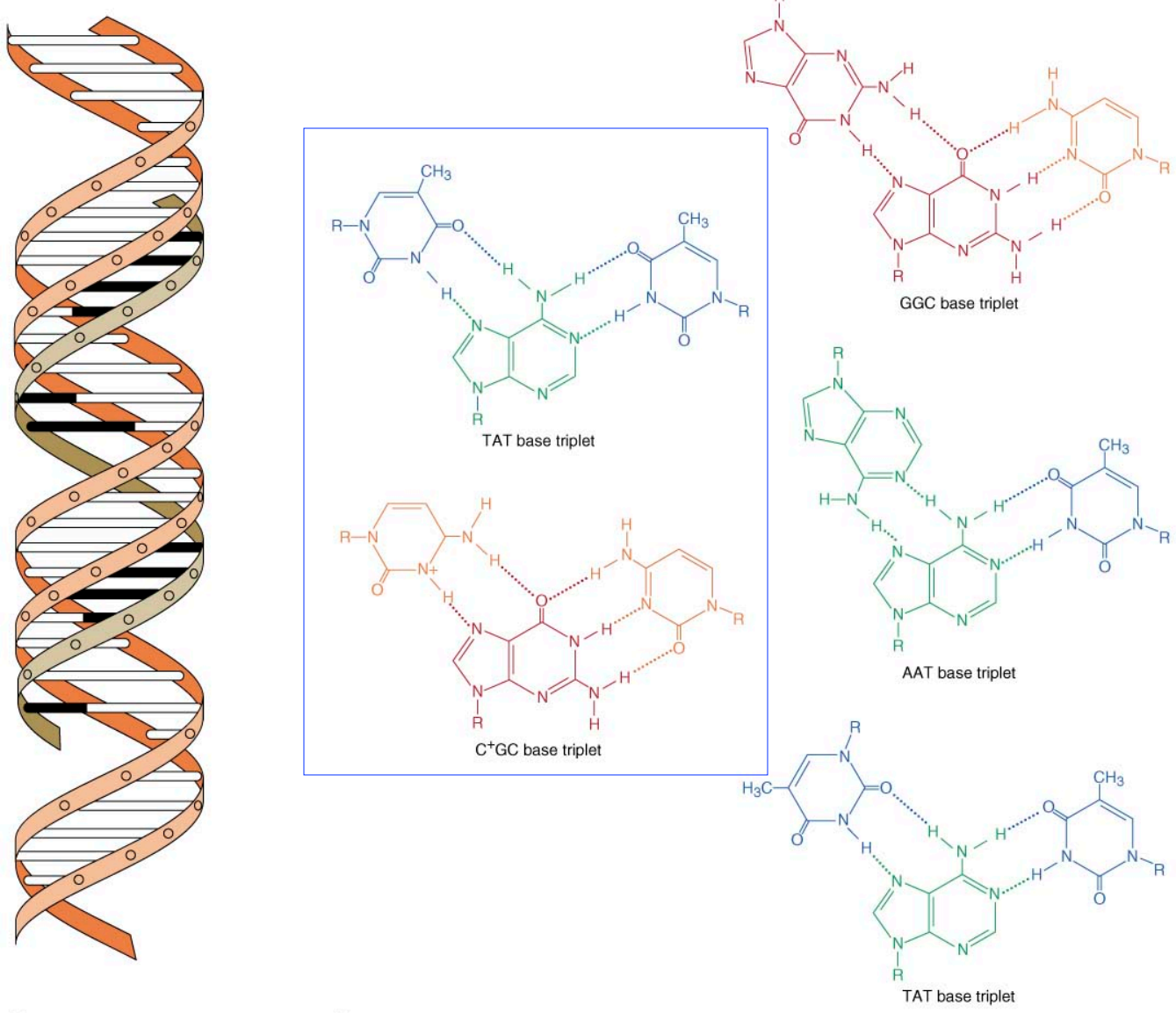



**Figure 2.28. Triple helices.**

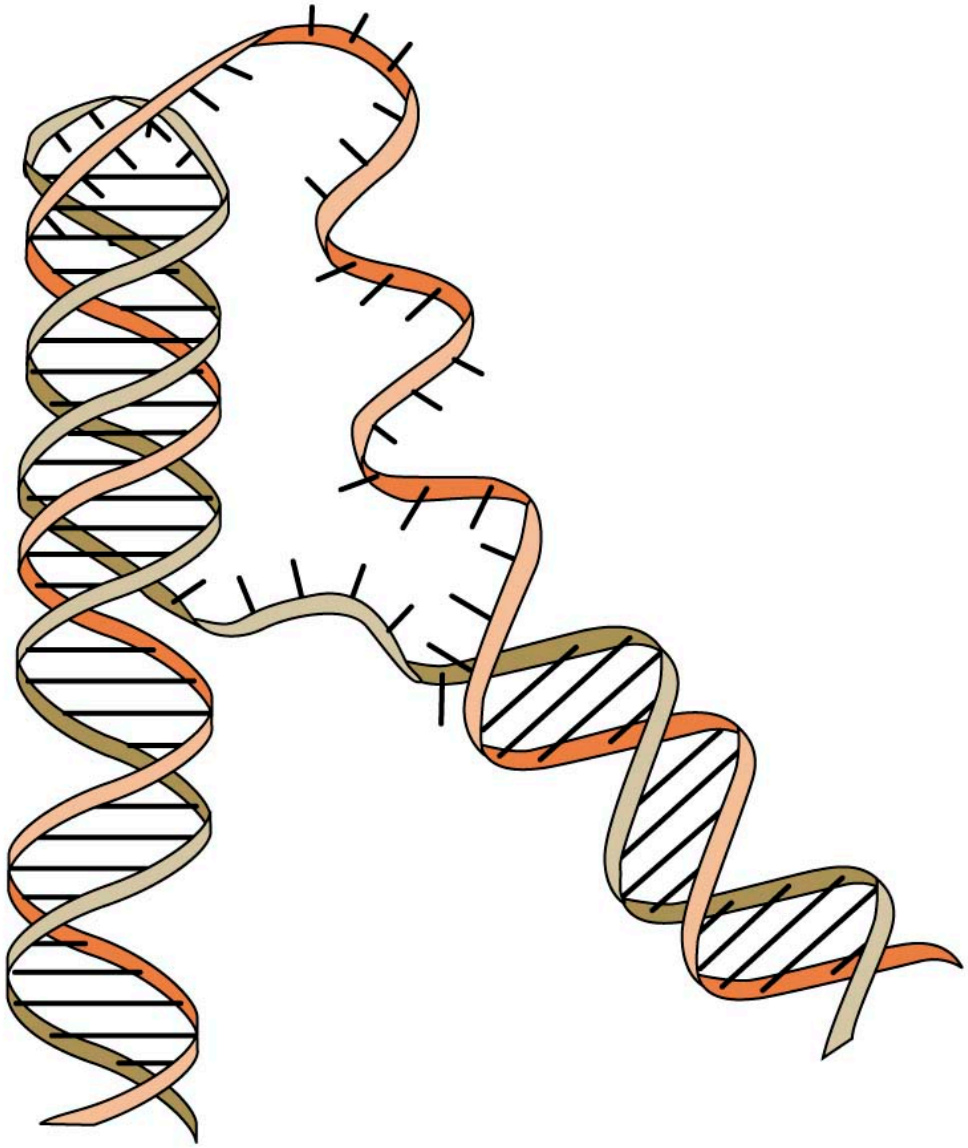
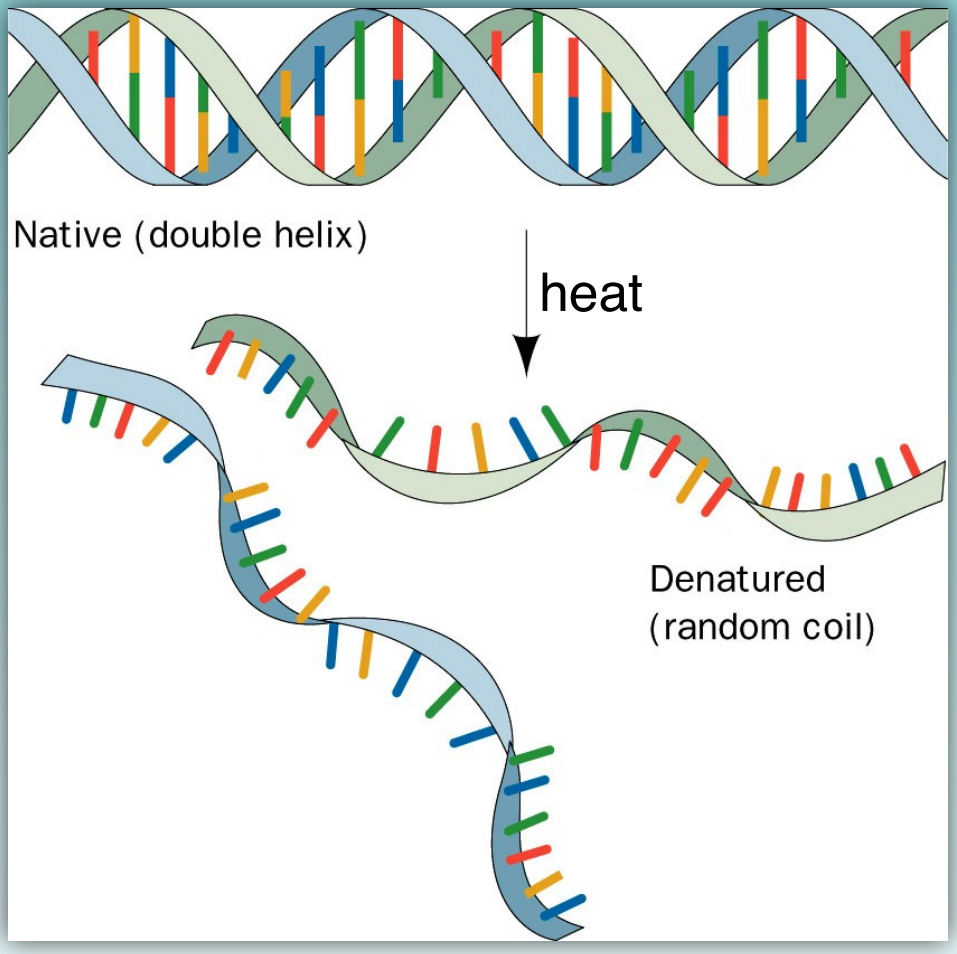


