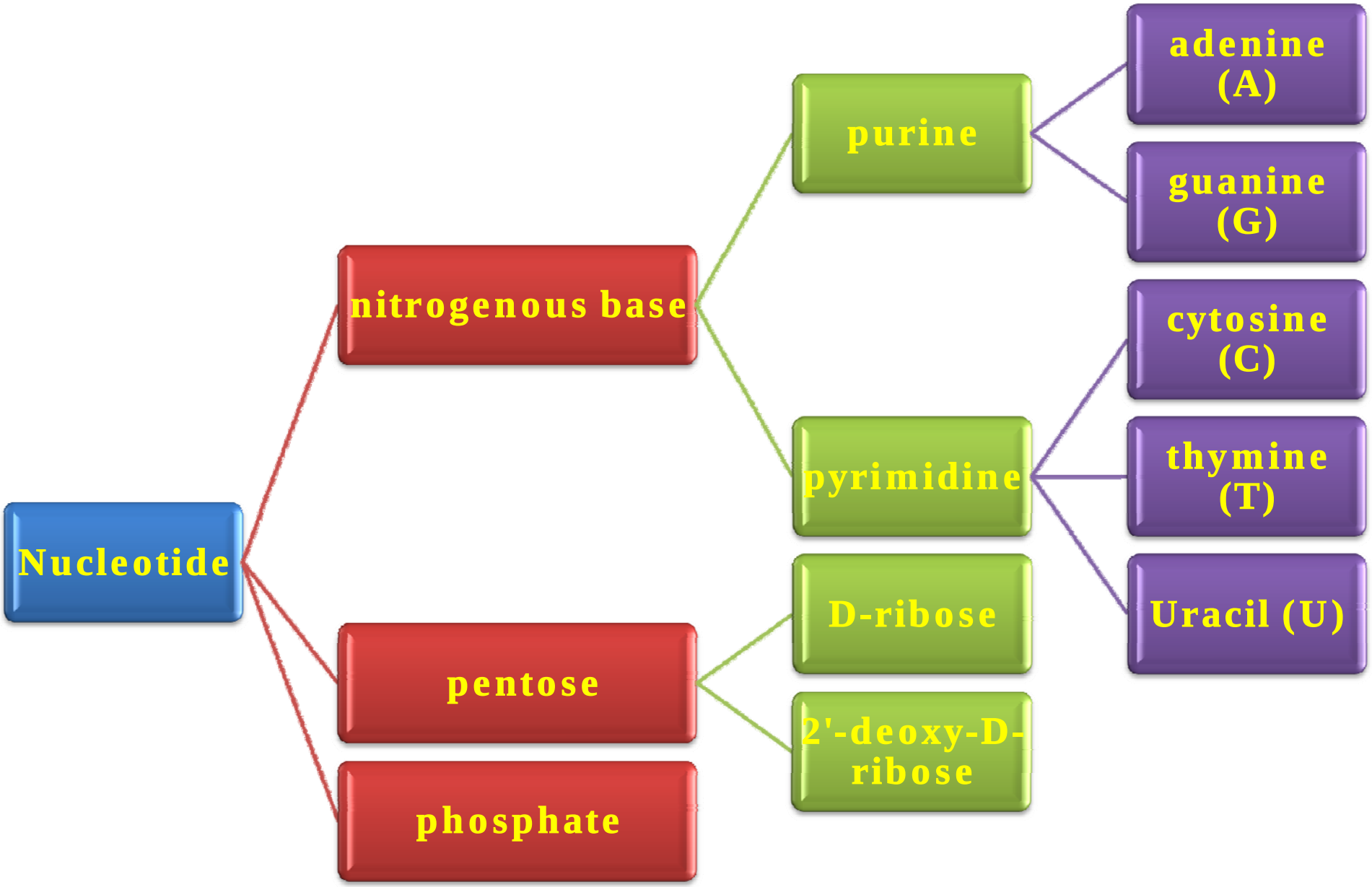
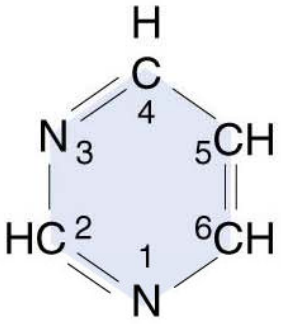
# NUCLEOTIDES AND NUCLEIC ACIDS

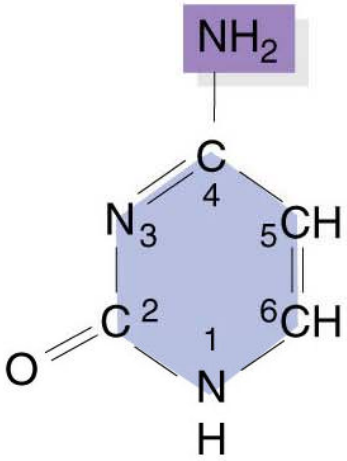



**Nucleotide = Nitrogenous base + pentose + phosphate**
**Nucleoside = Nitrogenous base + pentose**

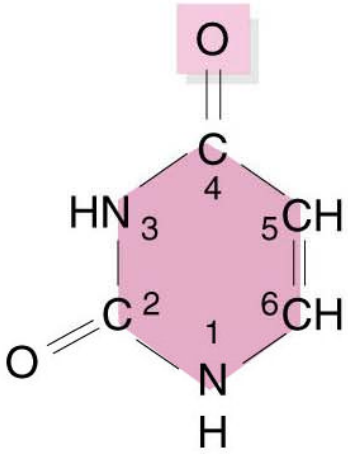
## Type of Nitrogenous base



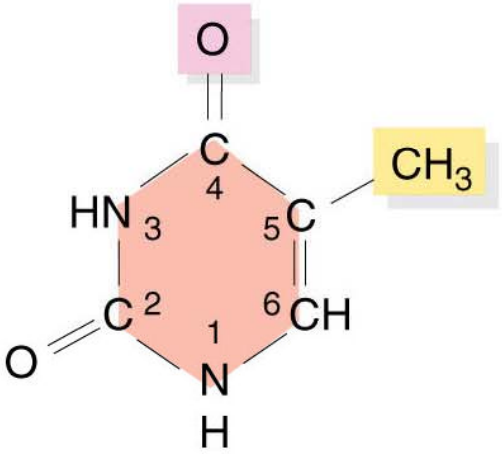
Pyrimidine



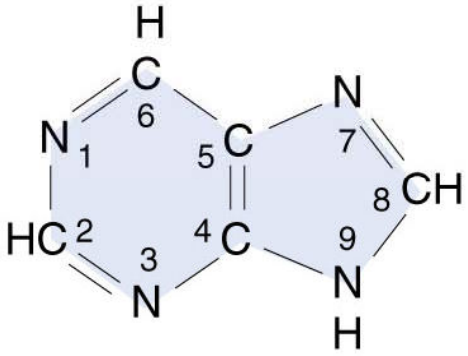
Cytosine (C)



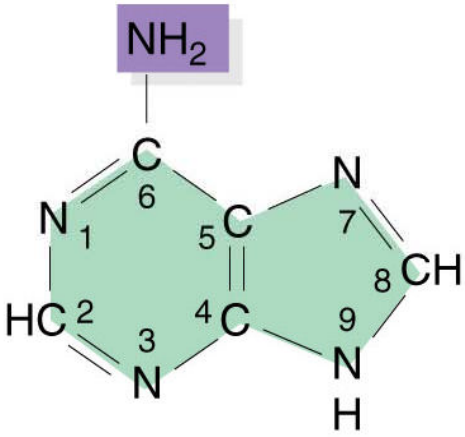
Uracil (U)
(found in RNA)



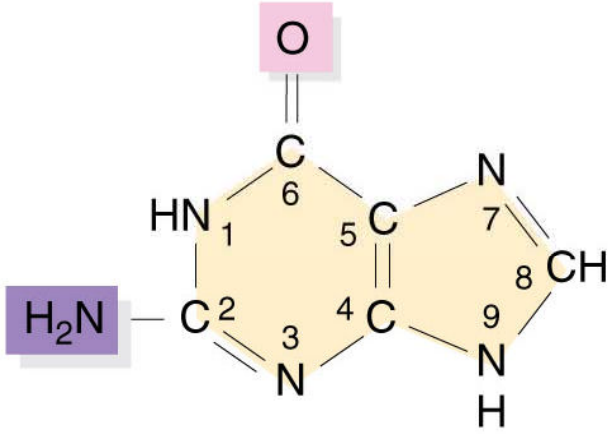
Thymine (T)
(found in DNA)



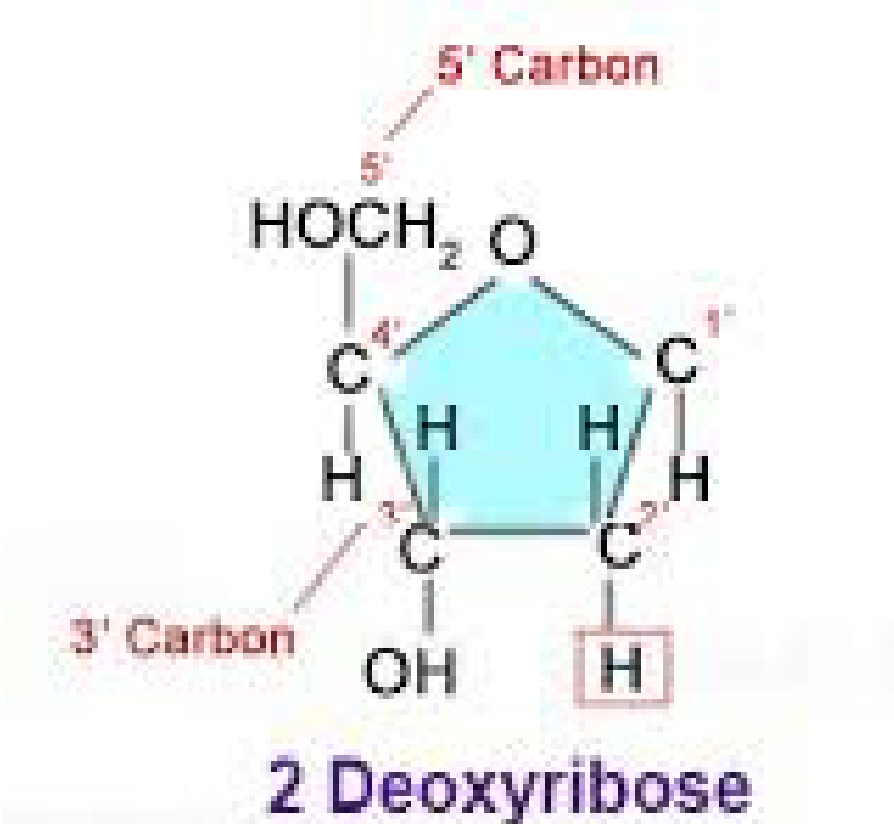
Purine



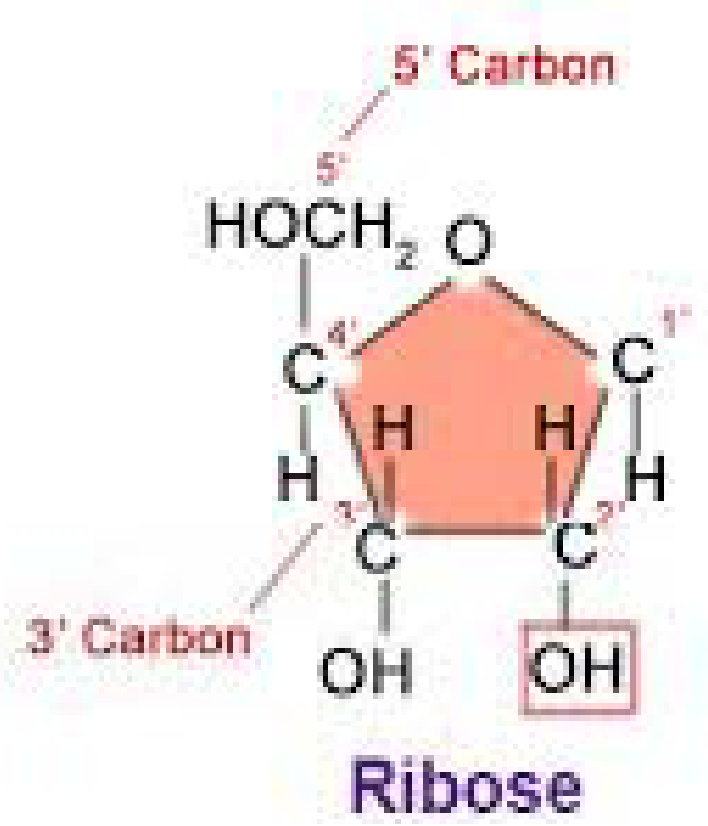
Adenine (A)



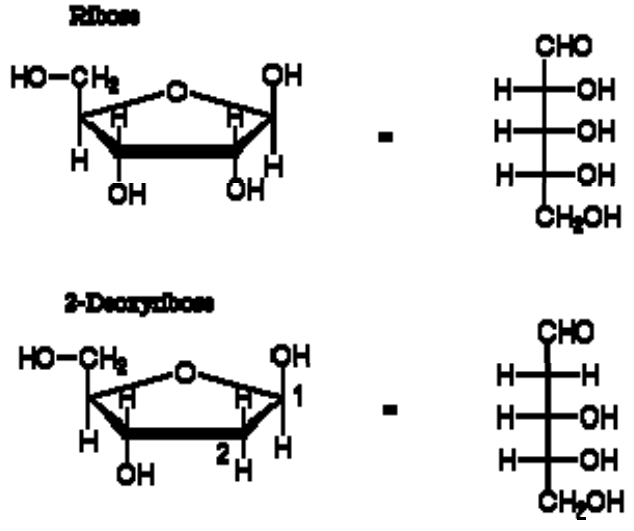
Guanine (G)

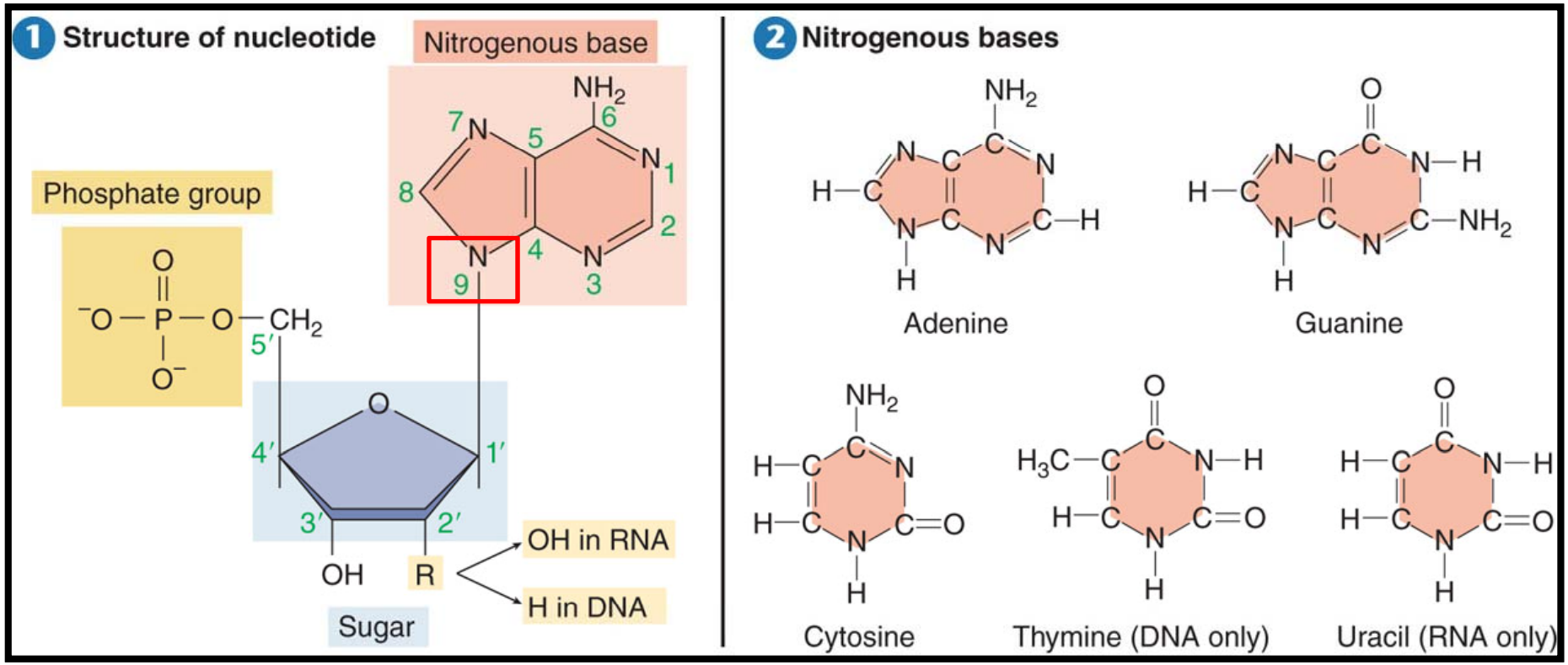


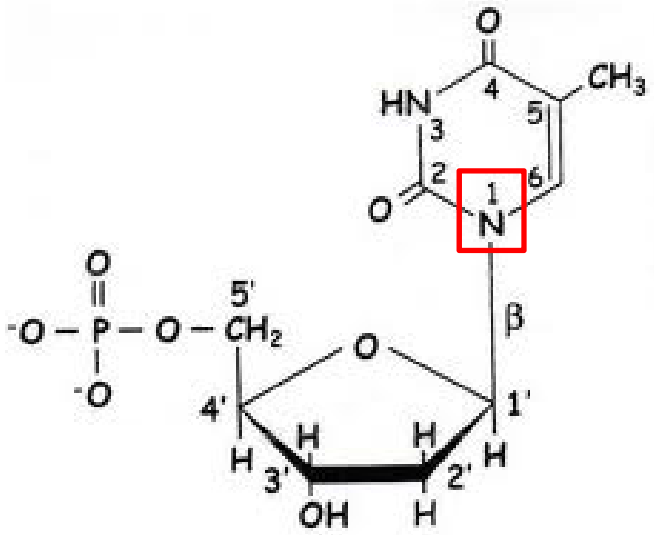

2 Deoxyribose

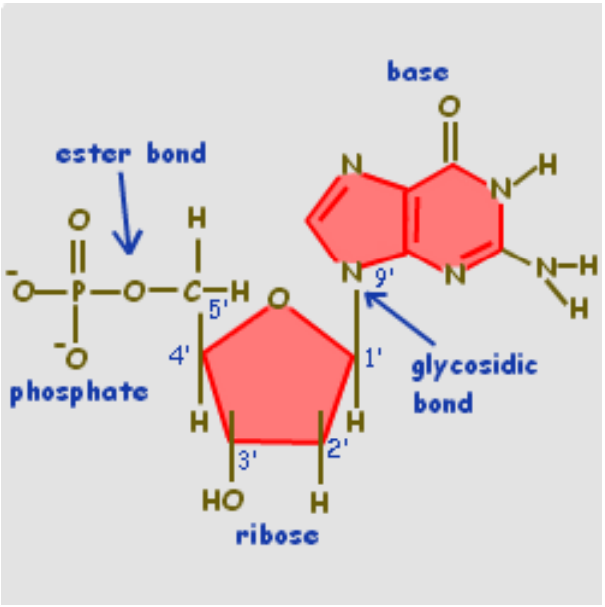


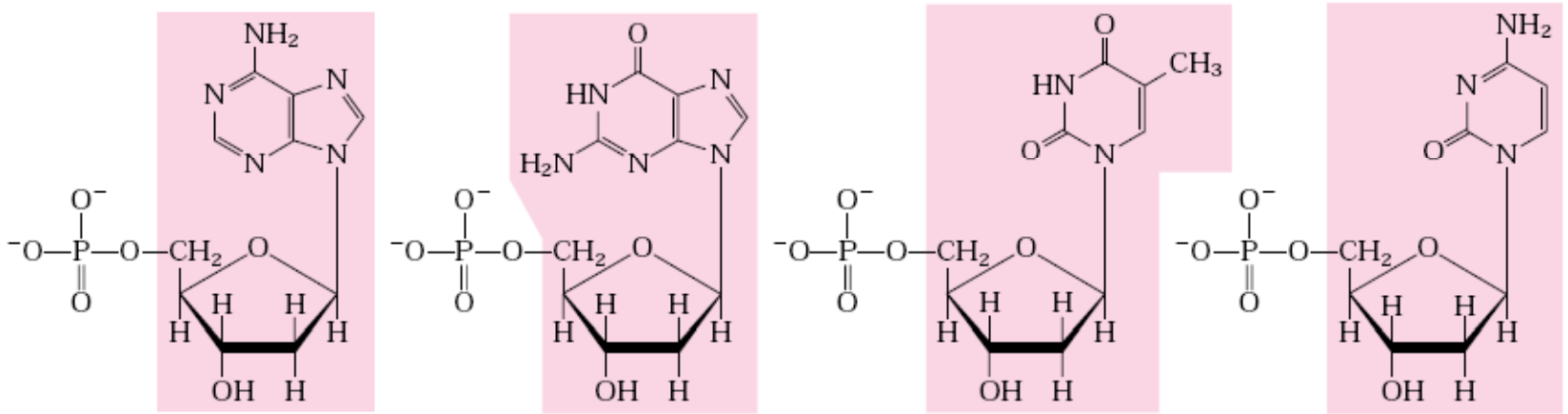

Ribose




1 Structure of nucleotide
Nitrogenous base
Phosphate group
Sugar
OH in RNA
H in DNA
2 Nitrogenous bases
Adenine
Guanine
Cytosine
Thymine (DNA only)
Uracil (RNA only)