**Figure 2.29. Intramolecular triple helices: H-DNA.** Redrawn based on figure in Sinden, R. R. *DNA Structure and Function*. New York: Academic Press, 1994.

# Energetics of denaturation




**Schematic representation of strand separation in duplex DNA resulting from heat denaturation**

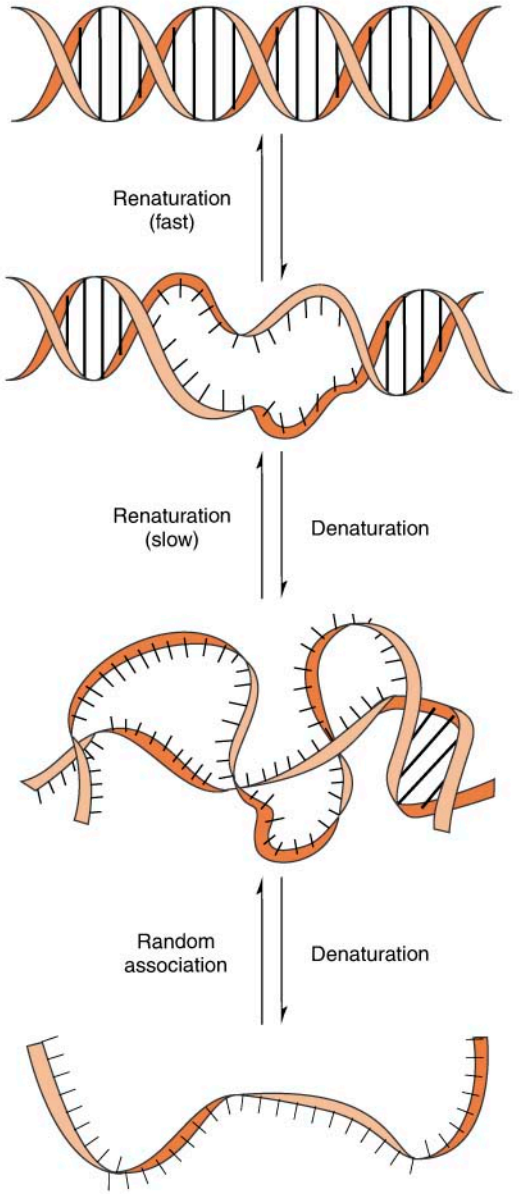
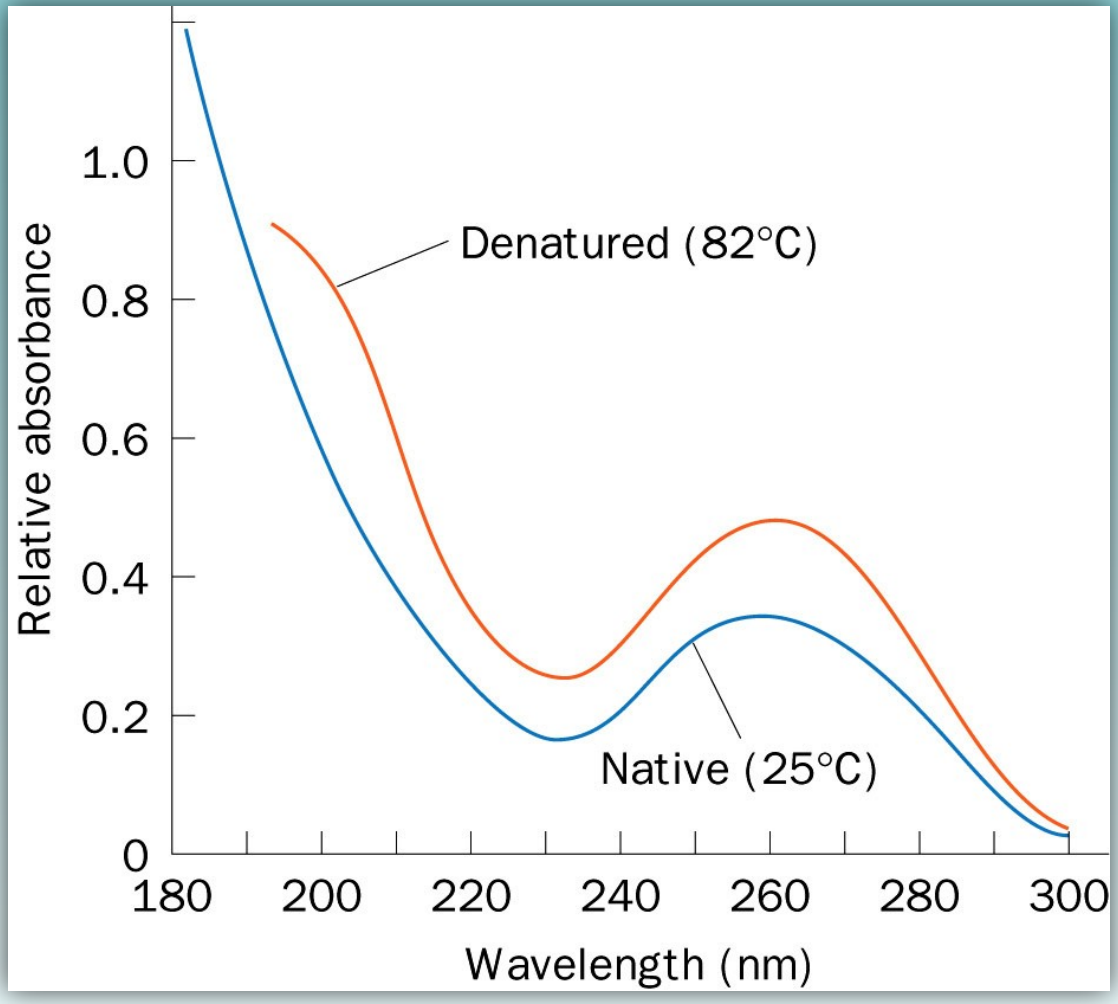
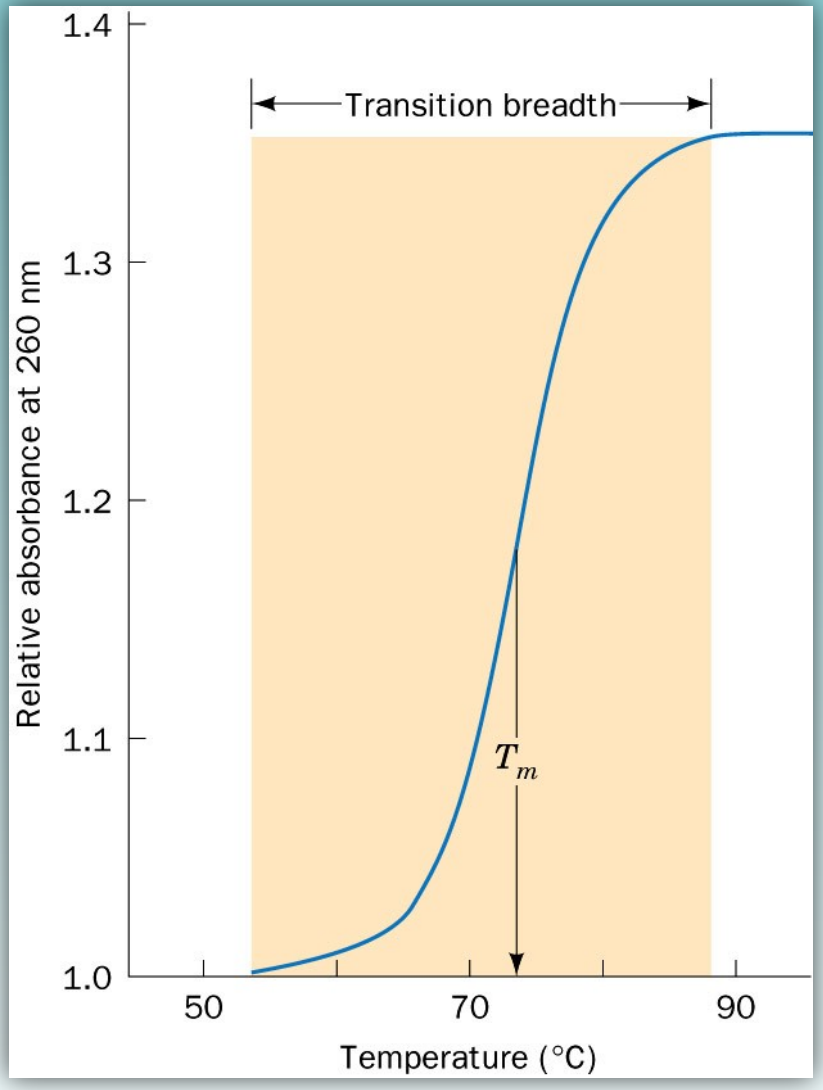



**Figure 2.19. Denaturation of DNA.**




**The hyperchromic effect**

**UV absorbance spectra of native and heat-denatured *E. coli* DNA**




**The melting temperature, $T_m$, is defined as the temperature at which half of the maximum absorbance increase is attained.**

Denaturation over a narrow T range implies a cooperative process.

**Example of a DNA melting curve**

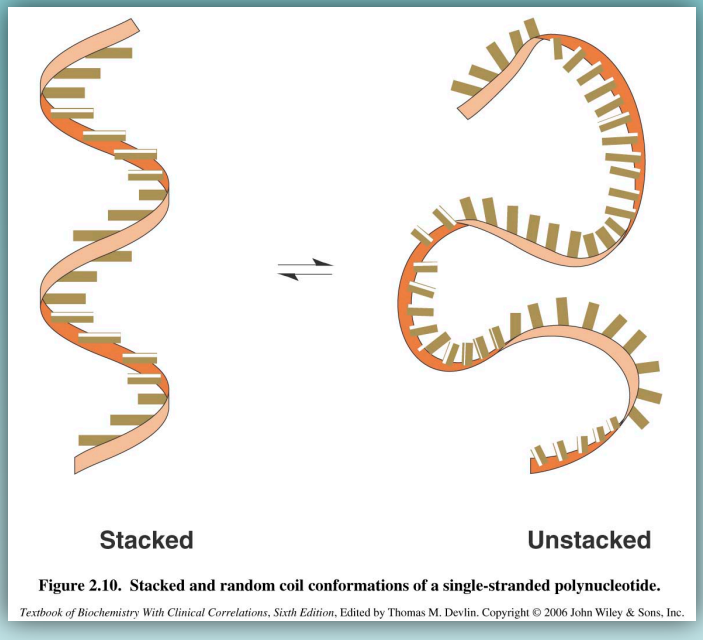



**Figure 2.10. Stacked and random coil conformations of a single-stranded polynucleotide.**

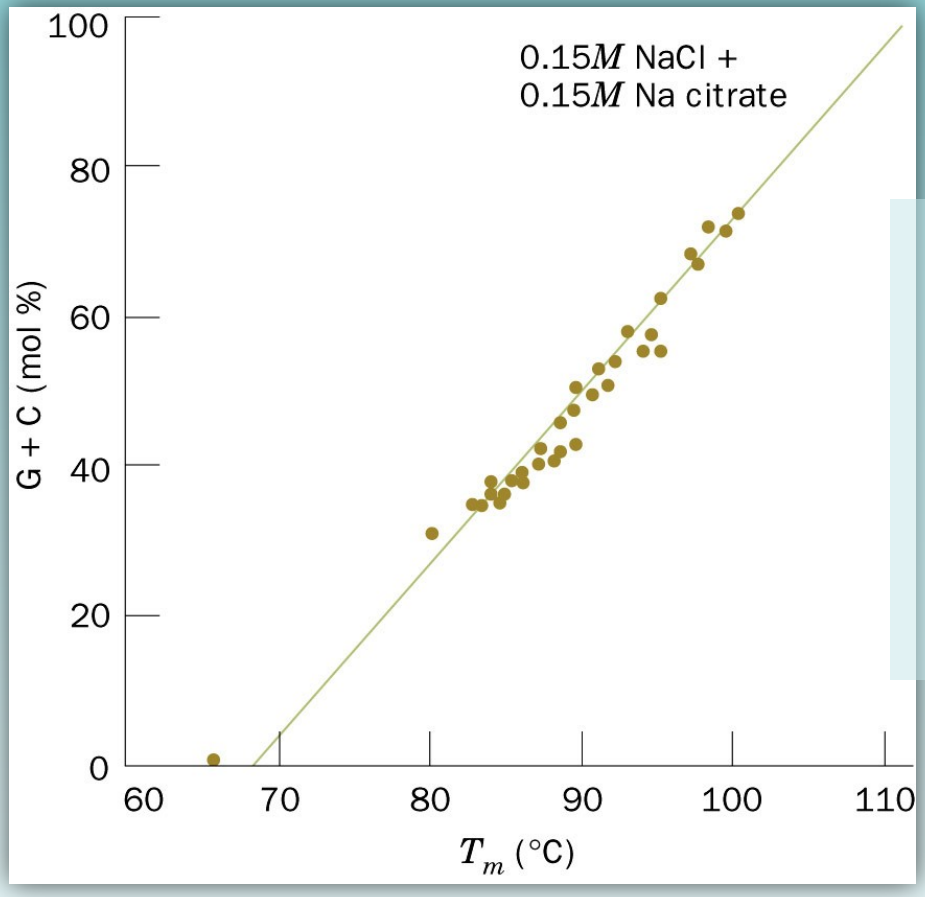


**Thermodynamic parameters for the stacking/unstacking reaction: stacking is enthalpically driven and entropically opposed (opposite to what is observed in protein stabilization)**

Dinucleoside phosphate $\rightleftharpoons$ dinucleoside phosphate
(*unstacked*) (*stacked*)

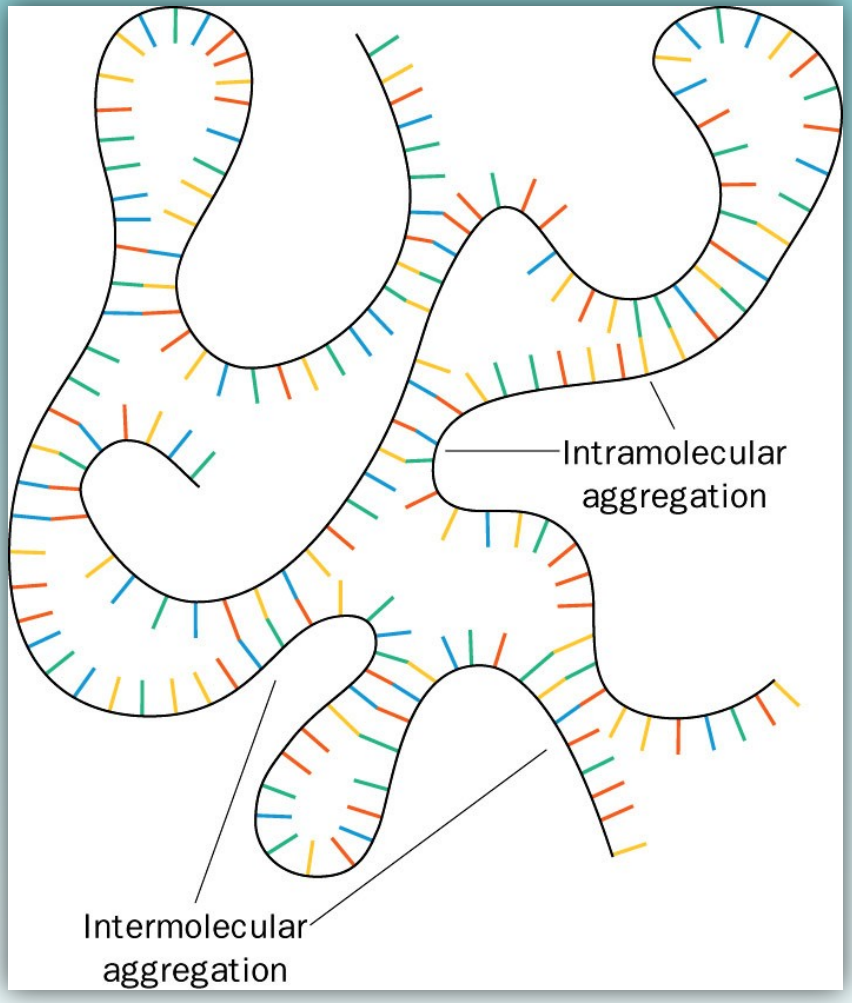
| Dinucleoside Phosphate | $\Delta H_{stacking}$ (kJ · mol$^{-1}$) | $-T\Delta S_{stacking}$ (kJ · mol$^{-1}$ at 25°C) |
|---|---|---|
| ApA | −22.2 | 24.9 |
| ApU | −35.1 | 39.9 |
| GpC | −32.6 | 34.9 |
| CpG | −20.1 | 21.2 |
| UpU | −32.6 | 36.2 |

*Source:* Davis, R.C. and Tinoco, I., Jr., *Biopolymers* **6,** 230 (1968).