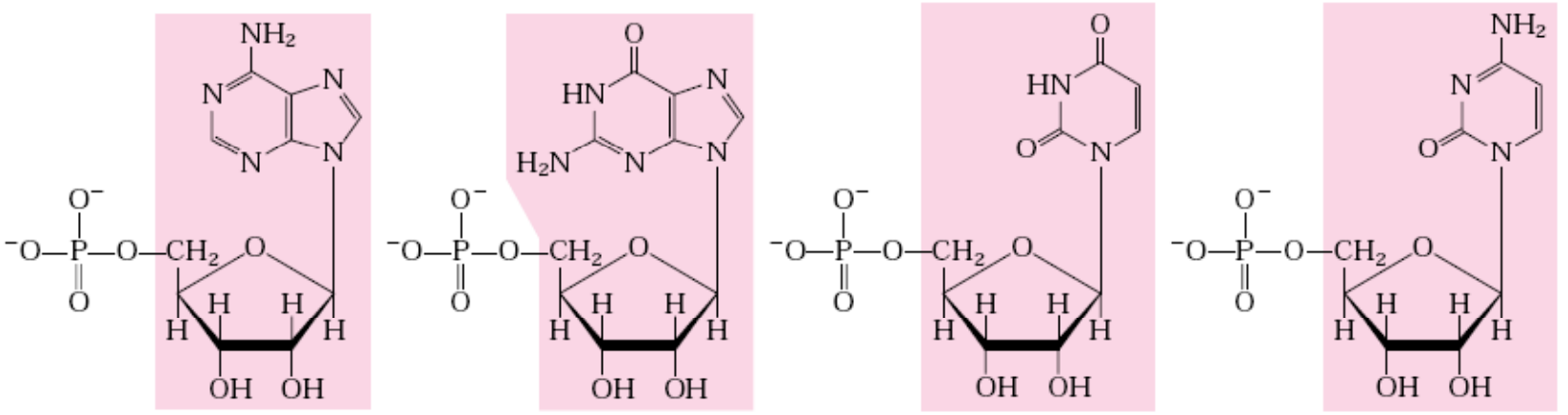



base
ester bond
phosphate
glycosidic bond
ribose



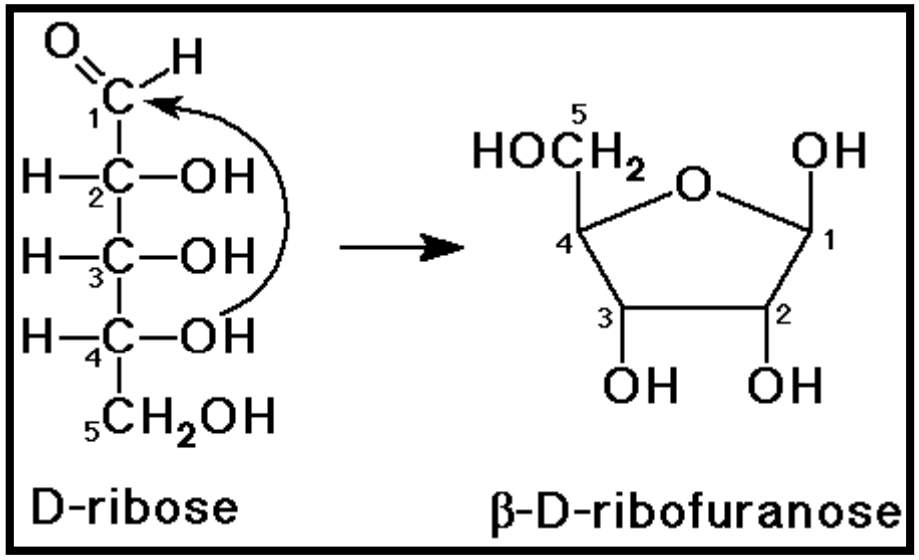
| | | | | |
|---|---|---|---|---|
| **Nucleotide:** | Deoxyadenylate (deoxyadenosine 5′-monophosphate) | Deoxyguanylate (deoxyguanosine 5′-monophosphate) | Deoxythymidylate (deoxythymidine 5′-monophosphate) | Deoxycytidylate (deoxycytidine 5′-monophosphate) |
| **Symbols:** | A, dA, dAMP | G, dG, dGMP | T, dT, dTMP | C, dC, dCMP |
| **Nucleoside:** | Deoxyadenosine | Deoxyguanosine | Deoxythymidine | Deoxycytidine |

**(a) Deoxyribonucleotides**



| | | | | |
|---|---|---|---|---|
| **Nucleotide:** | Adenylate (adenosine 5′-monophosphate) | Guanylate (guanosine 5′-monophosphate) | Uridylate (uridine 5′-monophosphate) | Cytidylate (cytidine 5′-monophosphate) |
| **Symbols:** | A, AMP | G, GMP | U, UMP | C, CMP |
| **Nucleoside:** | Adenosine | Guanosine | Uridine | Cytidine |

**(b) Ribonucleotides**

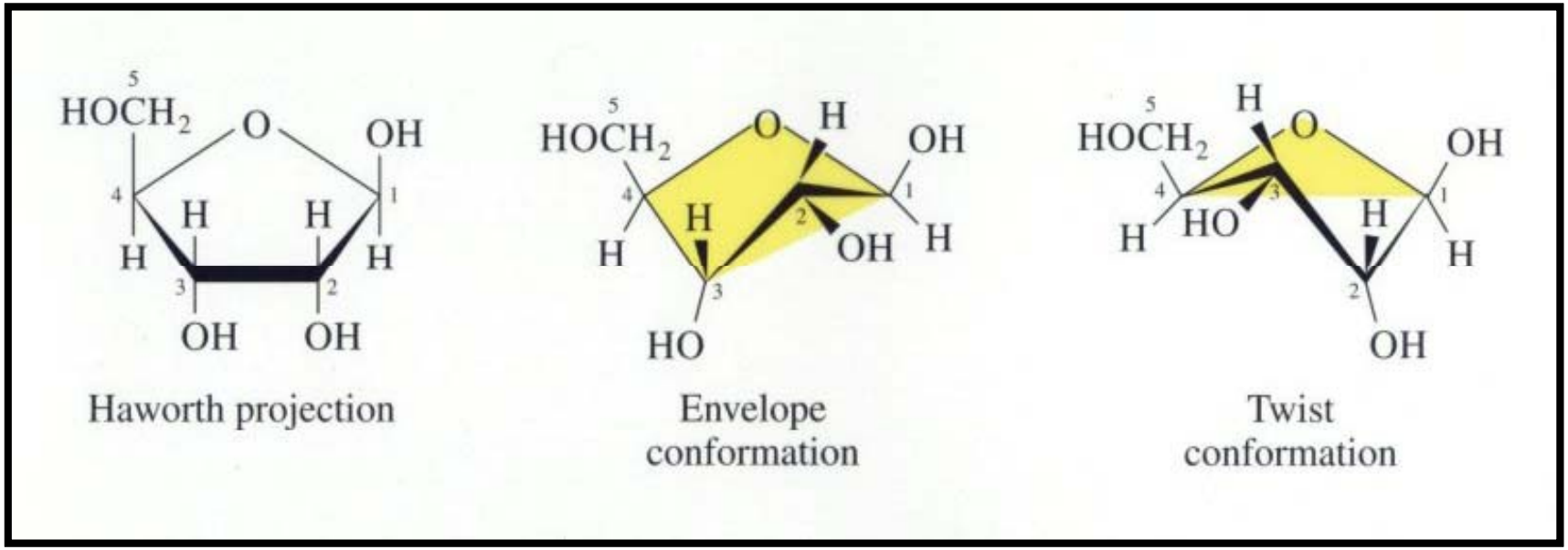


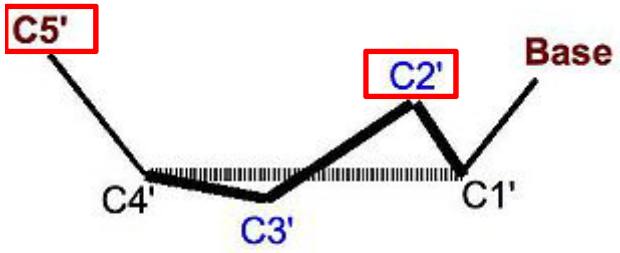

**In solution**, the straightchain (aldehyde) and ring (β-D-furanose) forms of **free** ribose are in equilibrium.

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

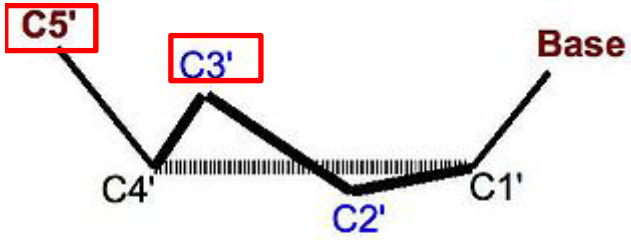
**In DNA**, exists solely as 2'-β-D-deoxyribofuranose.

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

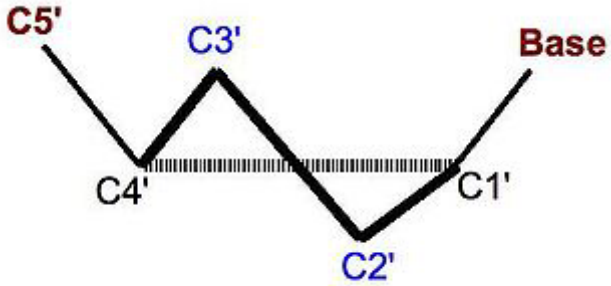




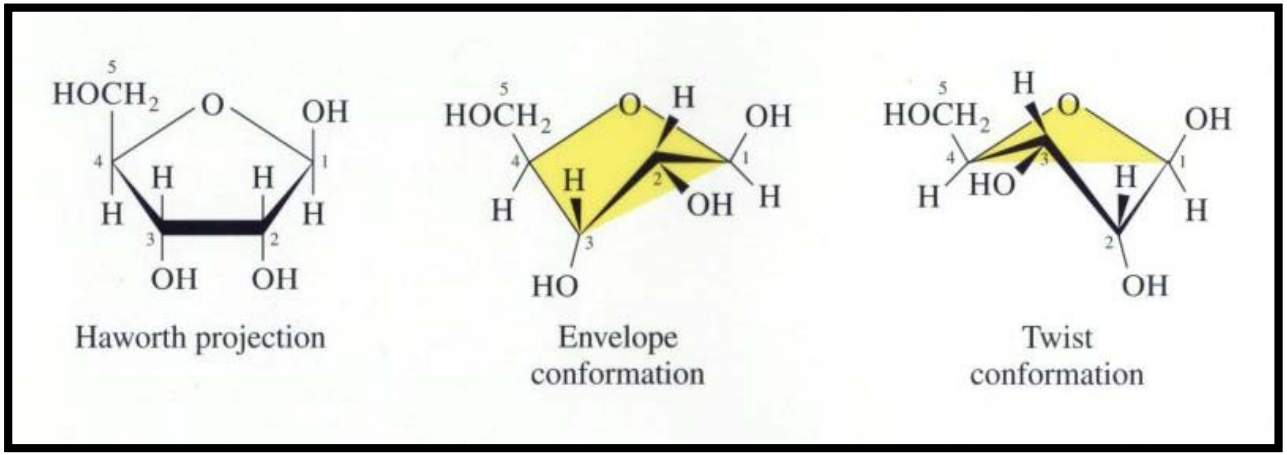

*E C2'-endo*

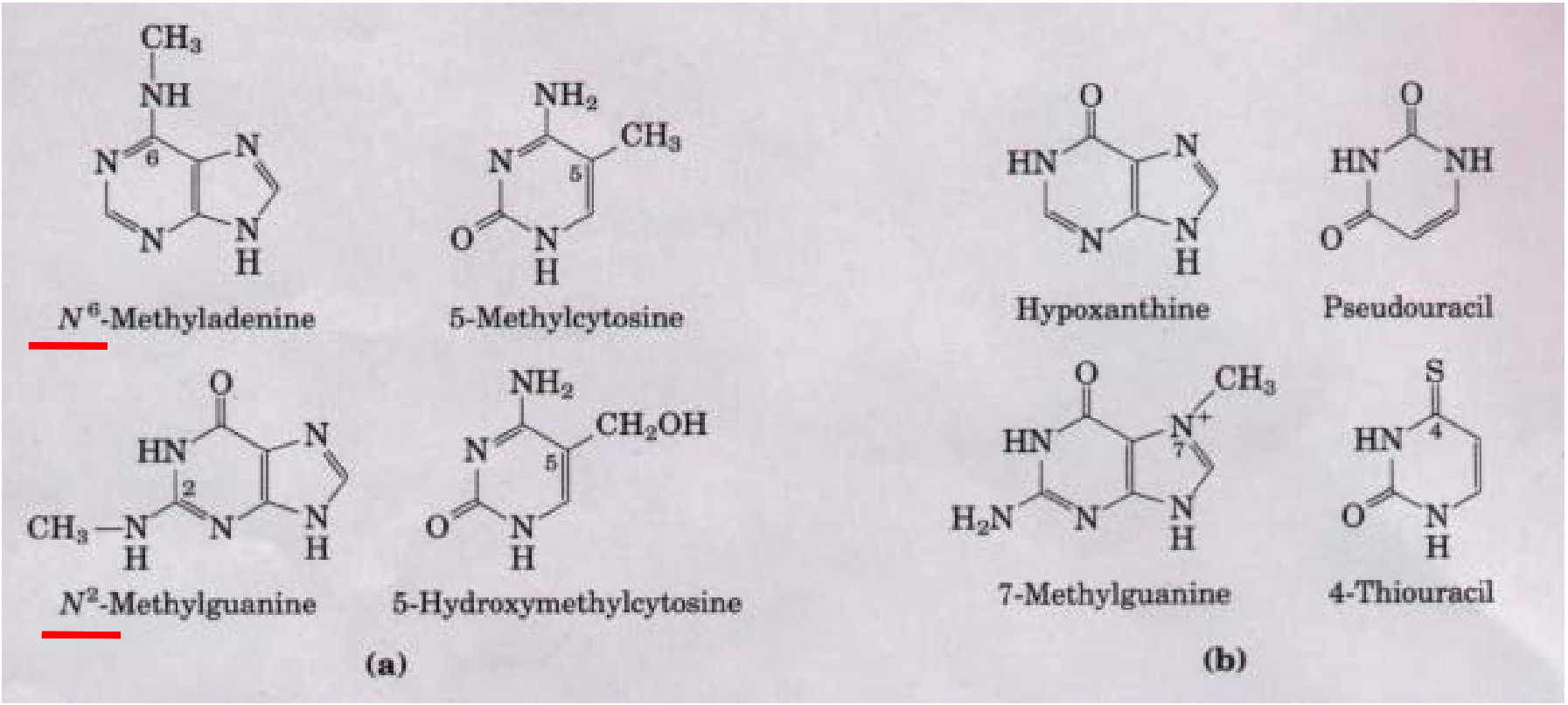



*E C3'-endo*




*T C2'-exo-C3'-endo*

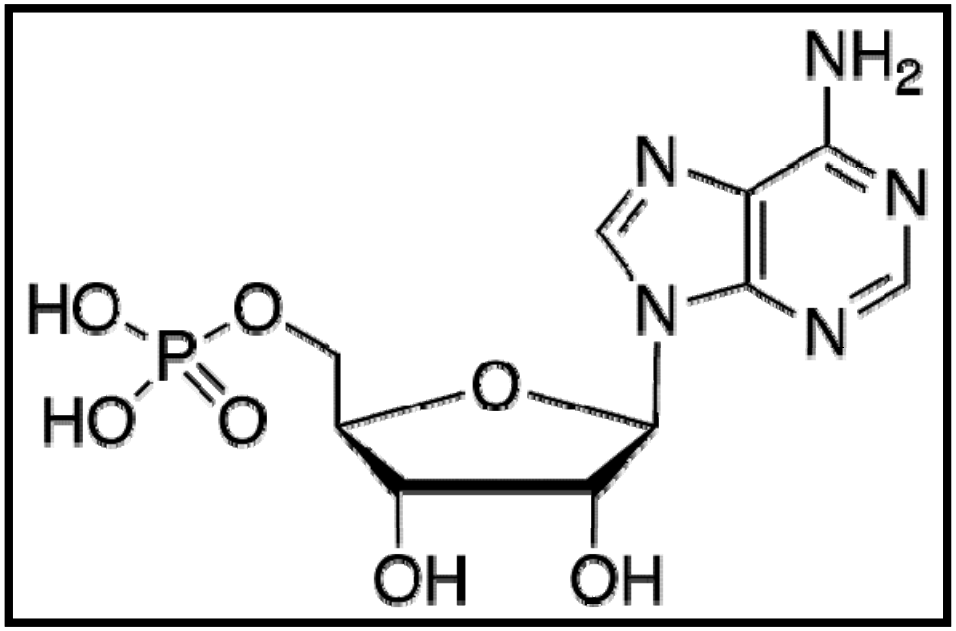





**(a) Minor bases of DNA**

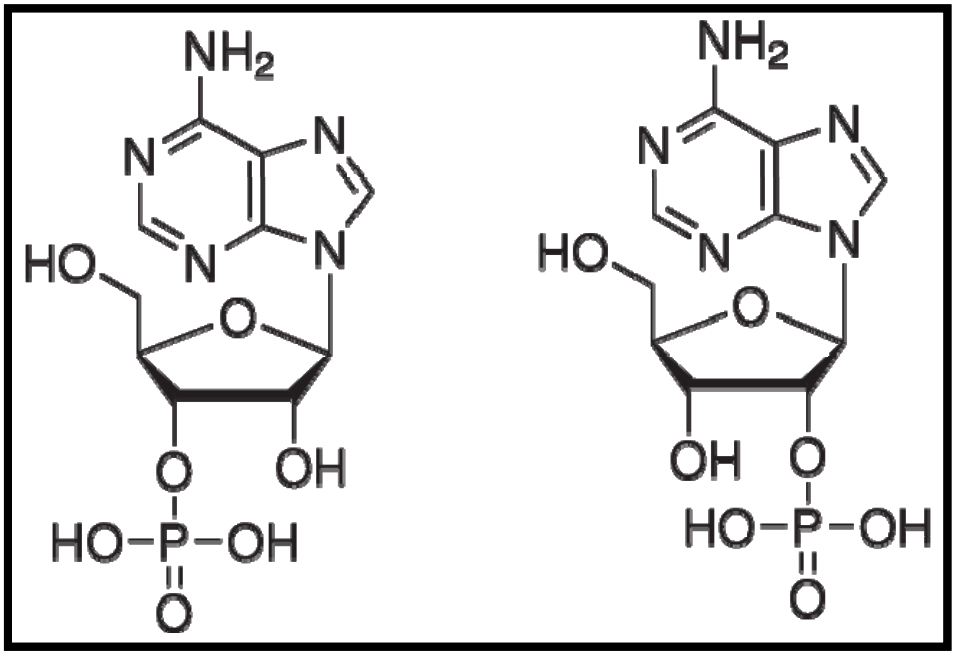
- 5-Methylcytidine occurs in the DNA of animals and higher plants,
- *N6-methyladenosine in bacterial* DNA,
- 5-hydroxymethylcytidine in the DNA of bacteria infected with certain bacteriophages.
- N2-methylguanine is present in a wide variety of RNAs