**$T_m$ increases linearly with the mole fraction of G-C content (G-C base-pairs are more stable than A-T base pairs due mainly to stronger base stacking interactions, not to increased H-bonding).**

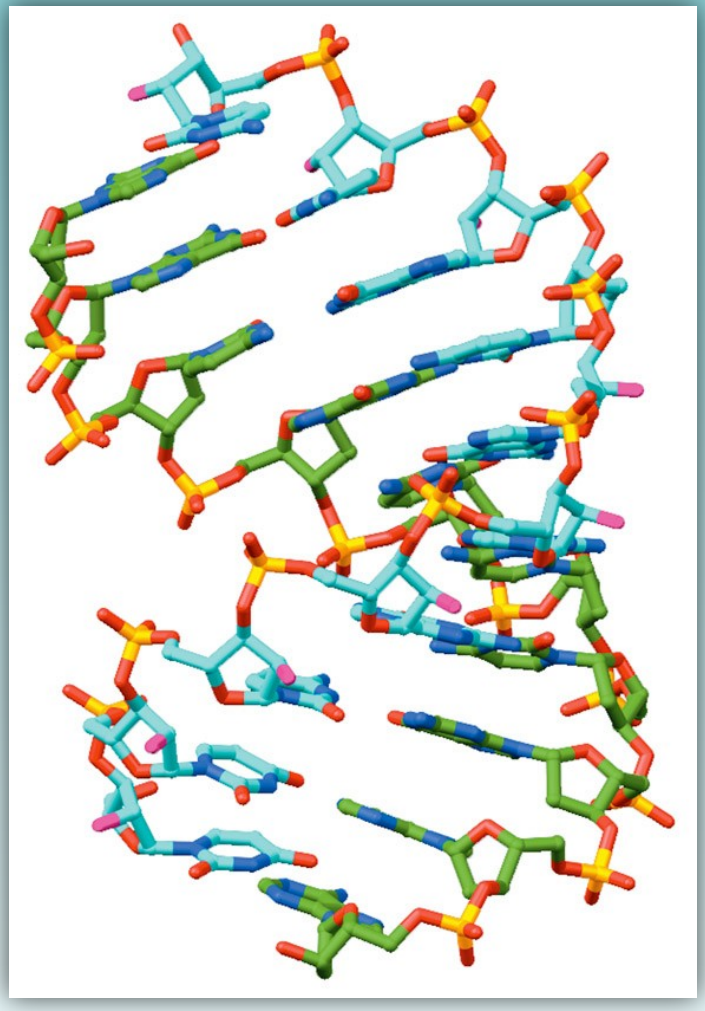
**Variation of the melting temperatures, $T_m$, of various DNAs with their G + C content**




**DNA that has been heat denatured then rapidly cooled to well below its $T_m$**

**Partially renatured DNA**

The RNA strand has an A-DNA-like conformation



X-ray structure of a 10-bp RNA–DNA hybrid helix consisting of d(GGCGCCCGAA) in complex with r(UUCGGGCGCC)

## Table 6–1 Principal Types of RNAs Produced in Cells

| TYPE OF RNA | FUNCTION |
|---|---|
| mRNAs | messenger RNAs, code for proteins |
| rRNAs | ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis |
| tRNAs | transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids |
| snRNAs | small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA |
| snoRNAs | small nucleolar RNAs, used to process and chemically modify rRNAs |
| scaRNAs | small cajal RNAs, used to modify snoRNAs and snRNAs |
| miRNAs | microRNAs, regulate gene expression typically by blocking translation of selective mRNAs |
| siRNAs | small interfering RNAs, turn off gene expression by directing degradation of selective mRNAs and the establishment of compact chromatin structures |
| Other noncoding RNAs | function in diverse cell processes, including telomere synthesis, X-chromosome inactivation, and the transport of proteins into the ER |

Table 6-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

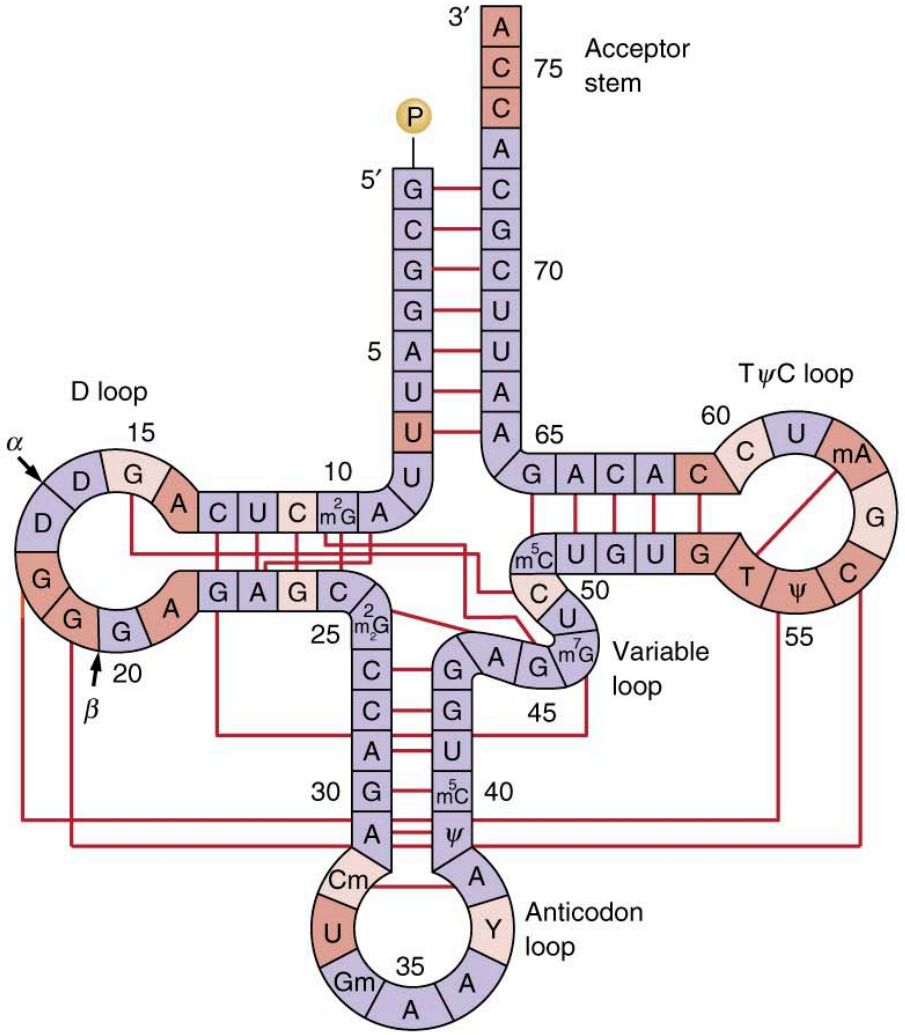
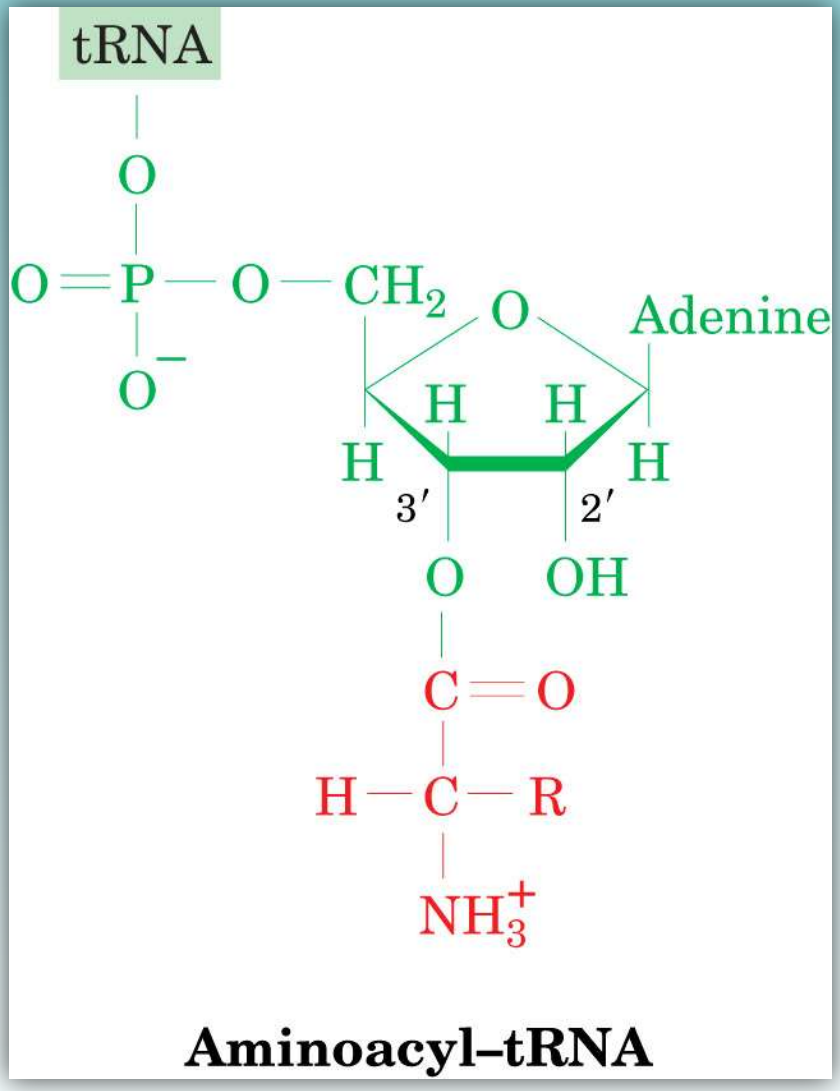
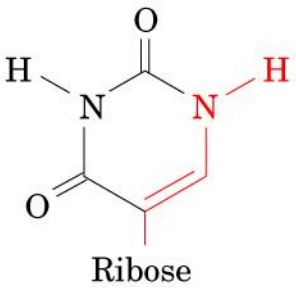



**Figure 2.53. Cloverleaf structure of tRNA.** Redrawn with permission from Quigley, G. J. and Rich, A. *Science* 194:797, 1976. Copyright (1976) AAAS.

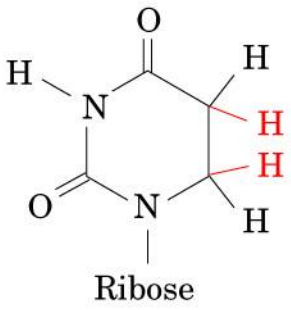



**Aminoacyl–tRNA**

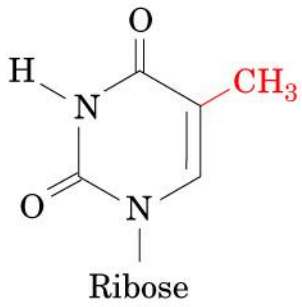
**Uracil derivatives**



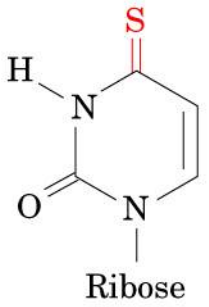

**Pseudouridine (ψ)**




**Dihydrouridine (D)**

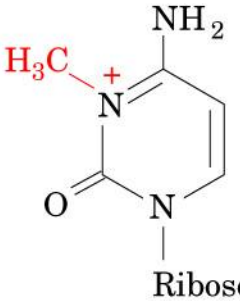



**Ribothymidine (T)**

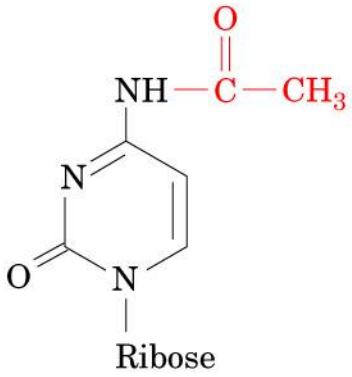



**4-Thiouridine ($s^4U$)**

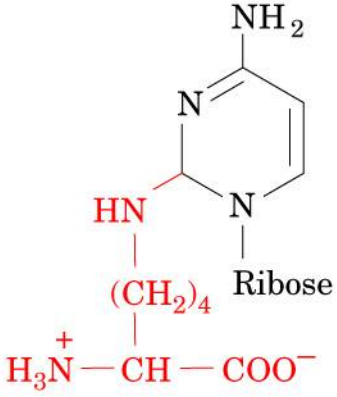
**Cytosine derivatives**




**3-Methylcytidine ($m^3C$)**




***N*⁴-Acetylcytidine ($ac^4C$)**




**Lysidine (L)**

**A selection of the modified nucleosides that occur in tRNAs together with their standard abbreviations.**

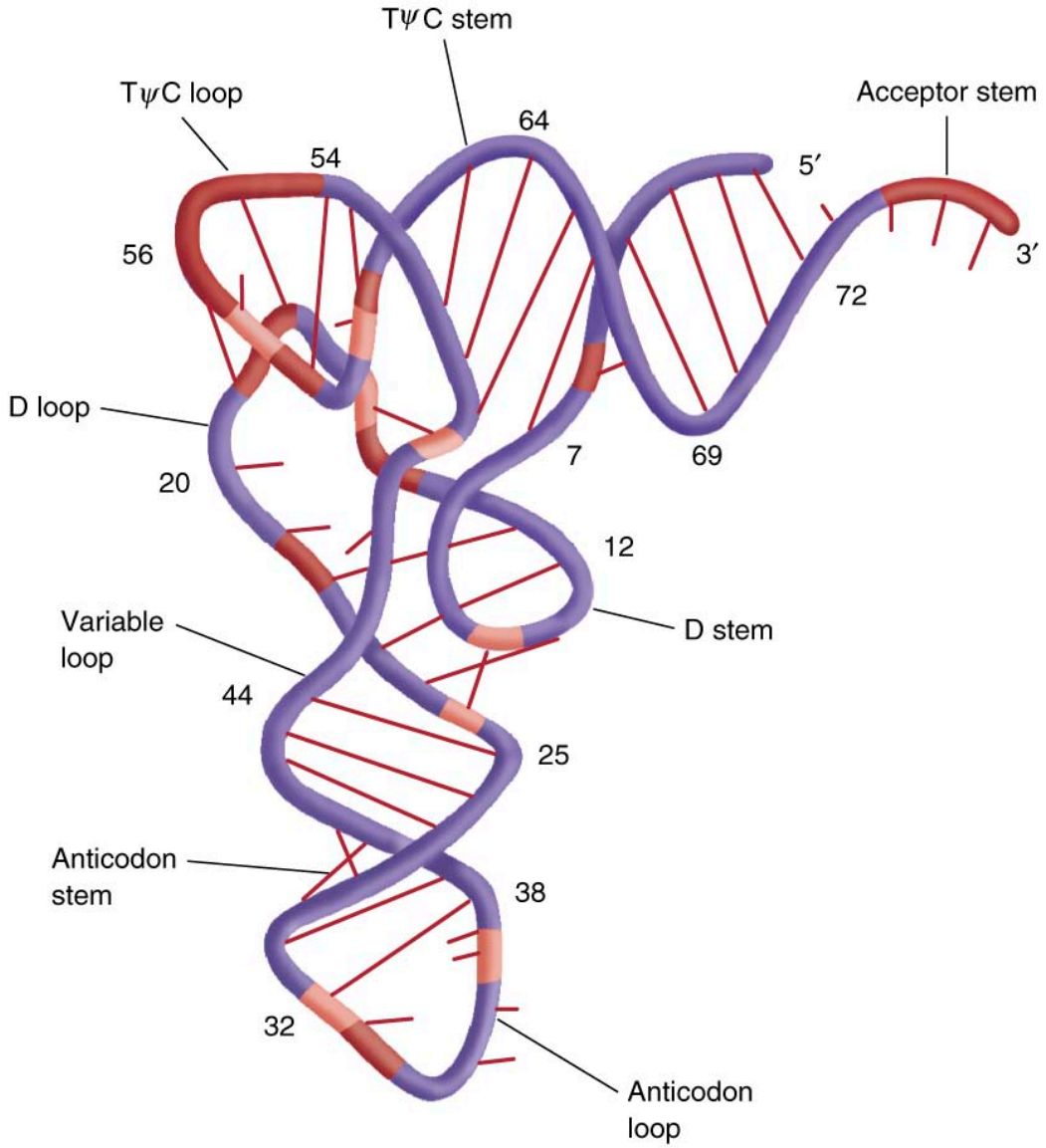



**Figure 2.54. Tertiary structure on tRNA.** Redrawn with permission from Quigley, G. J. and Rich, A. *Science* 194:797, 1976. Copyright (1976) AAAS.