**(b) Some minor bases of tRNAs**




**Adenosine 5‘ monophosphate**

*Cells also contain nucleotides with phosphate groups in positions other than on the 5' carbon.*




**Adenosine 3‘ monophosphate**
(end products of the hydrolysis of RNA by certain ribonucleases)

**Adenosine 2‘ monophosphate**

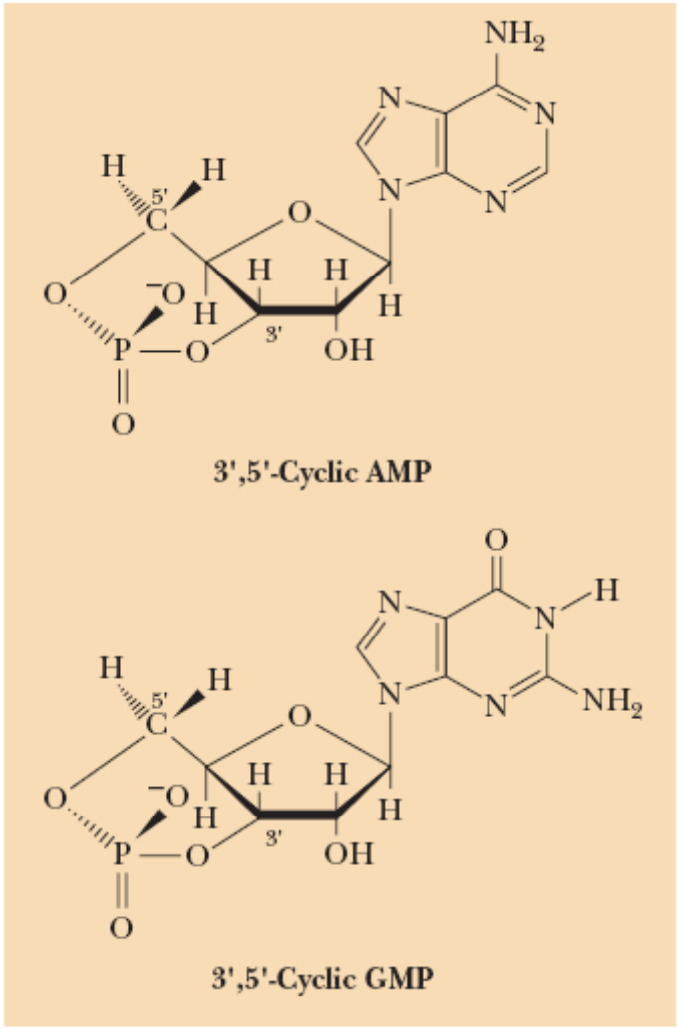
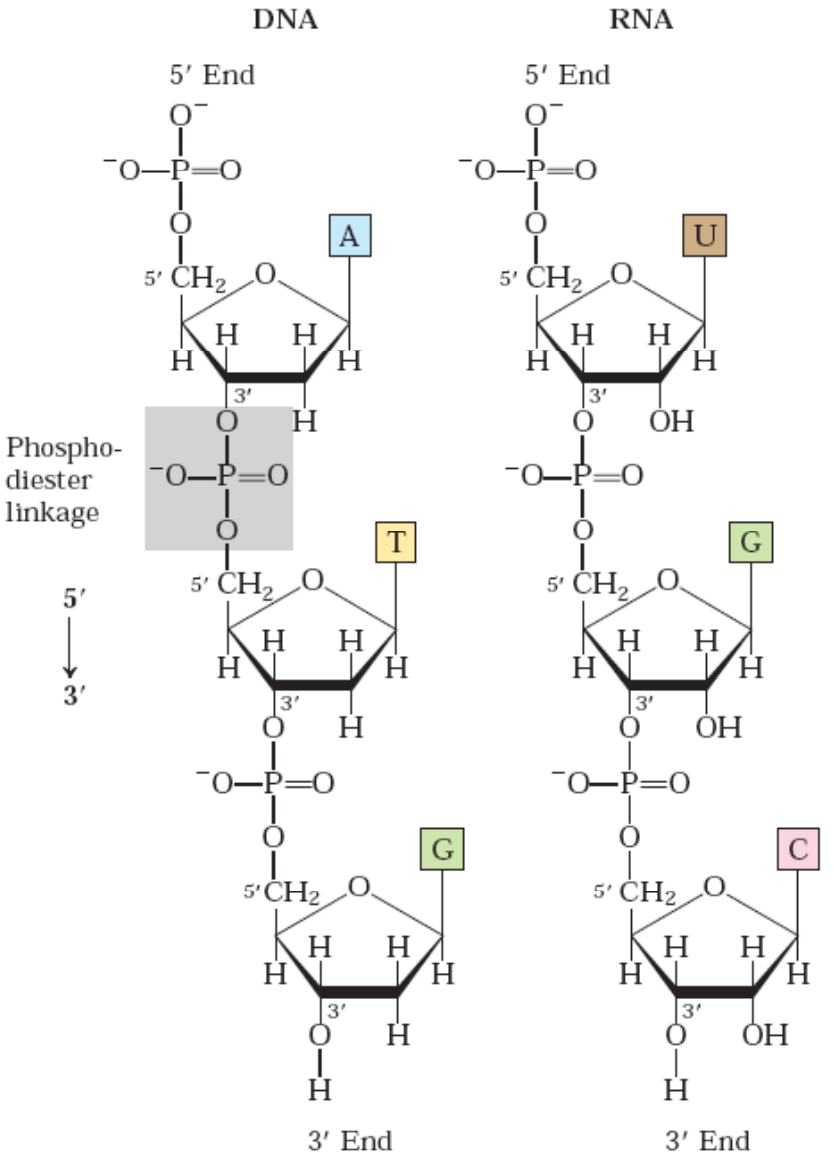



**FIGURE 10.12** The cyclic nucleotides cAMP and cGMP.



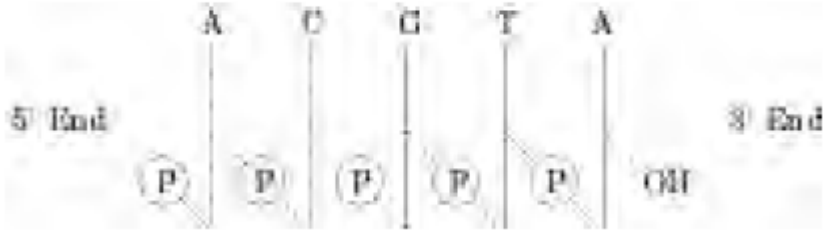




**FIGURE 8–7 Phosphodiester linkages in the covalent backbone of DNA and RNA.** The phosphodiester bonds (one of which is shaded in the DNA) link successive nucleotide units. The backbone of alternating pentose and phosphate groups in both types of nucleic acid is highly polar. The 5′ end of the macromolecule lacks a nucleotide at the 5′ position, and the 3′ end lacks a nucleotide at the 3′ position.

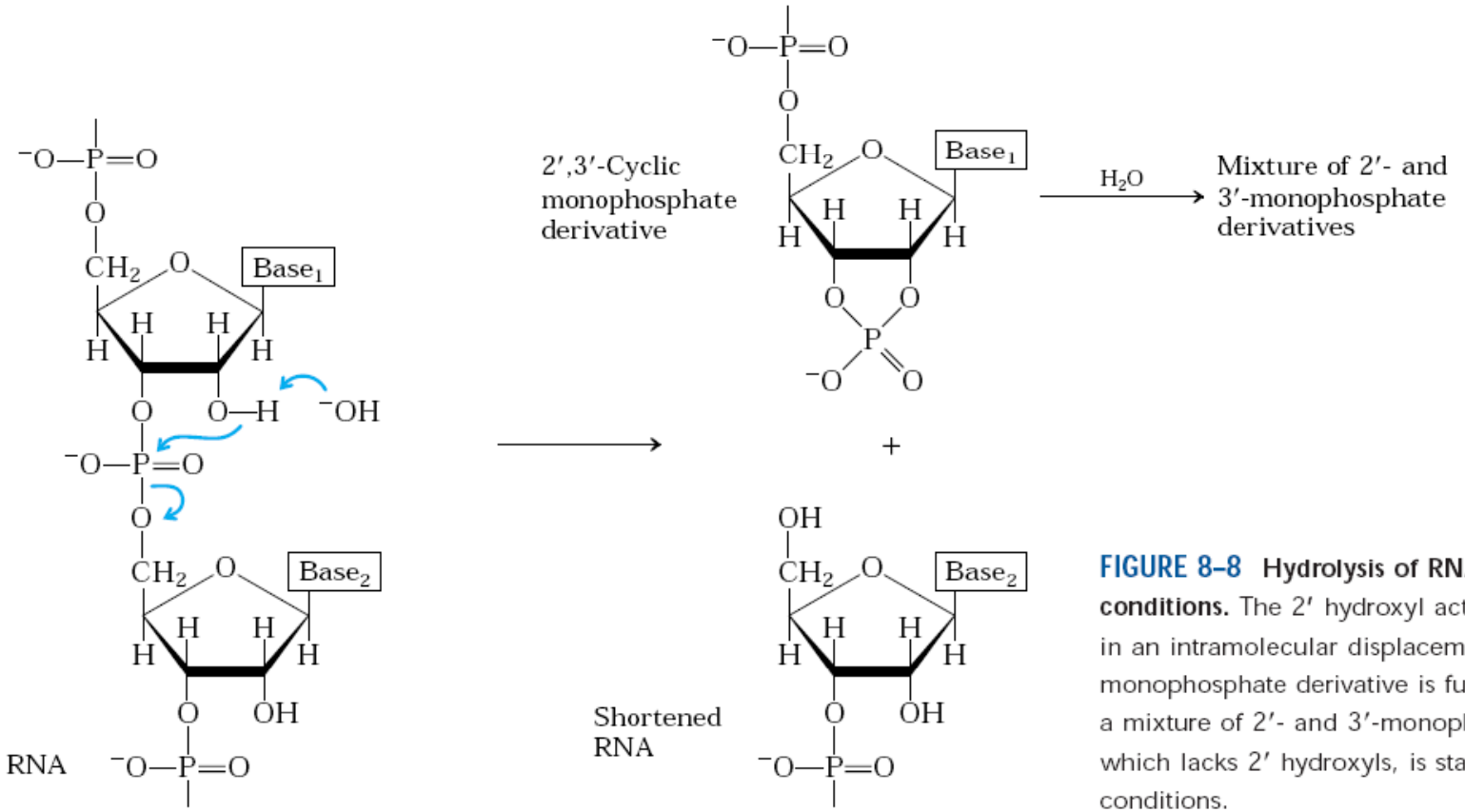
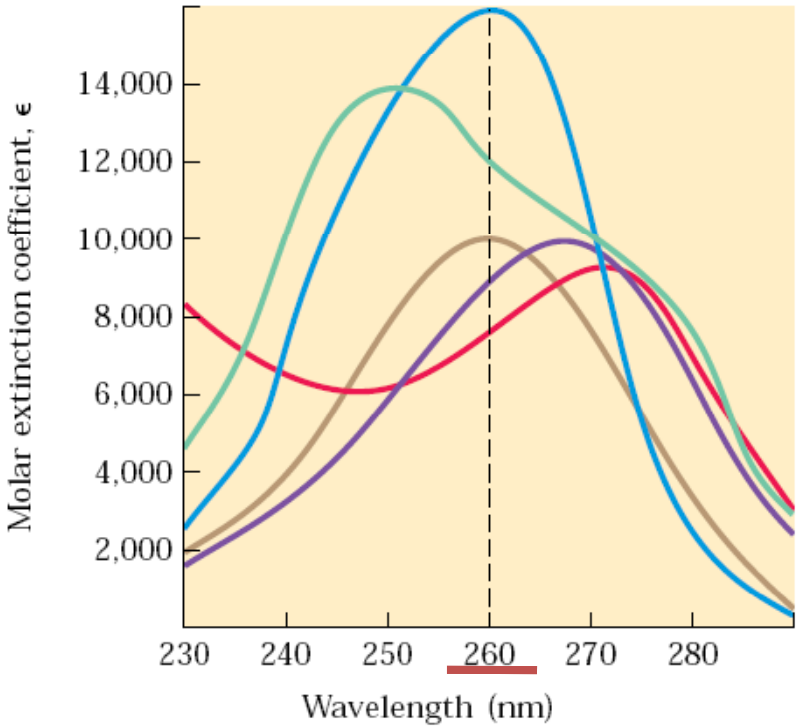



**FIGURE 8–8 Hydrolysis of RNA under alkaline conditions.** The 2′ hydroxyl acts as a nucleophile in an intramolecular displacement. The 2′,3′-cyclic monophosphate derivative is further hydrolyzed to a mixture of 2′- and 3′-monophosphates. DNA, which lacks 2′ hydroxyls, is stable under similar conditions.




| Molar extinction coefficient at 260 nm, $\epsilon_{260}$ ($M^{-1}cm^{-1}$) | |
|---|---|
| AMP | 15,400 |
| GMP | 11,700 |
| UMP | 9,900 |
| dTMP | 9,200 |
| CMP | 7,500 |

**FIGURE 8–10 Absorption spectra of the common nucleotides.** The spectra are shown as the variation in molar extinction coefficient with wavelength. The molar extinction coefficients at 260 nm and pH 7.0 ($\varepsilon_{260}$) are listed in the table. The spectra of corresponding ribonucleotides and deoxyribonucleotides, as well as the nucleosides, are essentially identical. For mixtures of nucleotides, a wavelength of 260 nm (dashed vertical line) is used for absorption measurements.