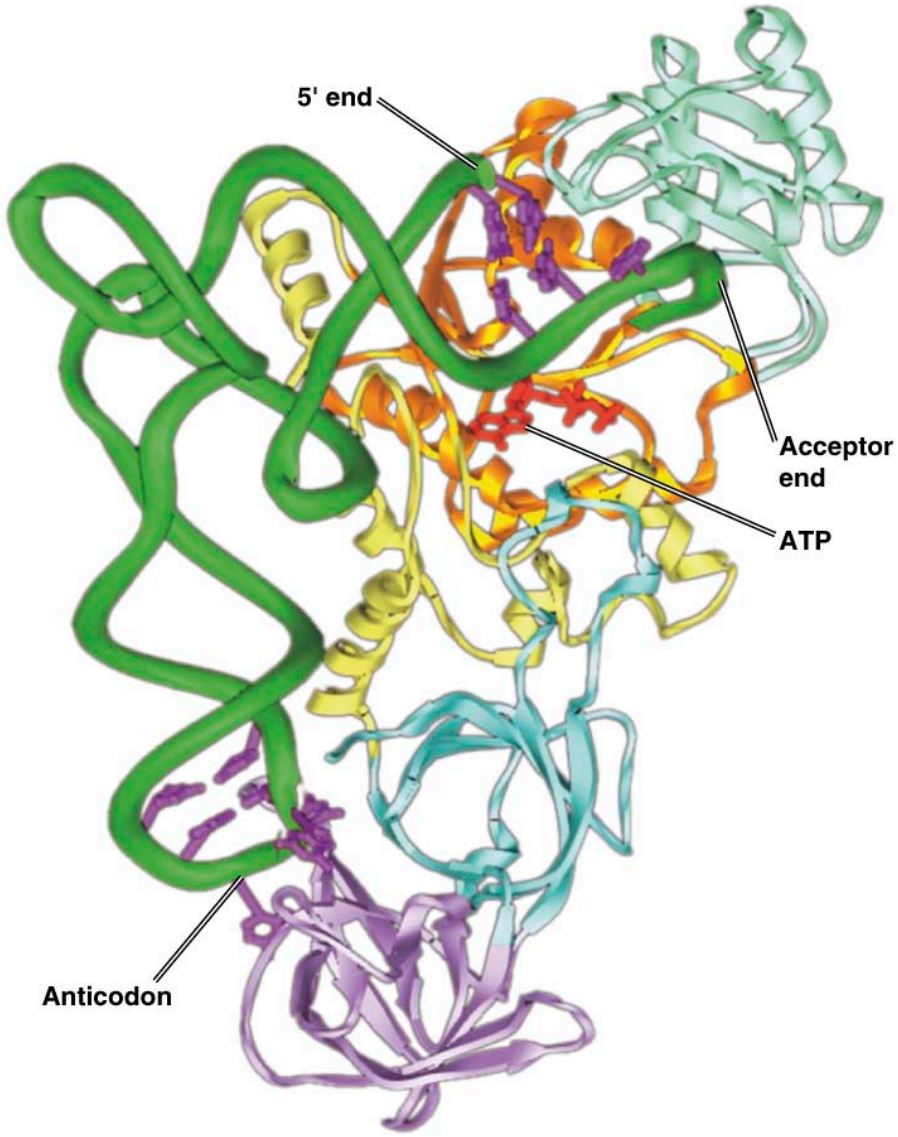



**Figure 6.3. Interaction of a tRNA with its aminoacyl-tRNA synthetase.** Adapted from Perona, J., Rould, M., and Steitz, T. *Biochemistry* 32:8758, 1993.

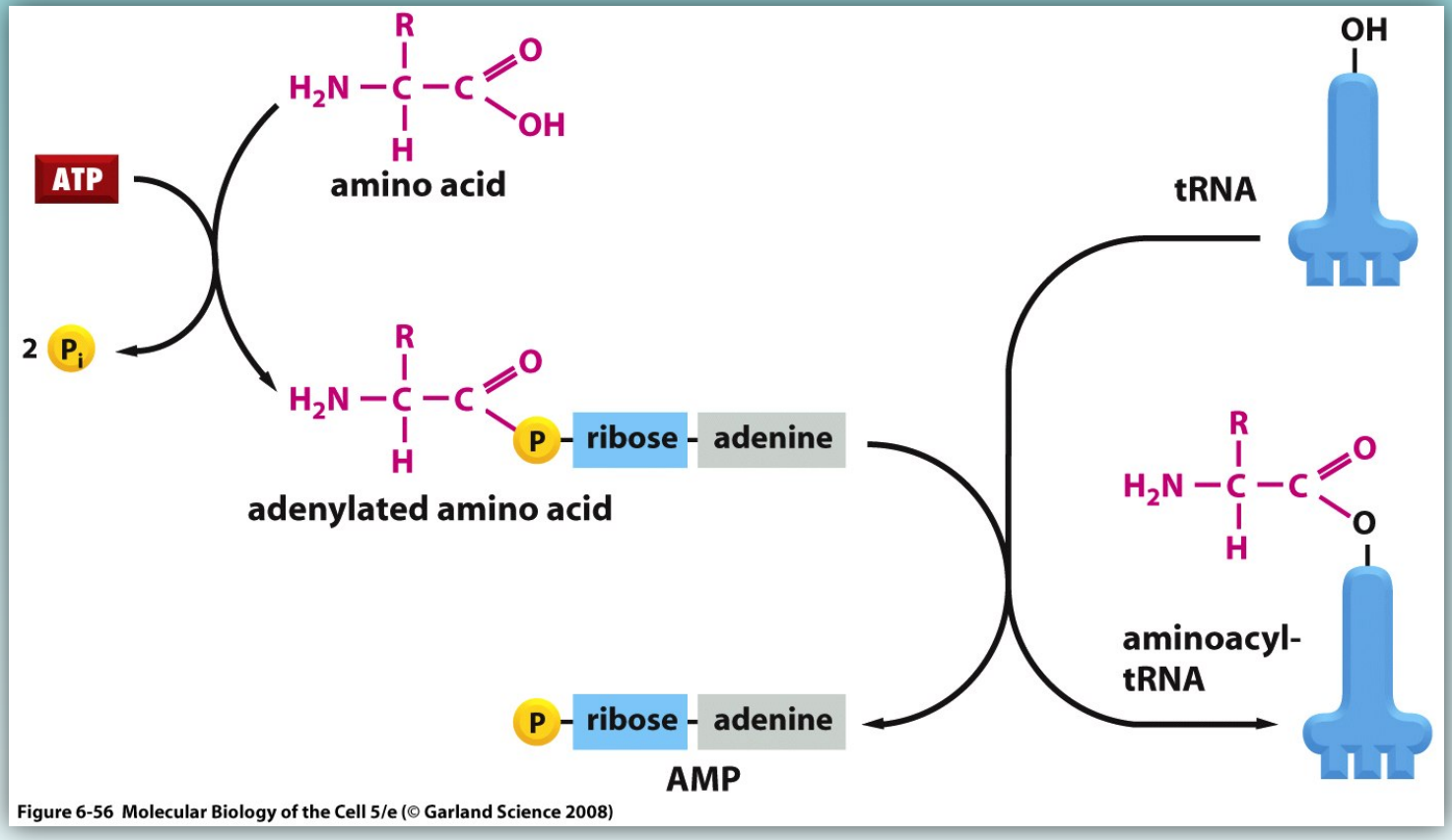



Figure 6-56 Molecular Biology of the Cell 5/e (© Garland Science 2008)

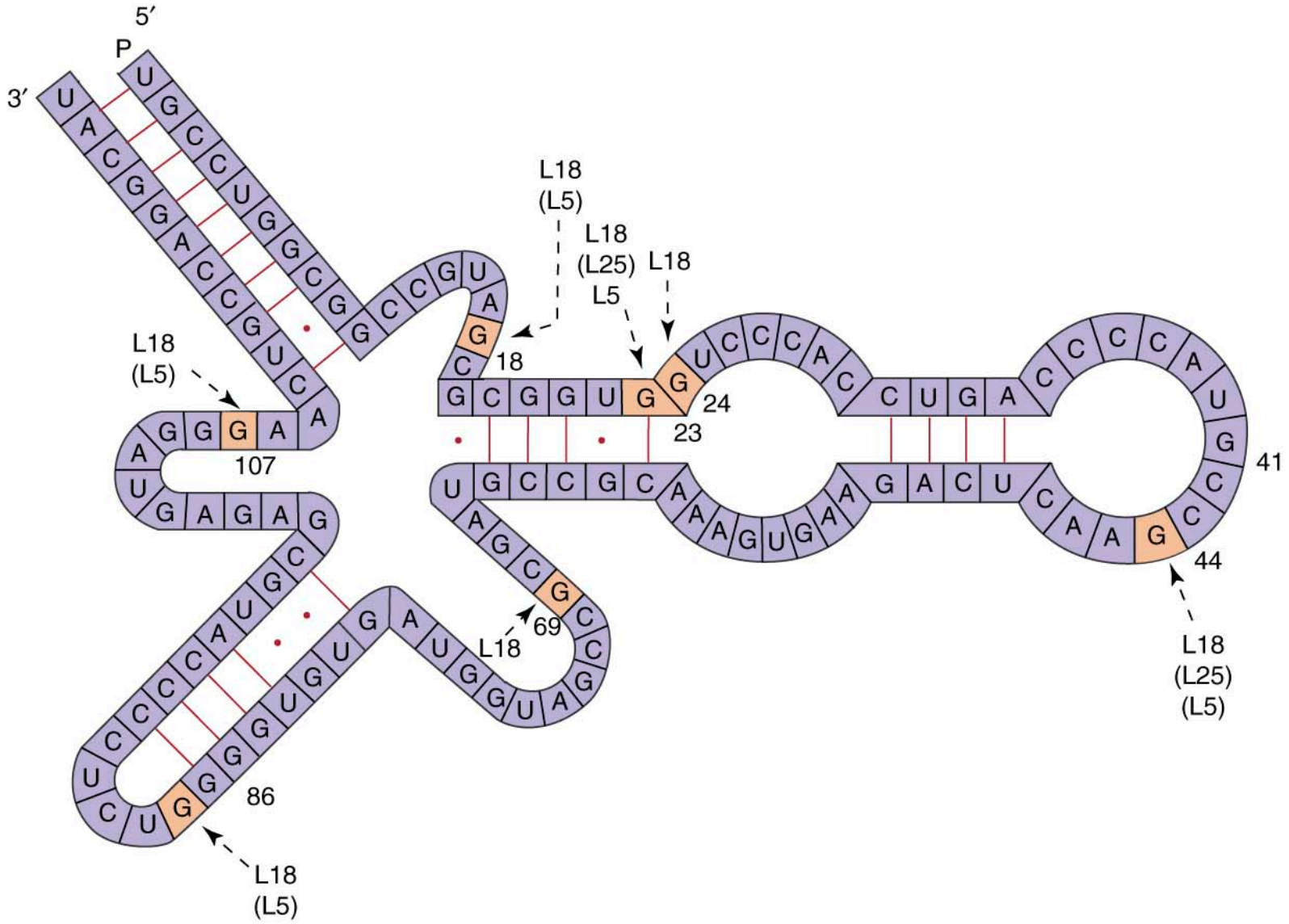
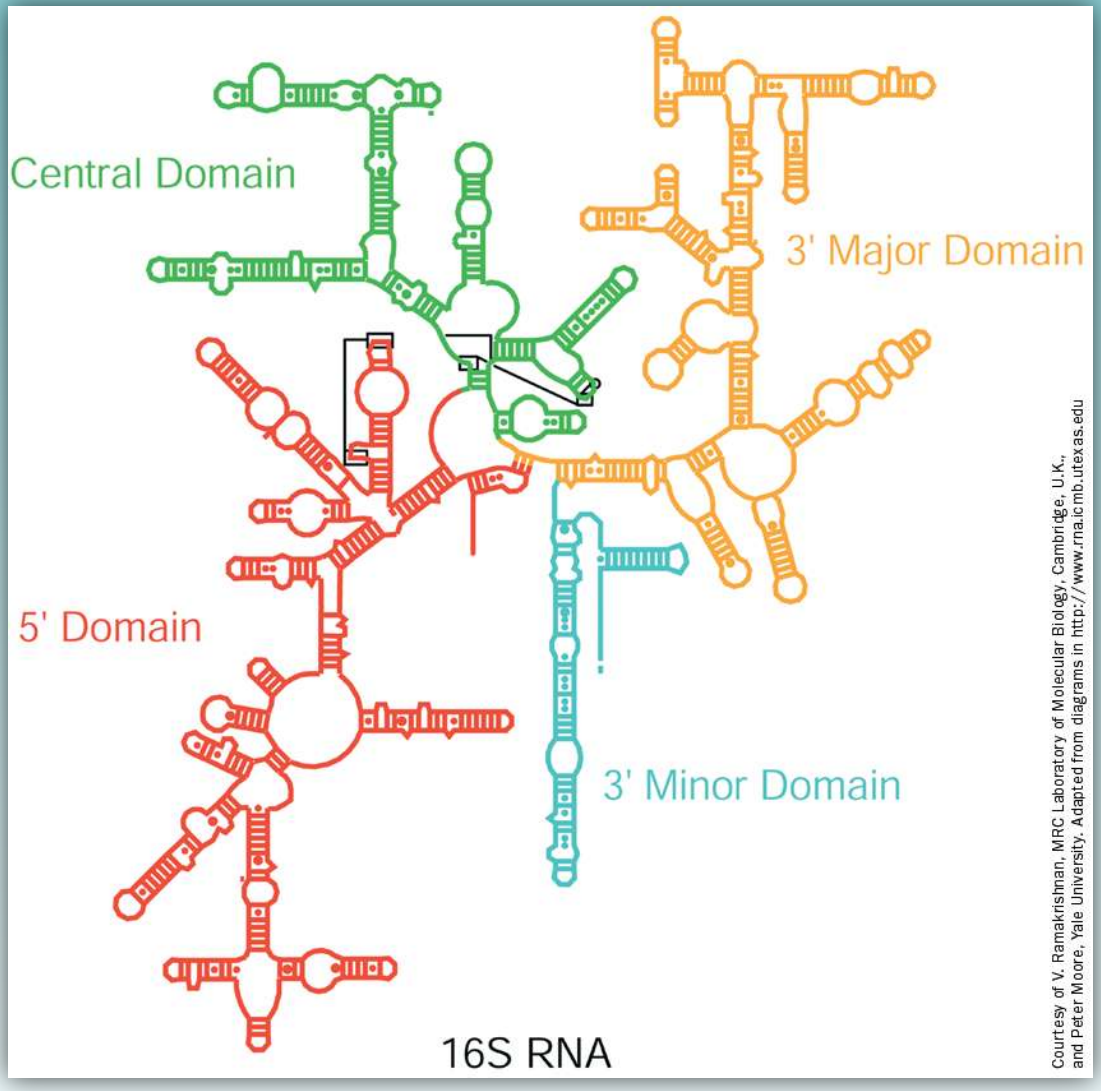
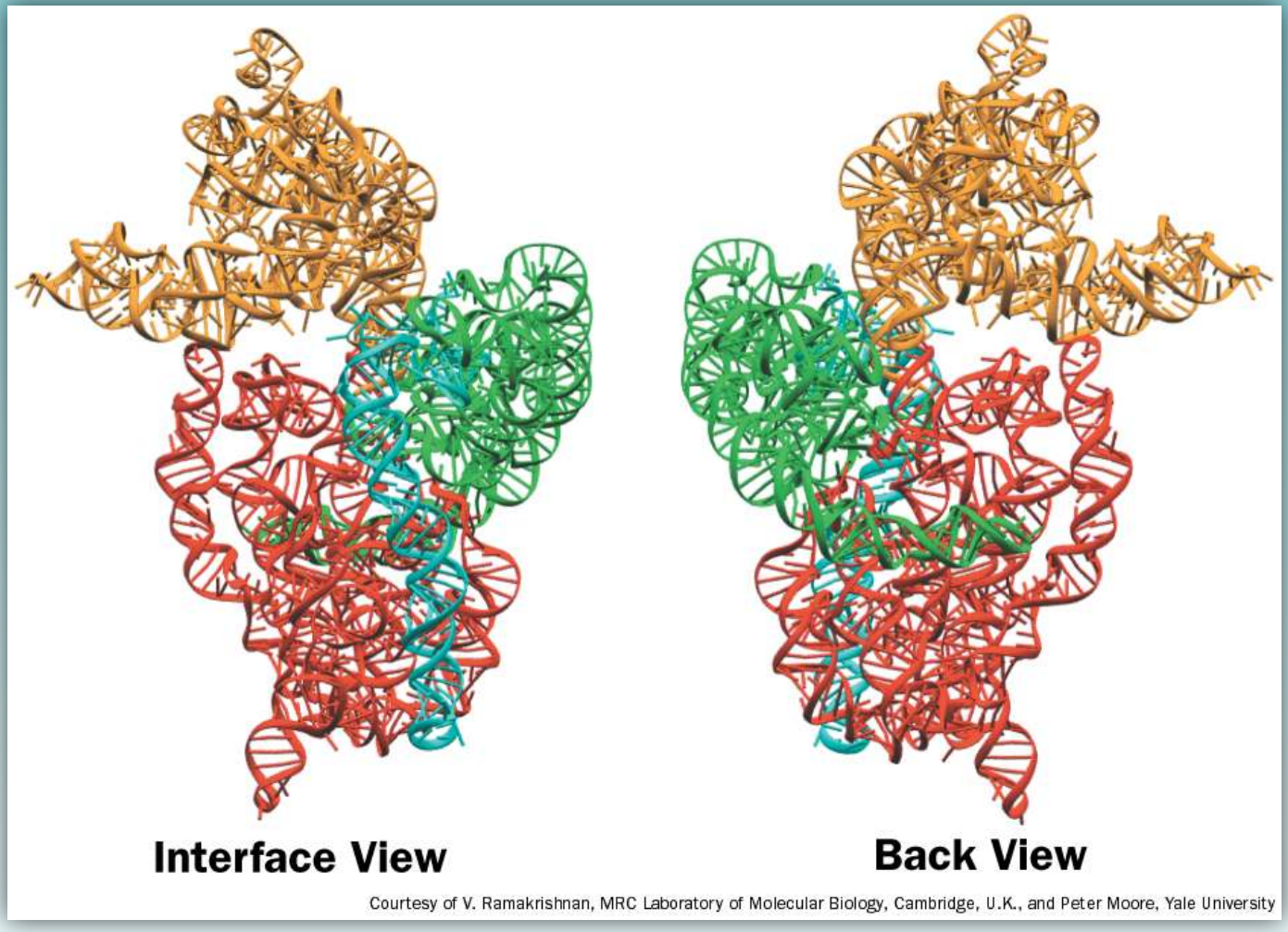



**Figure 2.56. Secondary, base-paired structure proposed for 5S rRNA.** Combined information from Fox, G. E. and Woese, C. R. *Nature (London)* 256:505, 1975, and Gray, P. N.et al. J. Mol. 73:133, 1973.