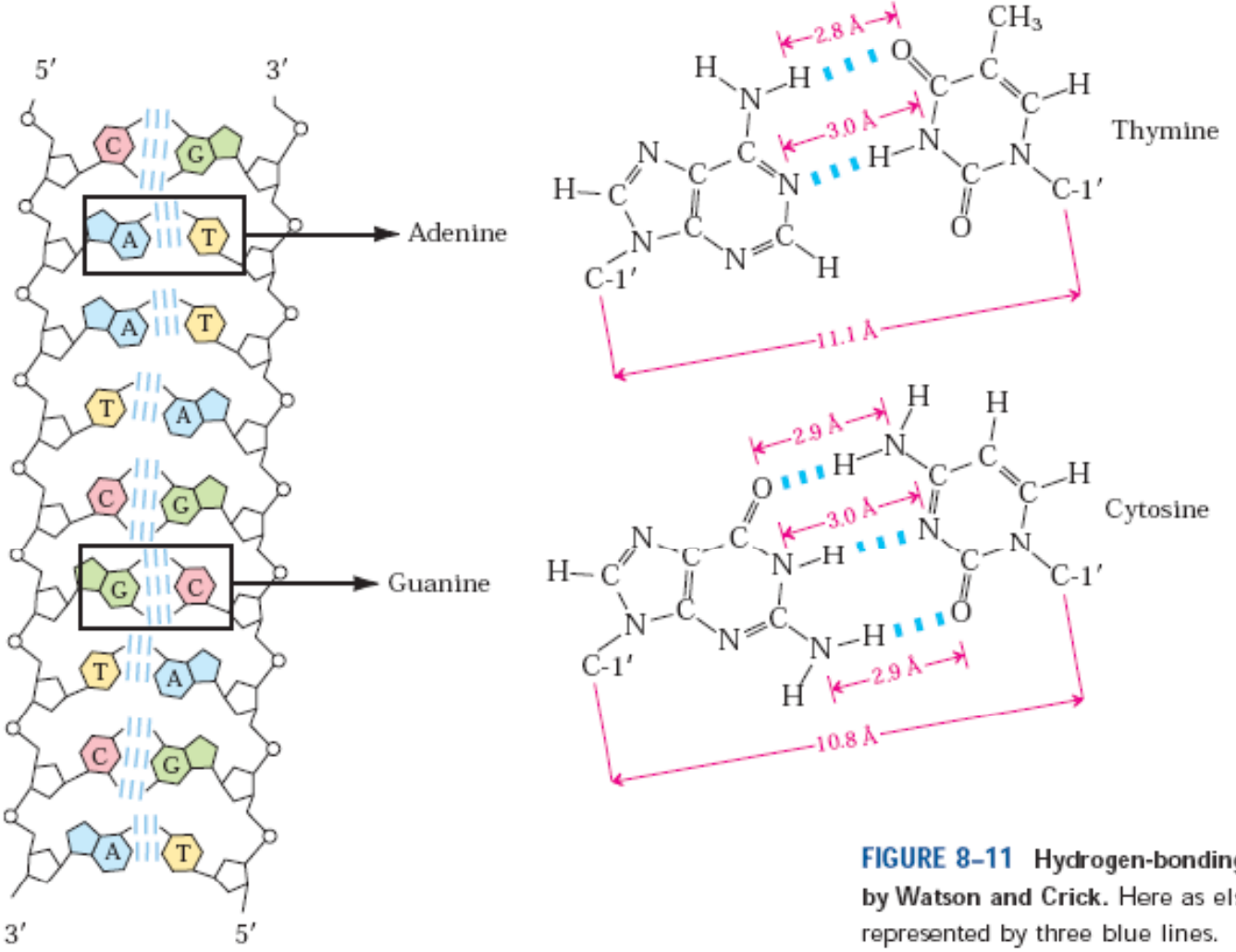
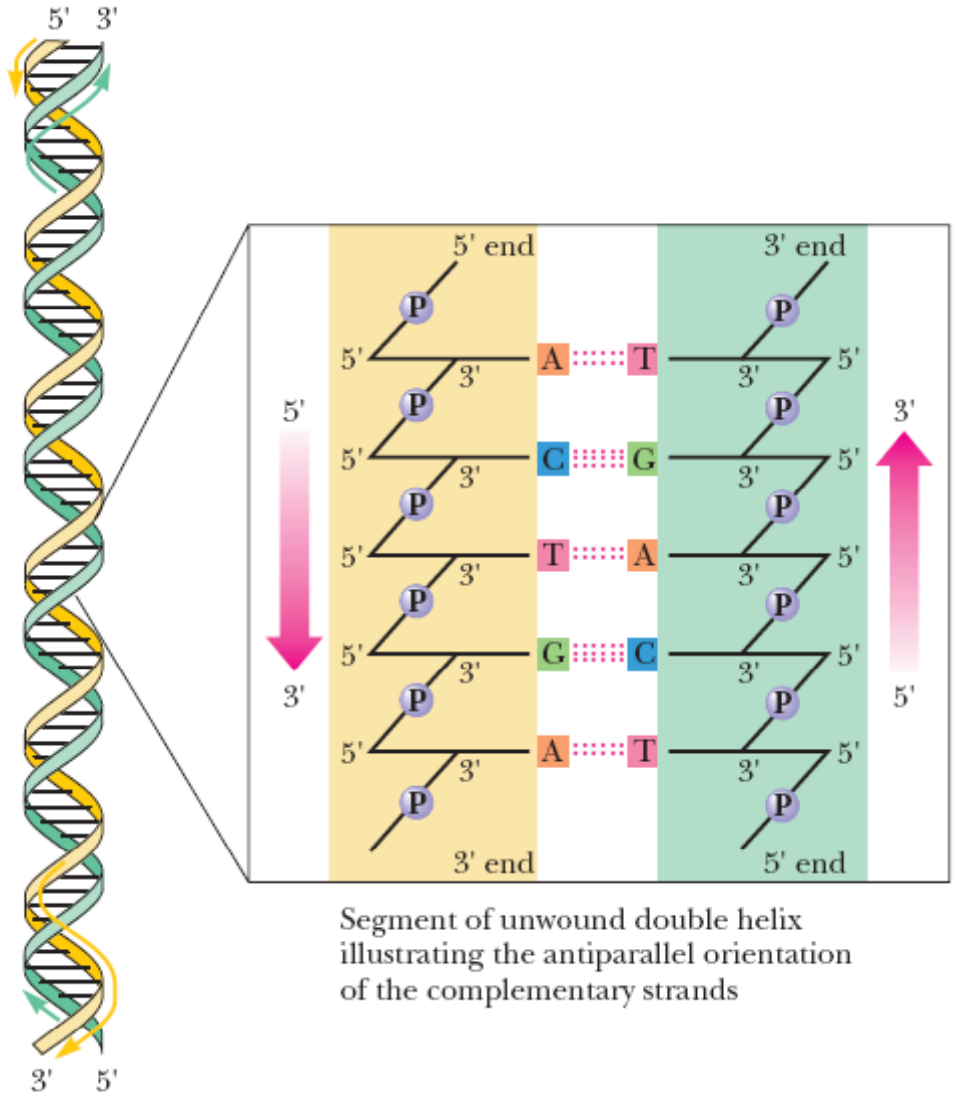



**FIGURE 8–11 Hydrogen-bonding patterns in the base pairs defined by Watson and Crick.** Here as elsewhere, hydrogen bonds are represented by three blue lines.

# Nucleic Acid Structure

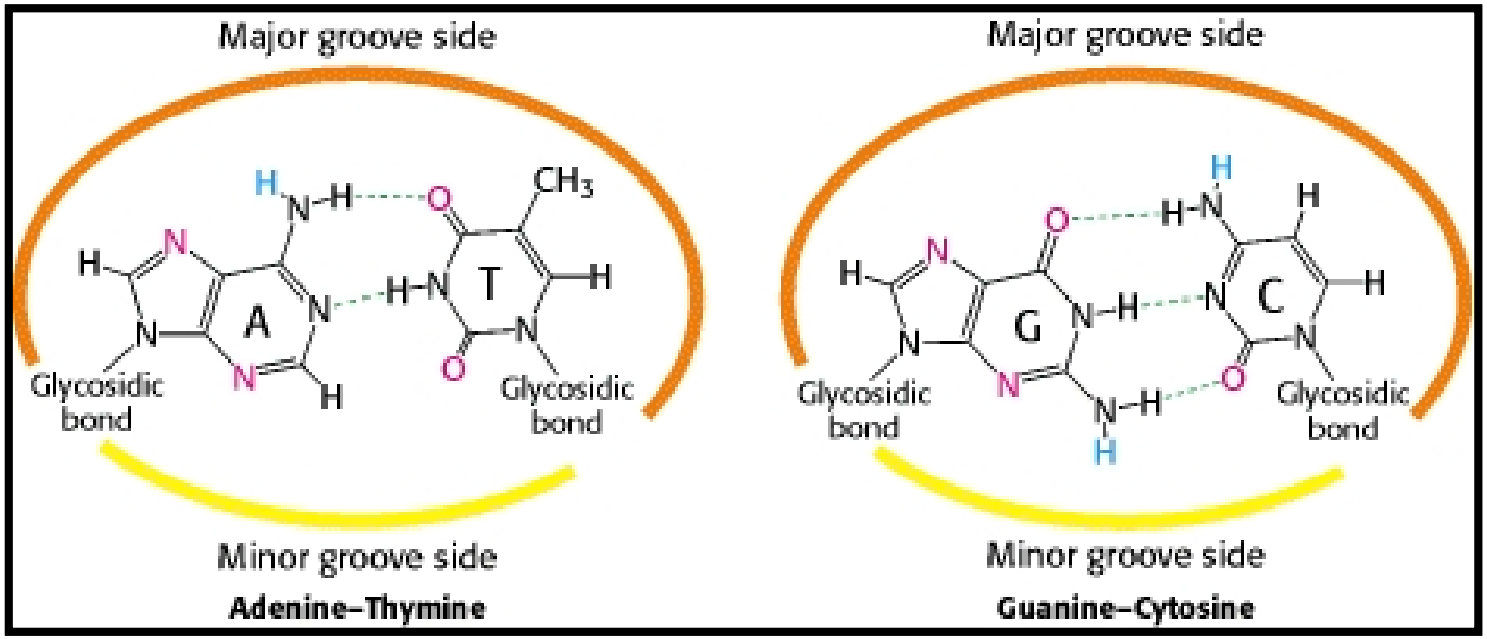
**The discovery of the structure of DNA by Watson and Crick in 1953**

**The Fundamental Structure of DNA Is a Double Helix (antiparallel)**




Segment of unwound double helix illustrating the antiparallel orientation of the complementary strands

**FIGURE 10.16** The antiparallel nature of the DNA double helix.



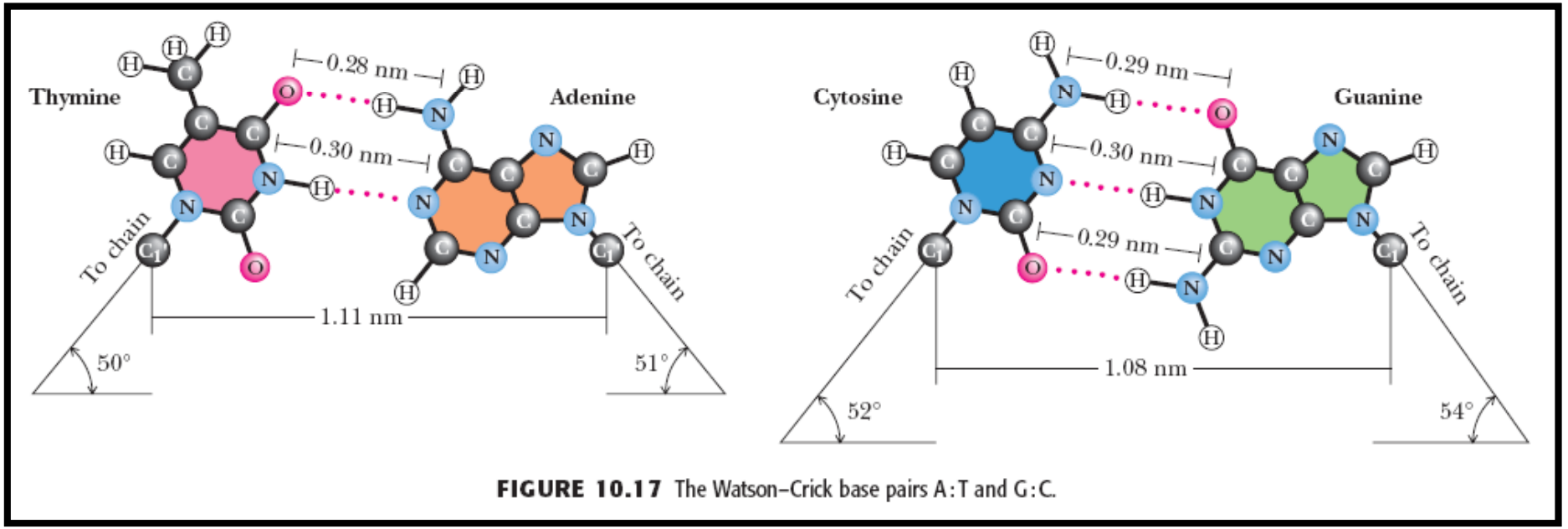




**FIGURE 10.17** The Watson–Crick base pairs A:T and G:C.

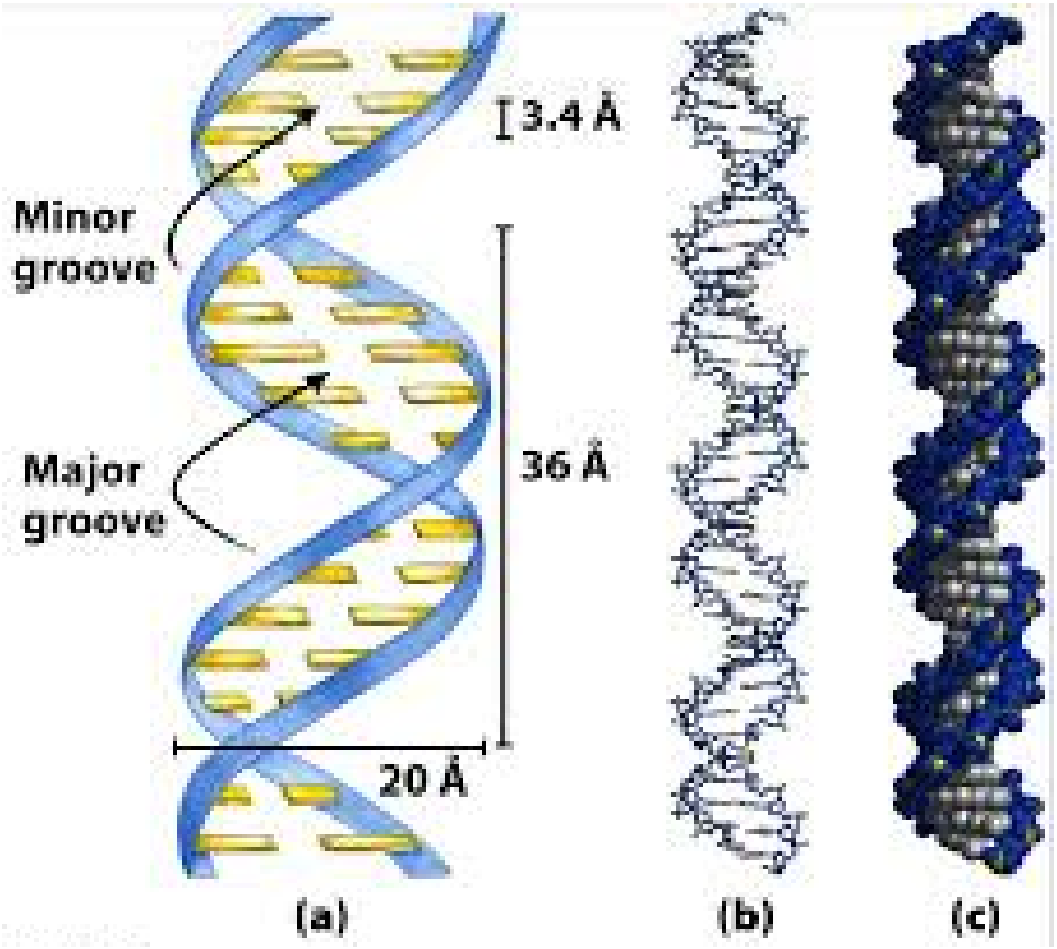
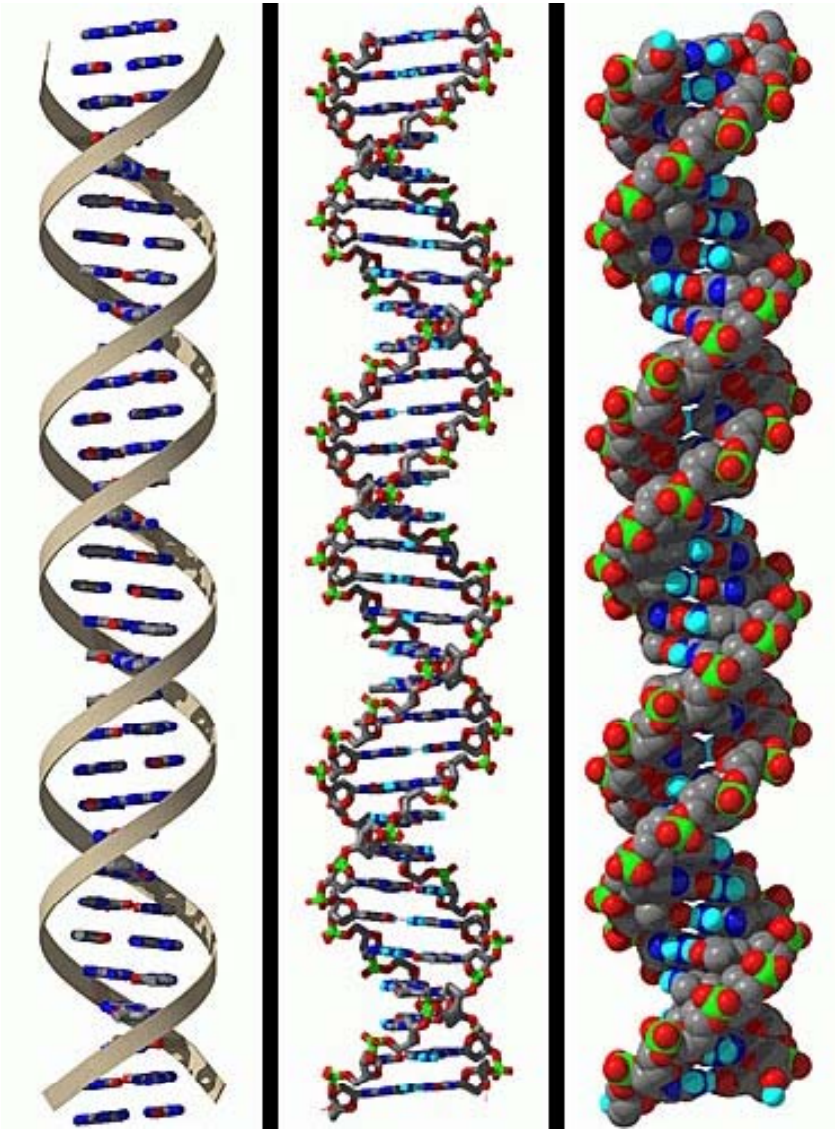
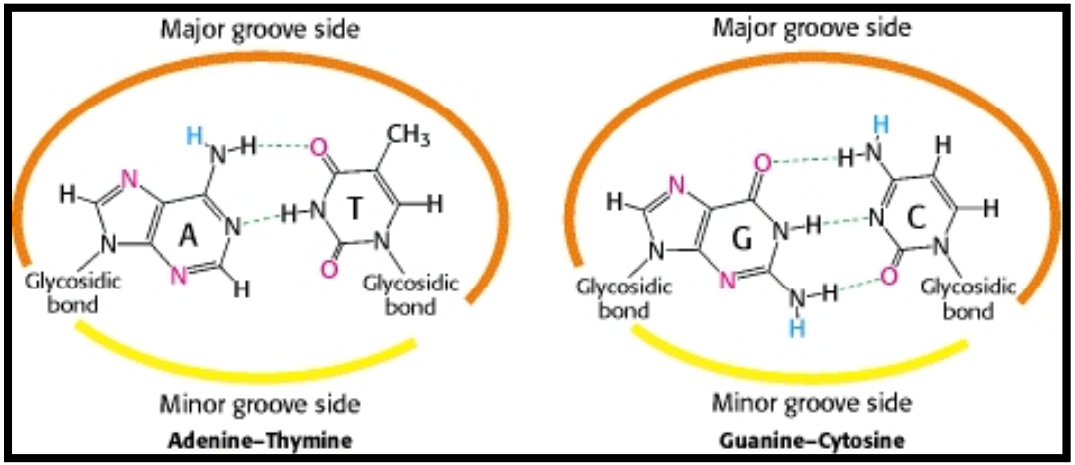


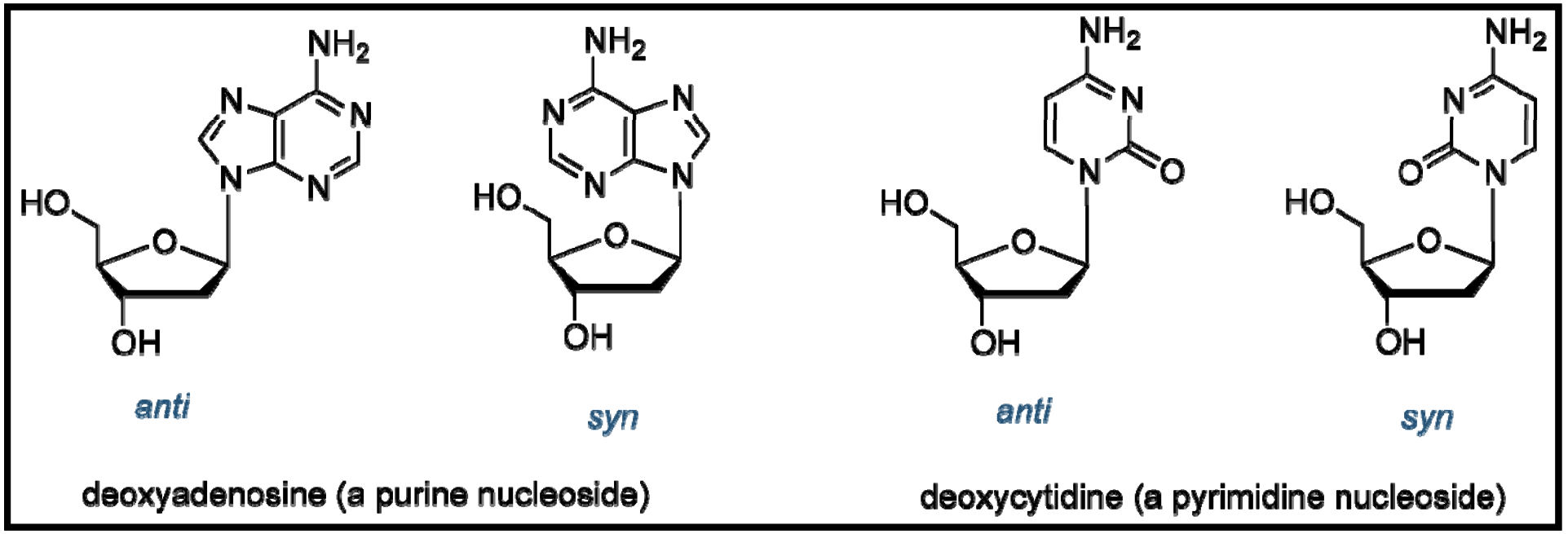

**FIGURE 8–15 Watson-Crick model for the structure of DNA.** The original model proposed by Watson and Crick had 10 base pairs, or 34 Å (3.4 nm), per turn of the helix; subsequent measurements revealed 10.5 base pairs, or 36 Å (3.6 nm), per turn. **(a)** Schematic representation, showing dimensions of the helix. **(b)** Stick representation showing the backbone and stacking of the bases. **(c)** Space-filling model.