**Secondary structures of the *E. coli* ribosomal RNAs.**
**16S RNA**




Tertiary structures of the ribosomal RNAs:
The 16S rRNA of *T. thermophilus*

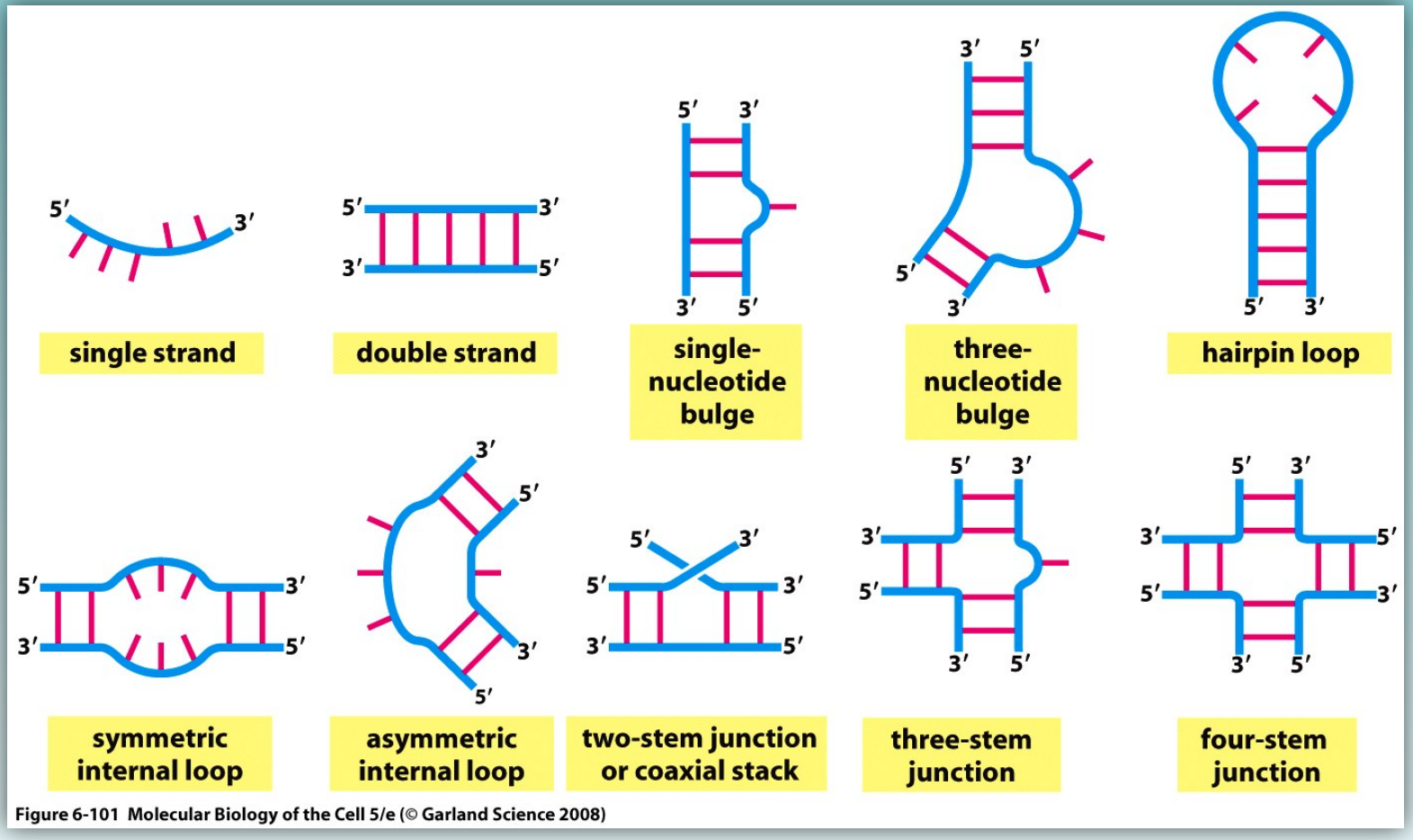
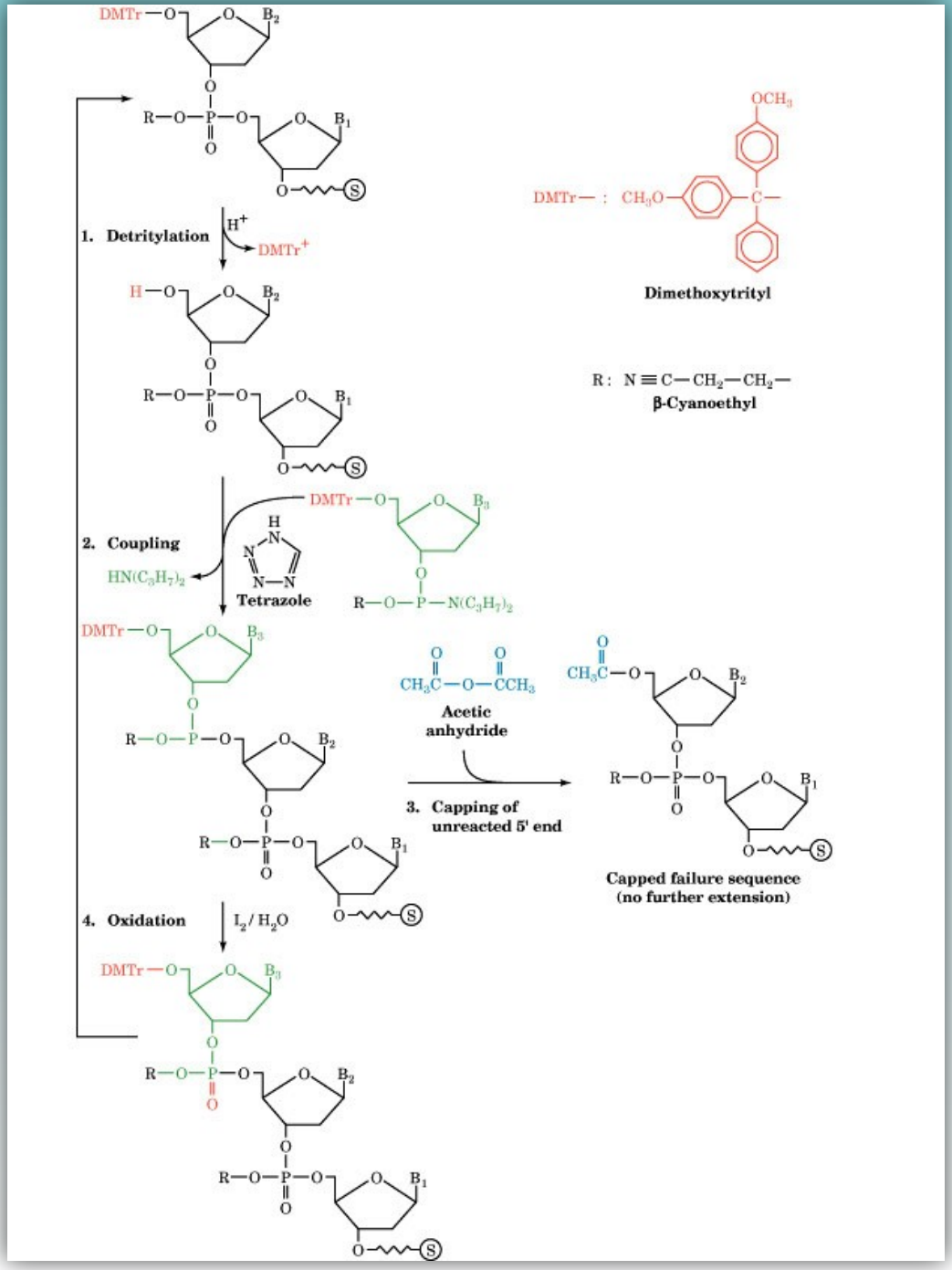



Figure 6-101 Molecular Biology of the Cell 5/e (© Garland Science 2008)

# Common elements of RNA secondary structure




Reaction cycle in the phosphoramidite method of solid-phase oligodeoxyribonucleotide chemical synthesis. Synthesis direction is 3 ' to 5 ' .