- Right handed double helix
- The furanose ring of each deoxyribose is in the C-2' endo conformation
- Major groove
- Minor groove
- Stacking force
- Hydrogen bond



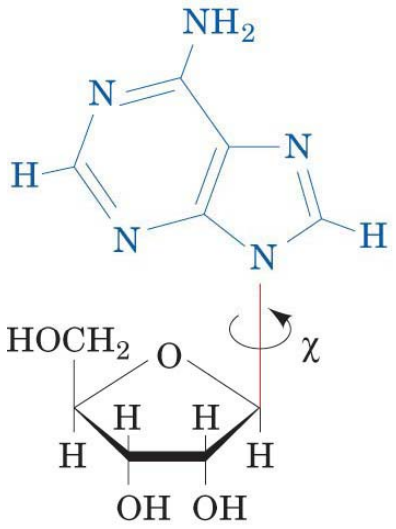

Major groove side
Major groove side
A
T
G
C
Glycosidic bond
Glycosidic bond
Glycosidic bond
Glycosidic bond
Minor groove side
Minor groove side
Adenine–Thymine
Guanine–Cytosine

# DNA Can Occur in Different Three-Dimensional Forms

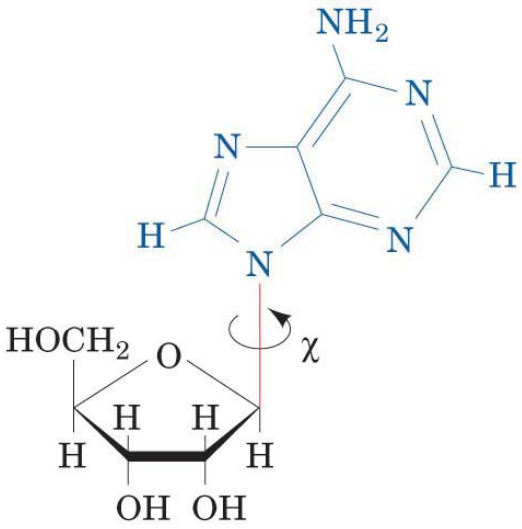

NH2
N
N
N
N
HO
O
OH
anti
NH2
N
N
N
N
HO
O
OH
syn
NH2
N
N
O
HO
O
OH
anti
NH2
N
O
N
HO
O
OH
syn
deoxyadenosine (a purine nucleoside)
deoxycytidine (a pyrimidine nucleoside)

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

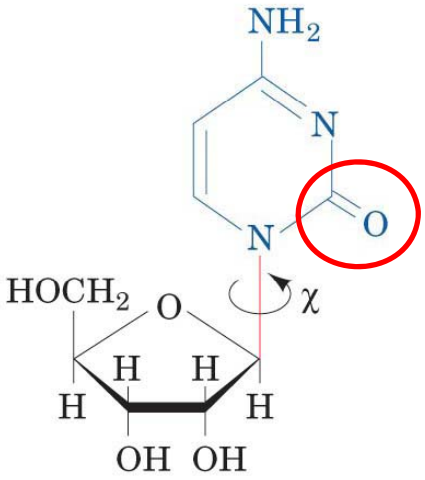
**Pyrimidines** are generally restricted to the *anti* conformation because of steric interference between the sugar and the carbonyl oxygen at C-2 of the pyrimidine.




**syn-Adenosine**




**anti-Adenosine**




**anti-Cytidine**

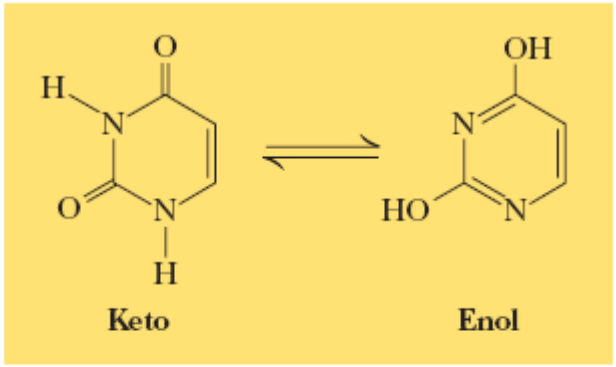



**FIGURE 10.6** The keto–enol tautomerization of uracil.

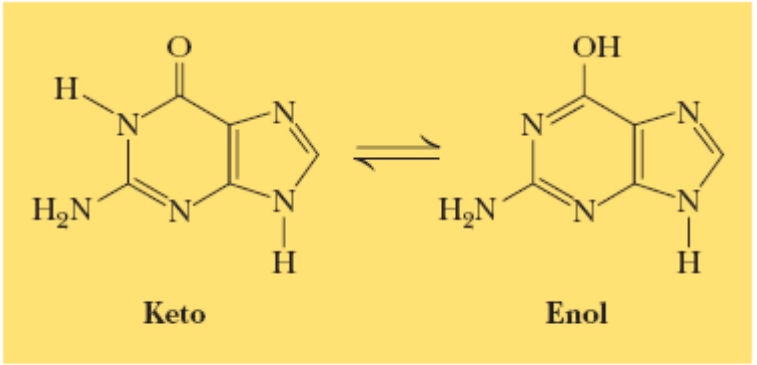
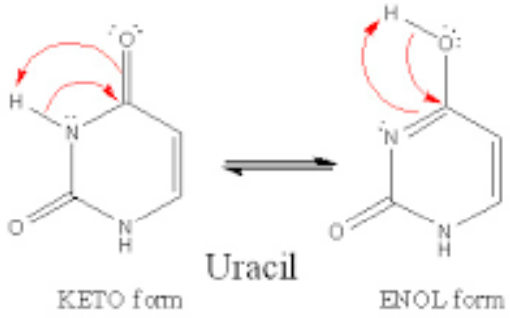
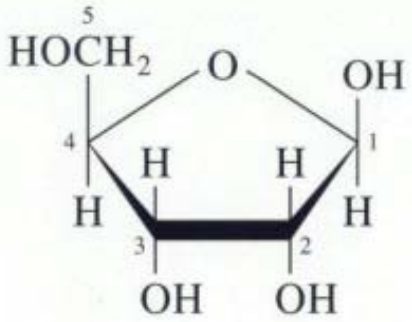
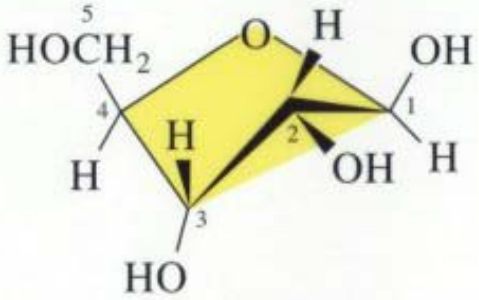



**FIGURE 10.7** The tautomerization of the purine guanine.

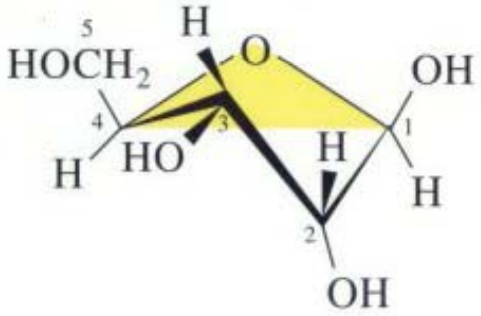





Haworth projection



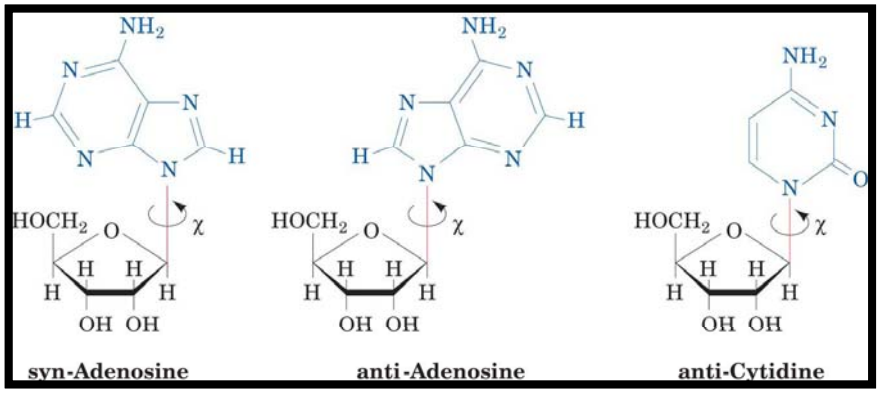

Envelope conformation

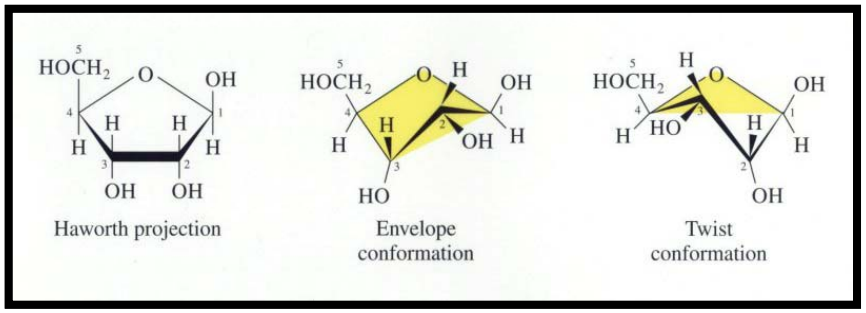


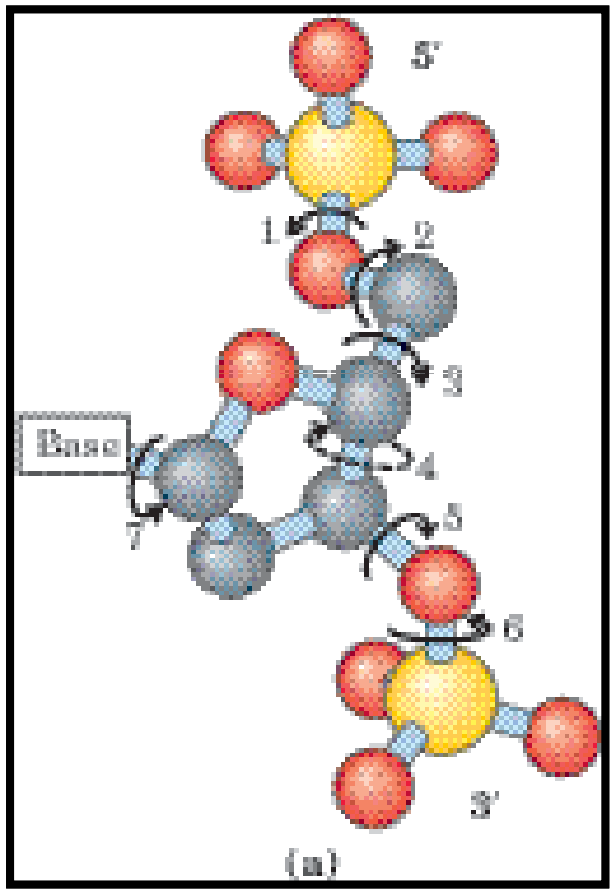

Twist conformation

# DNA Can Occur in Different Three-Dimensional Forms

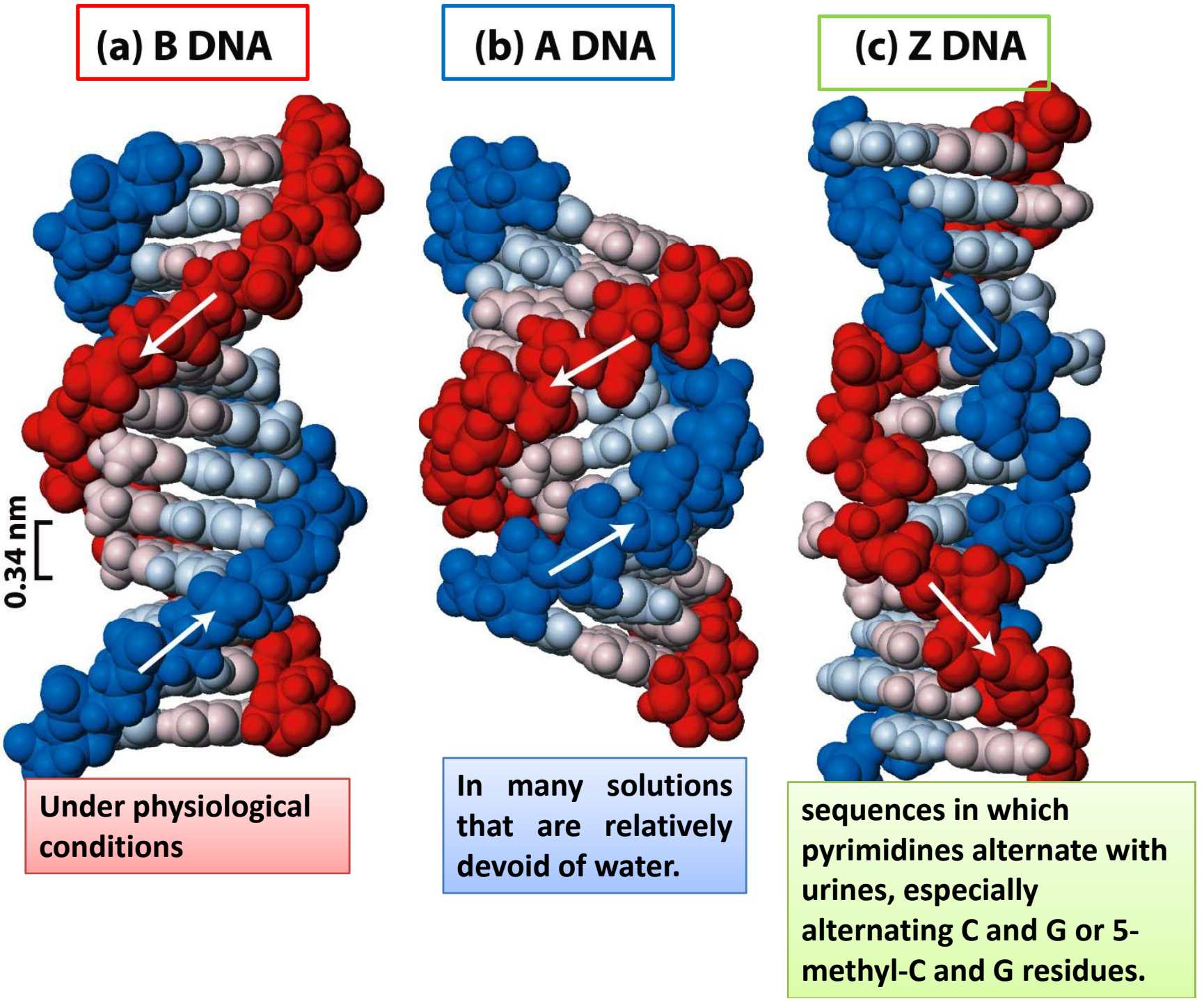
**Many significant deviations from the Watson-Crick DNA structure are found in cellular DNA.**

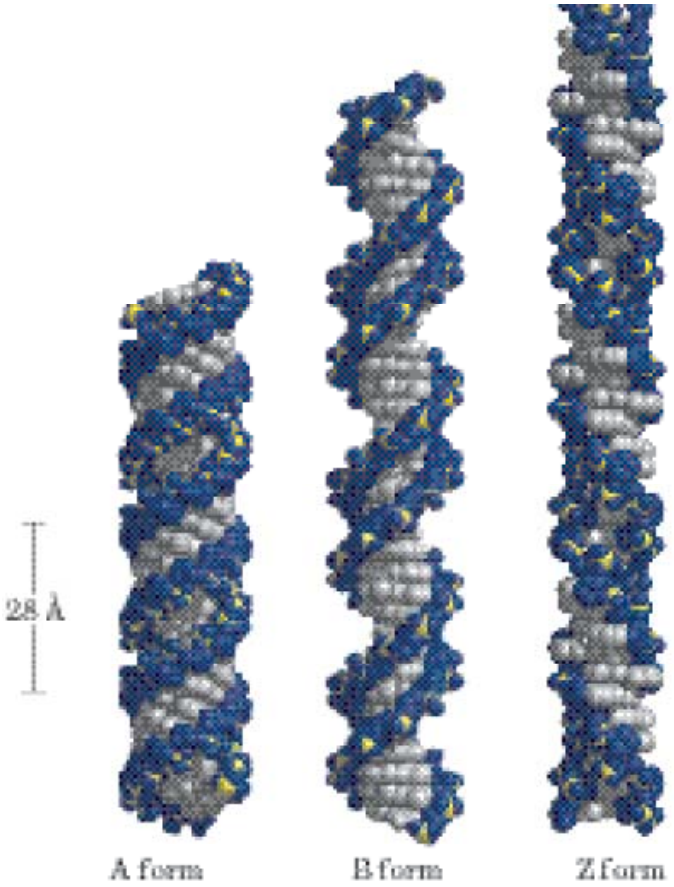



The conformation of a nucleotide in DNA is affected by rotation about seven different bonds


(a) B DNA
(b) A DNA
(c) Z DNA
0.34 nm
Under physiological conditions
In many solutions that are relatively devoid of water.
sequences in which pyrimidines alternate with urines, especially alternating C and G or 5-methyl-C and G residues.




| | *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.

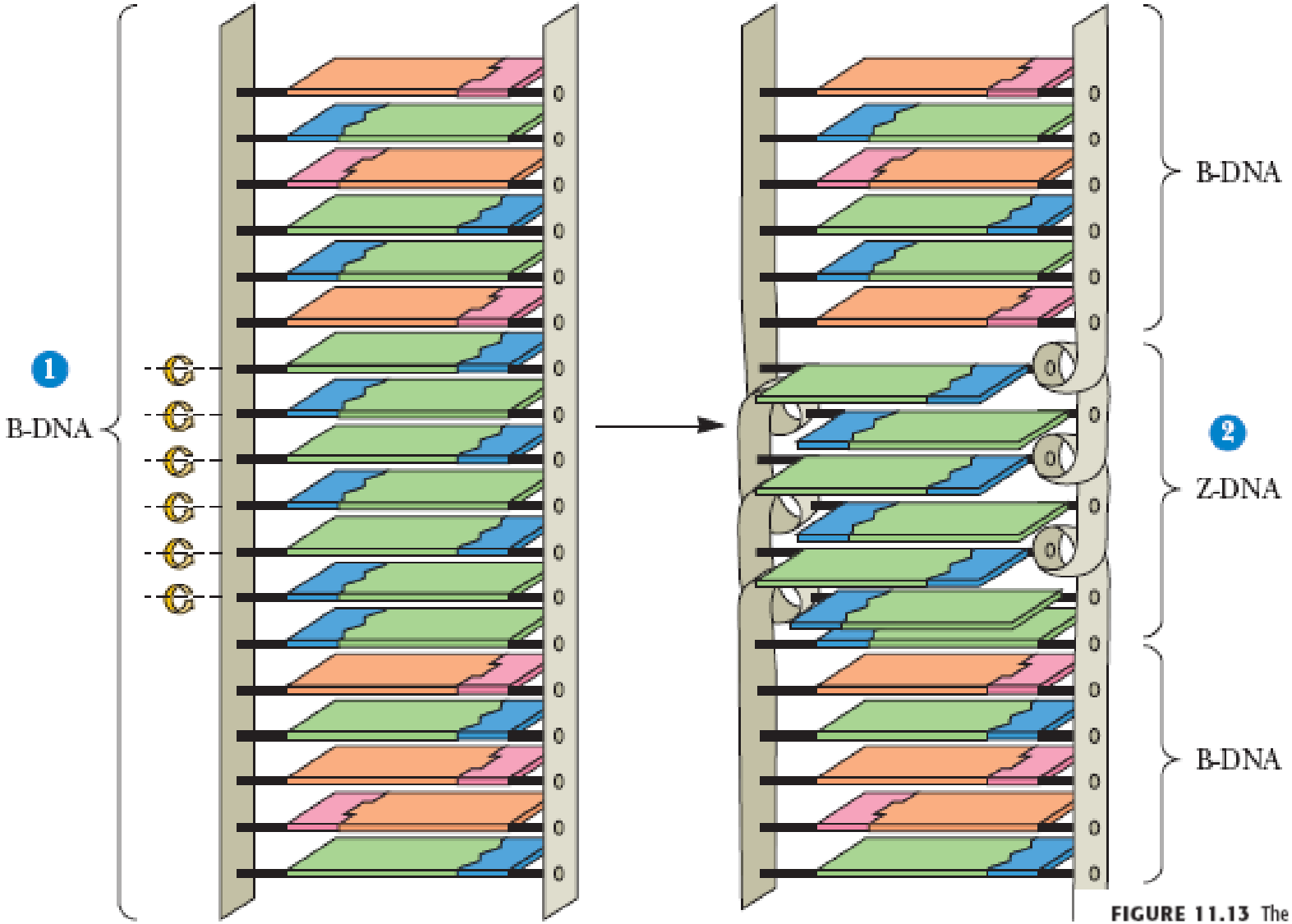
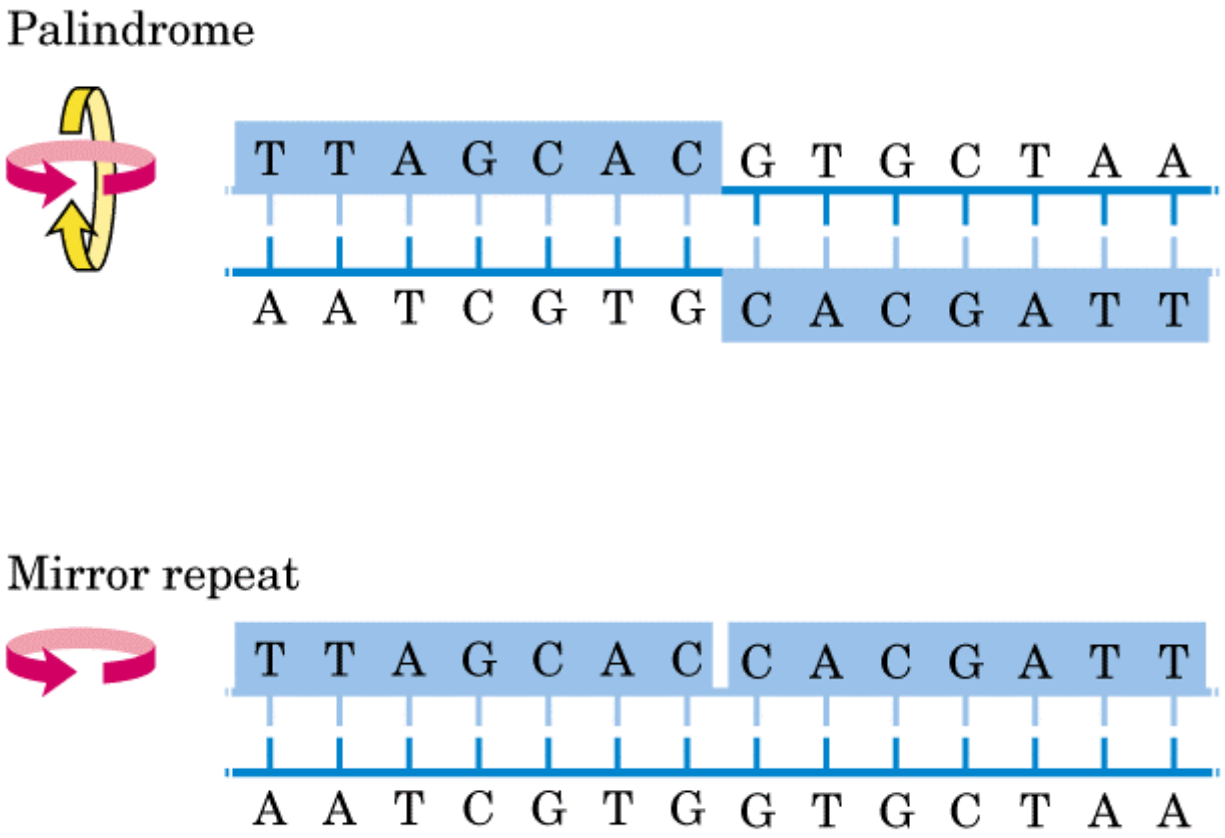


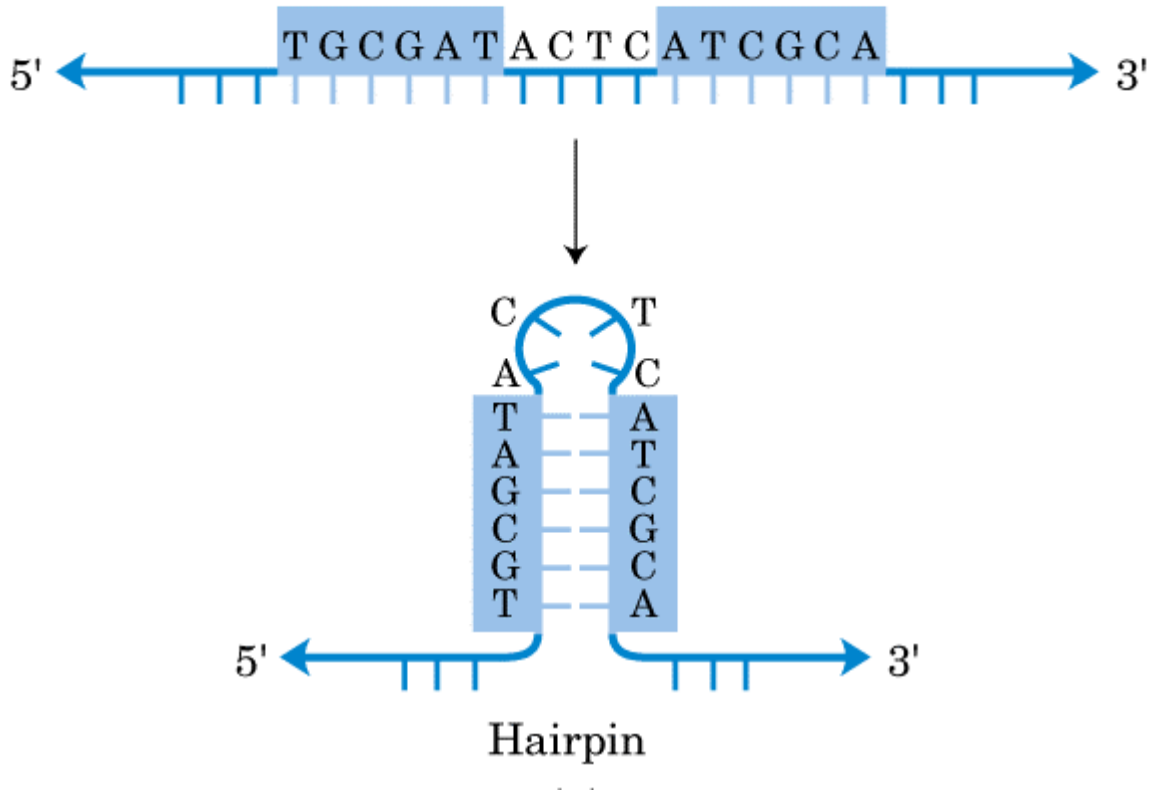

**FIGURE 11.13** The change in topological relationships of base pairs from B- to Z-DNA. A six-base-pair GCGCGC segment of B-DNA (1) is converted to Z-DNA (2) through rotation of the base pairs, as indicated by the curved arrows. The purine rings (green) of the deoxyguanosine nucleosides rotate via an anti to syn change in the conformation of the guanine–deoxyribose glycosidic bond; the pyrimidine rings (blue) are rotated by flipping the entire deoxycytosine nucleoside (base *and* deoxyribose).

# Certain DNA Sequences Adopt Unusual Structures

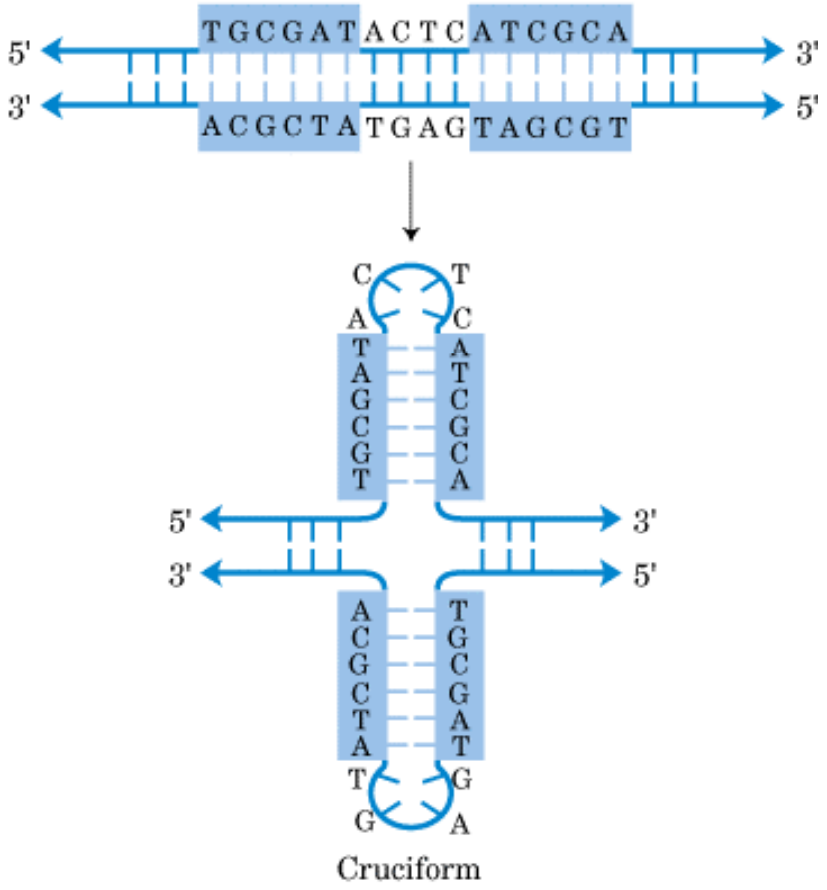
- ***Palindrome (Inverted repeats)***
- ***Hairpin***
- ***Cruciform***
- ***Mirror repeat***
- ***Triplex DNAs***
- ***Hoogsteen pairing***
- ***G tetraplex***



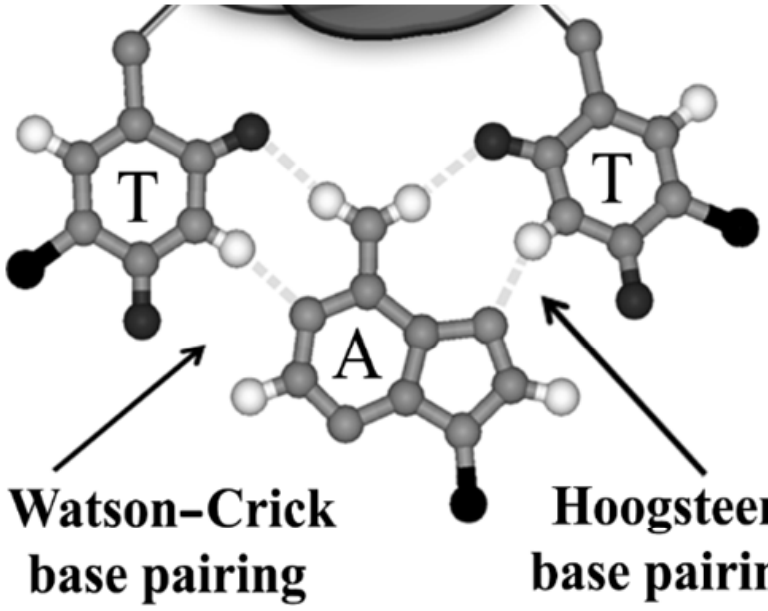

***Mirror repeats*** *do not have complementary sequences within the same strand and cannot form hairpin or cruciform structures. Sequences of these types are found in virtually every large DNA molecule and can encompass a few base pairs or thousands.*



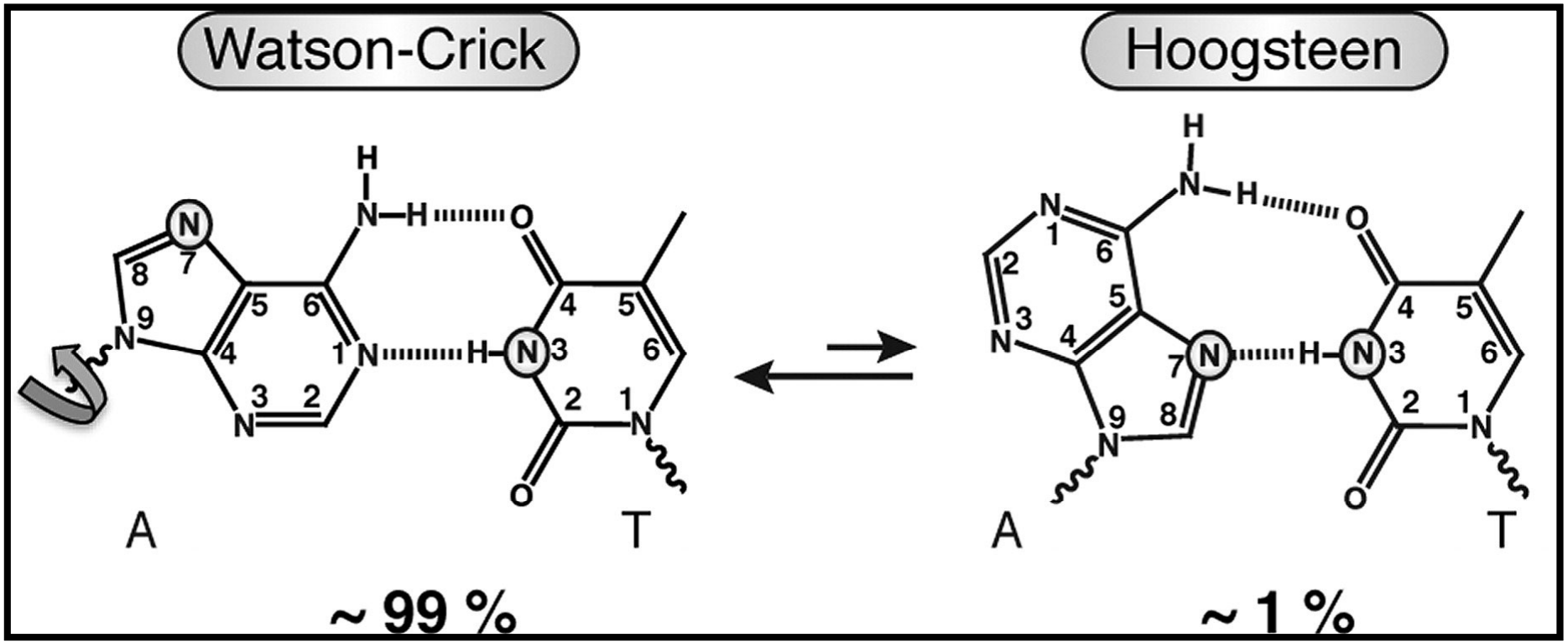

**When only a single DNA (or RNA) strand is involved, the structure is called a hairpin.**

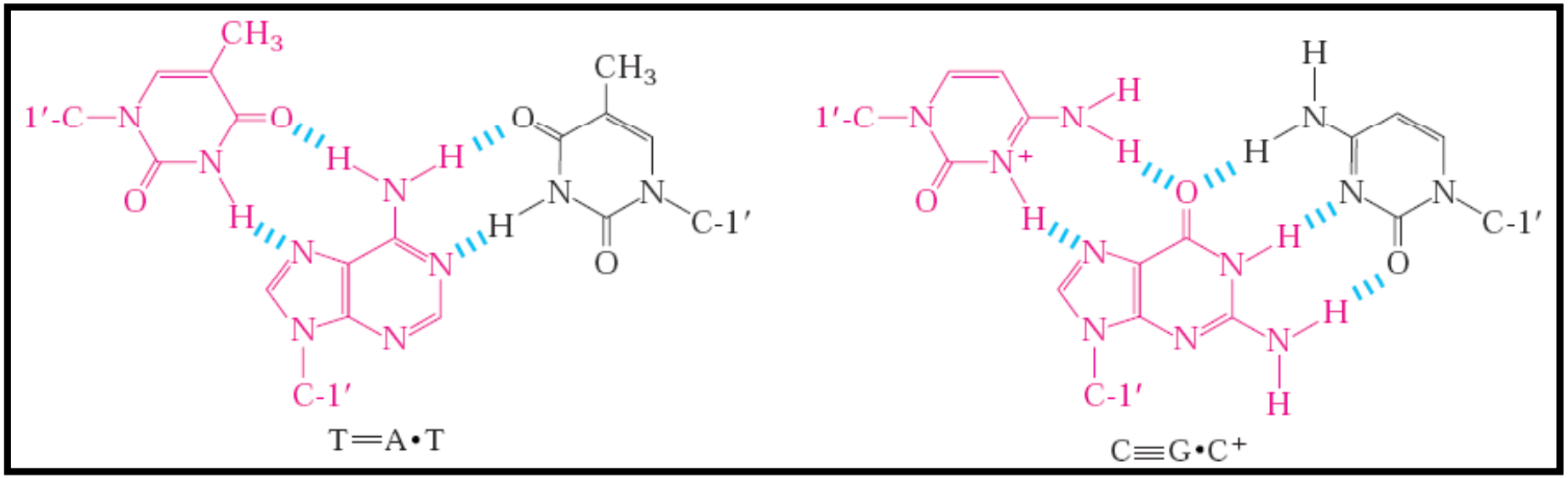



**When both strands of a duplex DNA are involved, it is called a cruciform**




non-Watson-Crick pairing is called
**Hoogsteen pairing**

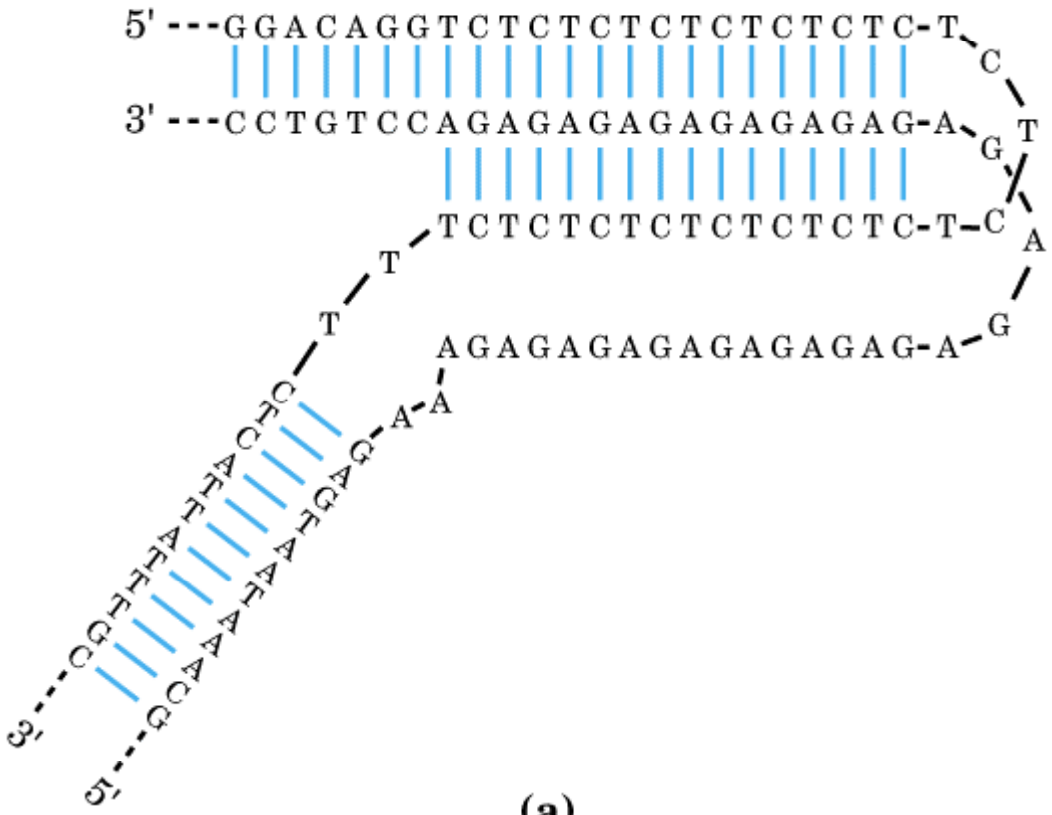




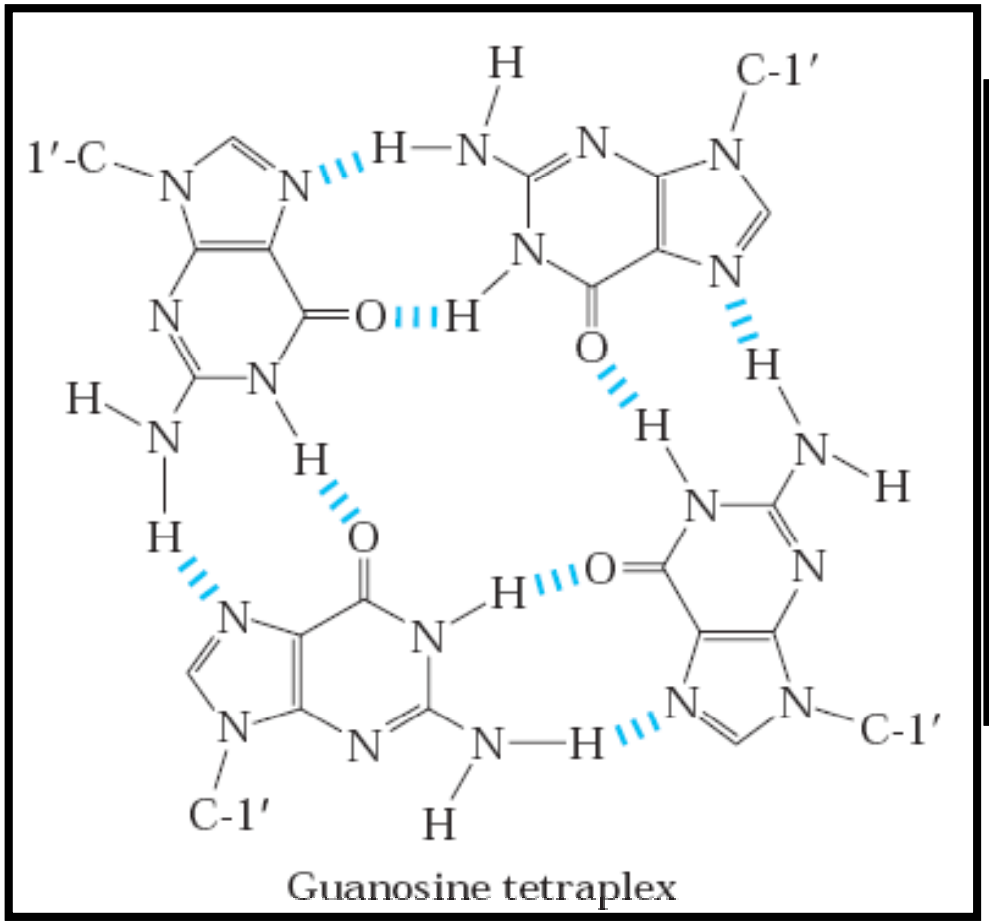

**Hoogsteen pairing**

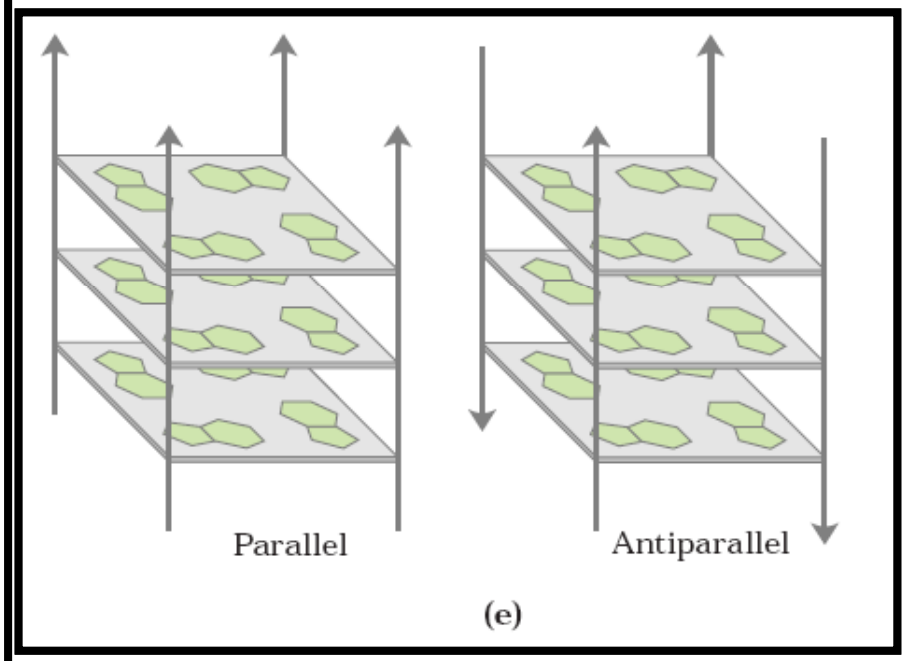
The N-7, *O6, and N6 of purines, the atoms* that participate in the hydrogen bonding of triplex DNA, are often referred to as **Hoogsteen positions**

# Triplex DNA




**(a)**

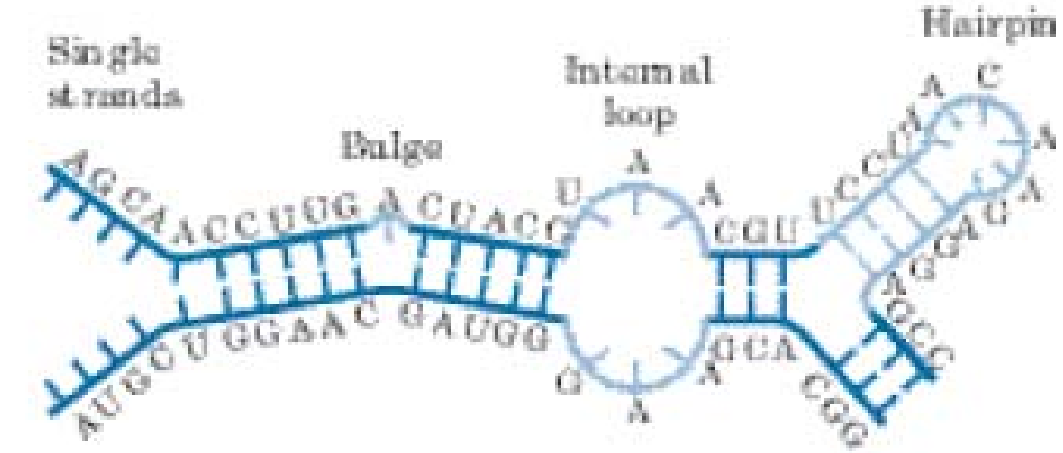


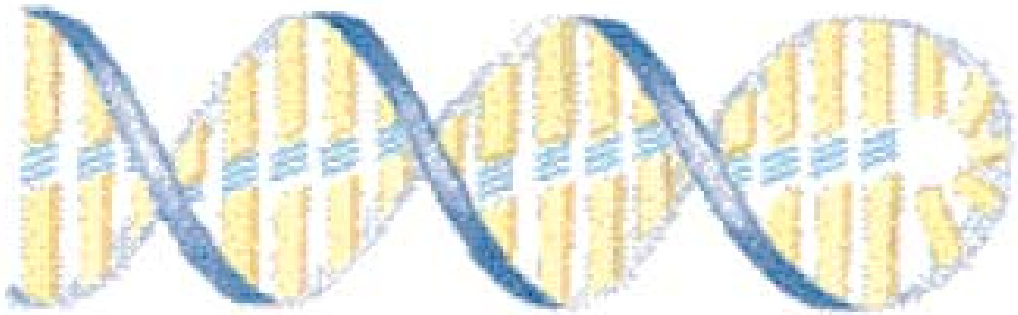

**Four DNA strands can also pair to form a tetraplex (quadruplex), but this occurs readily only for DNA sequences with a very high proportion of guanosine residues.**

# Many RNAs Have More Complex Three-Dimensional Structures

**FIGURE 8–25 Typical right-handed stacking pattern of single-stranded RNA.** The bases are shown in gray, the phosphate atoms in yellow, and the riboses and phosphate oxygens in green. Green is used to represent RNA strands in succeeding chapters, just as blue is used for DNA.




(a)



Hairpin double helix

(b)

**FIGURE 8–26** **Secondary structure of RNAs.** **(a)** Bulge, internal loop, and hairpin loop. **(b)** The paired regions generally have an A-form right-handed helix, as shown for a hairpin.

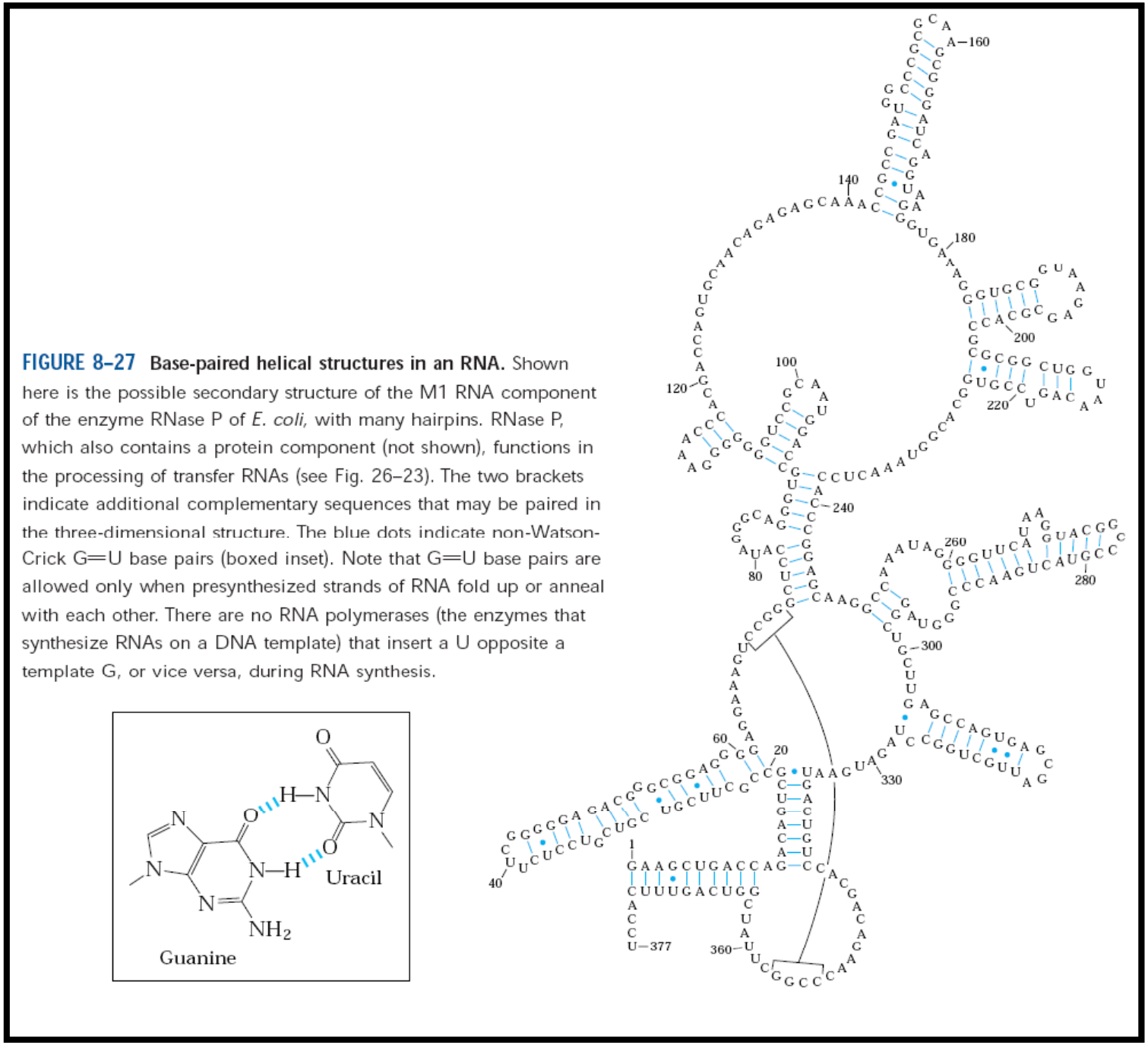
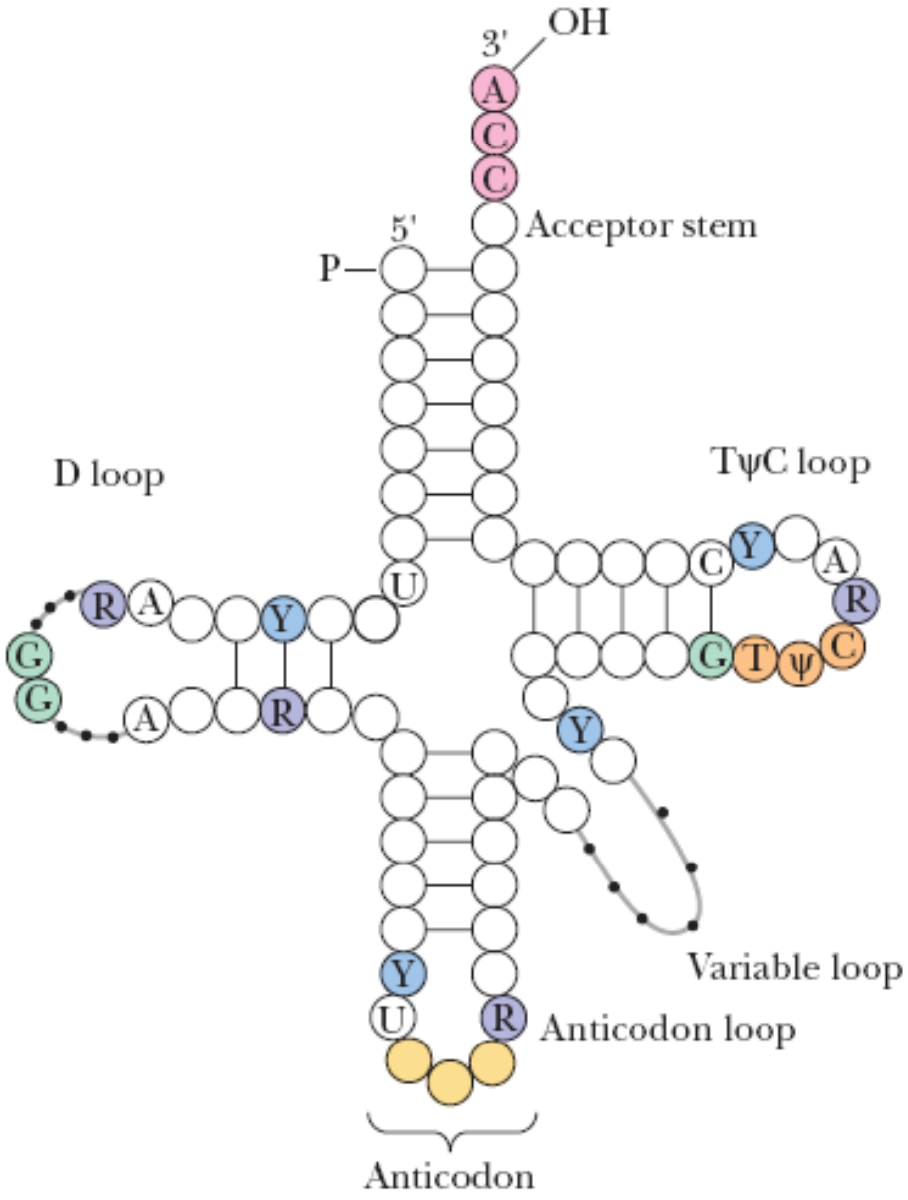
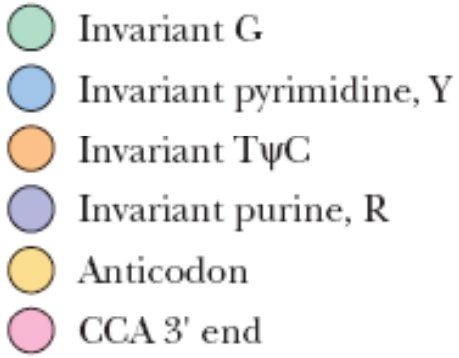



**FIGURE 8–27 Base-paired helical structures in an RNA.** Shown here is the possible secondary structure of the M1 RNA component of the enzyme RNase P of *E. coli,* with many hairpins. RNase P, which also contains a protein component (not shown), functions in the processing of transfer RNAs (see Fig. 26–23). The two brackets indicate additional complementary sequences that may be paired in the three-dimensional structure. The blue dots indicate non-Watson-Crick G═U base pairs (boxed inset). Note that G═U base pairs are allowed only when presynthesized strands of RNA fold up or anneal with each other. There are no RNA polymerases (the enzymes that synthesize RNAs on a DNA template) that insert a U opposite a template G, or vice versa, during RNA synthesis.



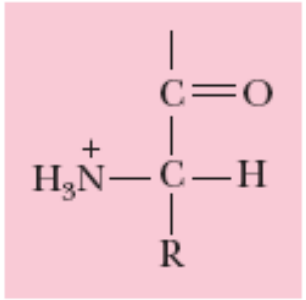
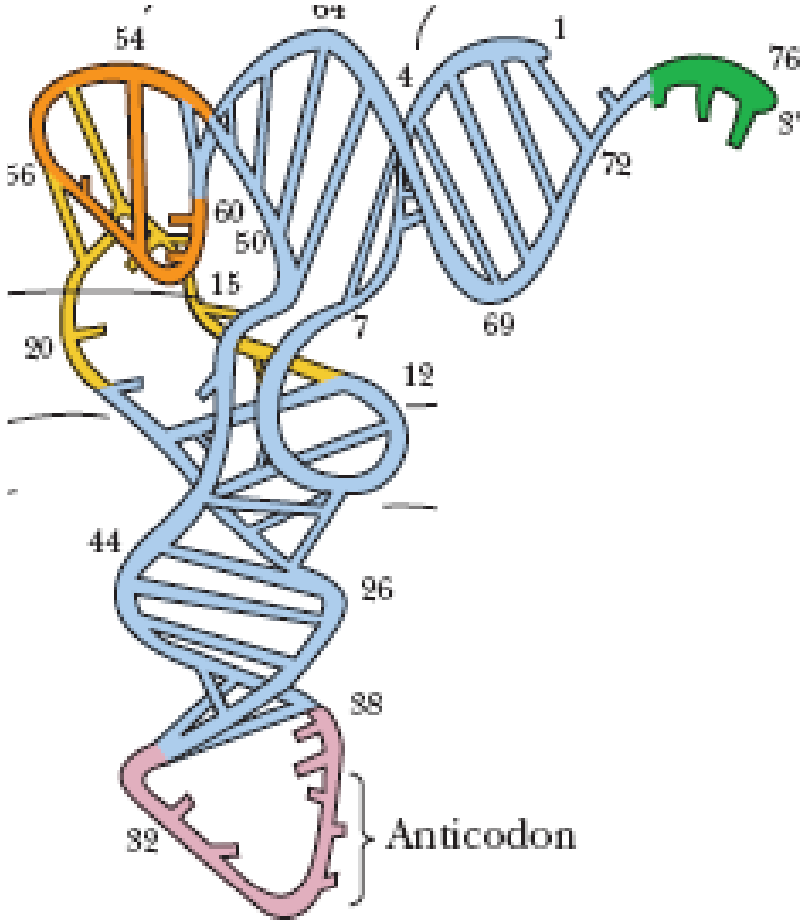
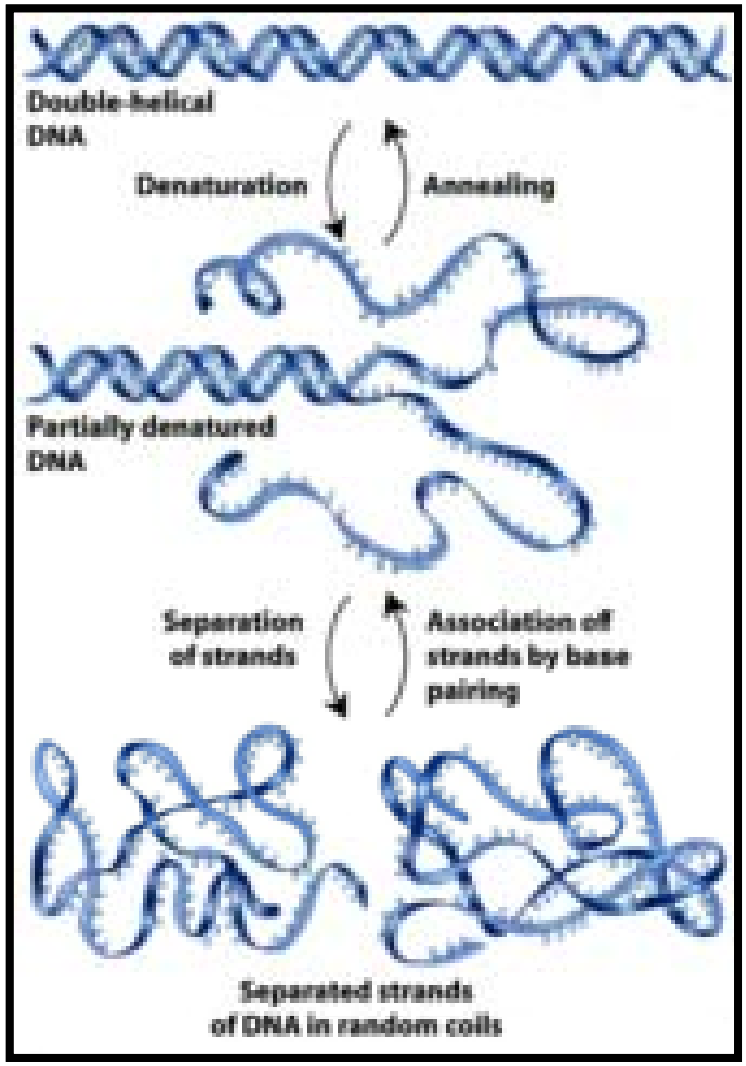




**FIGURE 11.36** A general diagram for the structure of tRNA. The positions of invariant bases as well as bases that seldom vary are shown in color. R = purine; Y = pyrimidine. Dotted lines denote sites in the D loop and variable loop regions where varying numbers of nucleotides are found in different tRNAs. Inset: An aminoacyl group can add to the 3′-OH to create an aminoacyl-tRNA.


54
1
4
76
5'
72
56
60
50
15
7
69
20
12
44
26
88
82
Anticodon

# Nucleic Acid Chemistry

## Double-Helical DNA and RNA Can Be Denatured




Denaturation

Renaturation

If the two strands are completely separated, renaturation occurs in two steps.

In the first, relatively slow step, the two strands "find" each other by random collisions and form a short segment of complementary double helix.

The second step is much faster: the remaining unpaired bases successively come into register as base pairs, and the two strands "zipper" themselves together to form the double helix.

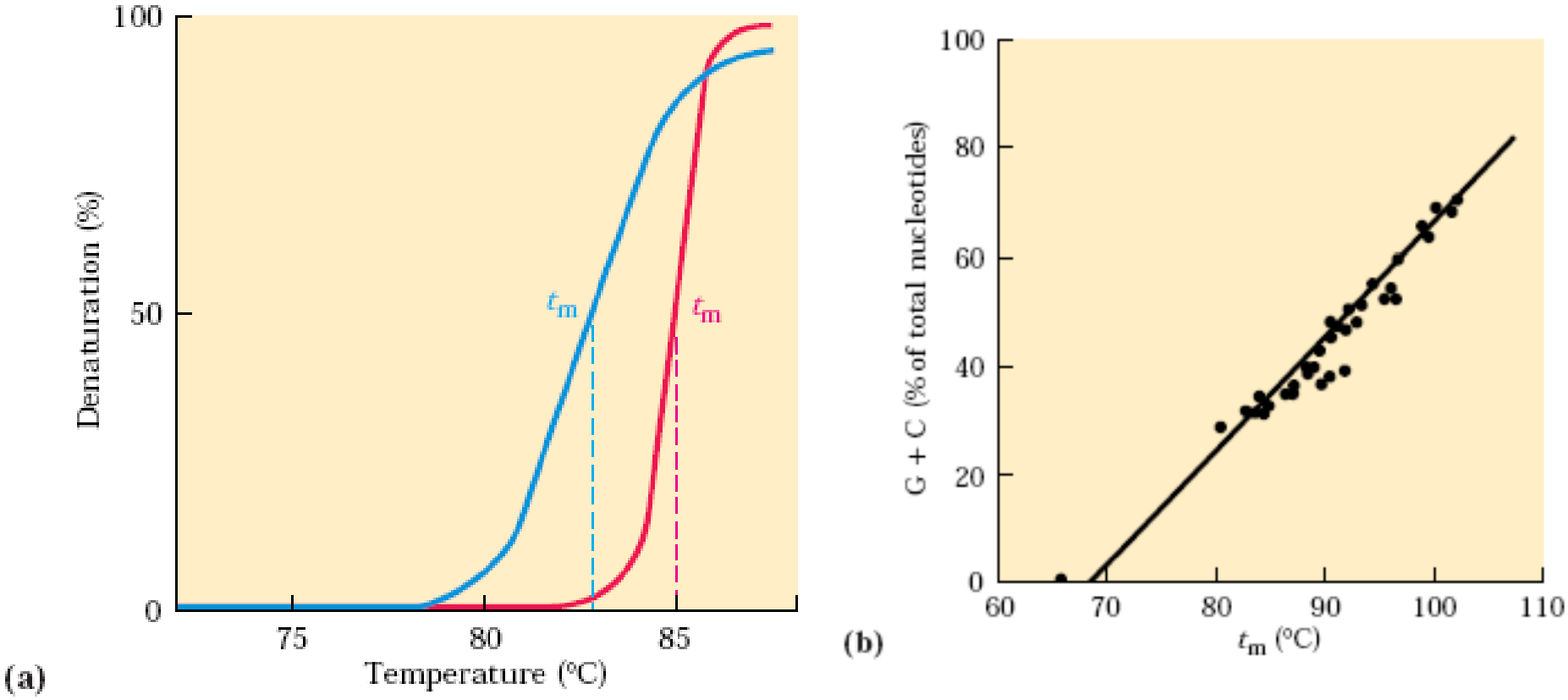
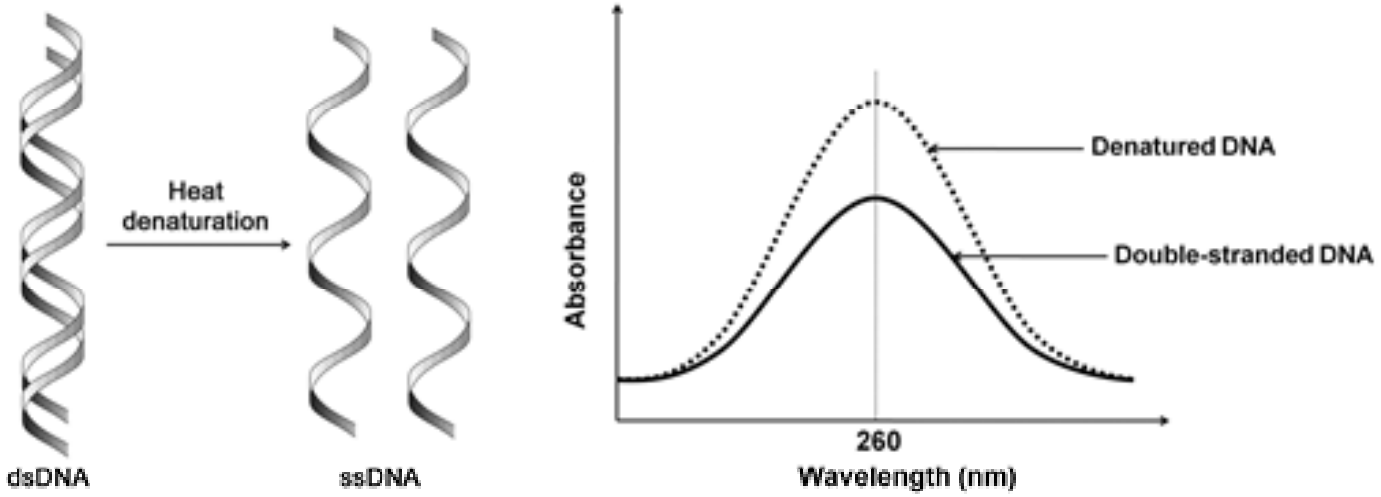
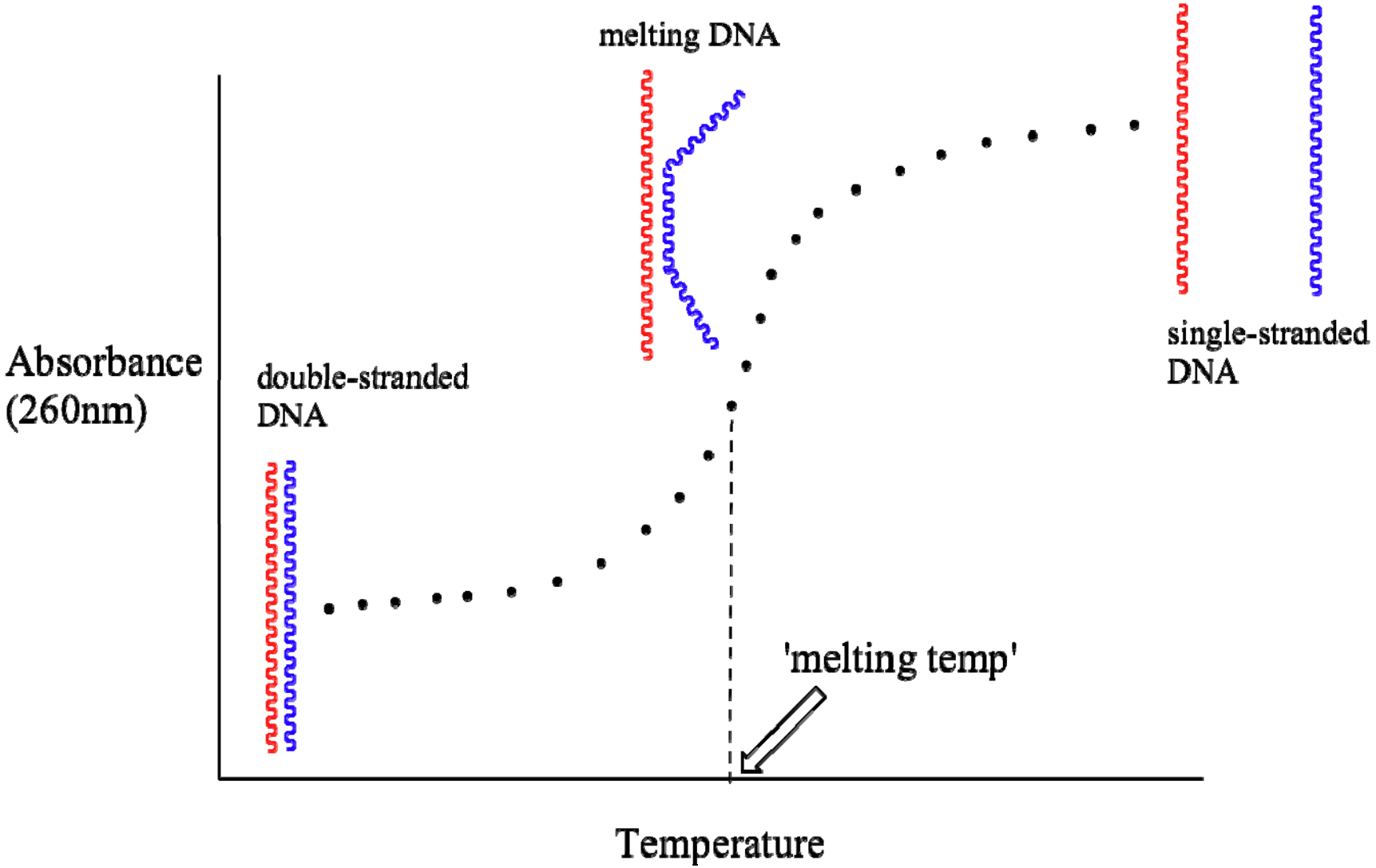



**FIGURE 8–30** **Heat denaturation of DNA.** **(a)** The denaturation, or melting, curves of two DNA specimens. The temperature at the midpoint of the transition ($t_m$) is the melting point; it depends on pH and ionic strength and on the size and base composition of the DNA. **(b)** Relationship between $t_m$ and the G≡C content of a DNA.




The transition from double-stranded DNA to the single-stranded, denatured form can thus be detected by monitoring the absorption of UV light.


melting DNA
Absorbance
(260nm)
double-stranded
DNA
single-stranded
DNA
'melting temp'
Temperature

## Stability

RNA duplexes > RNA-DNA hybrid > DNA duplexes

The physical basis for these differences in thermal stability is not known.

# Nucleotides and Nucleic Acids Undergo Nonenzymatic Transformations

# Mutation

A permanent change of the nucleotide sequence of the genome of an organism.

Deamination (1/10^7 C → U / 24 hours)

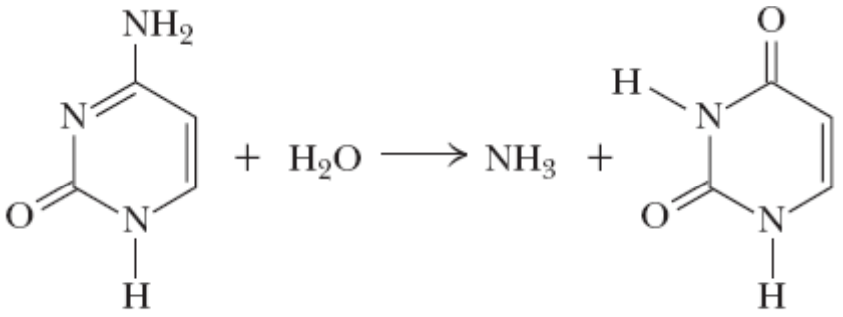
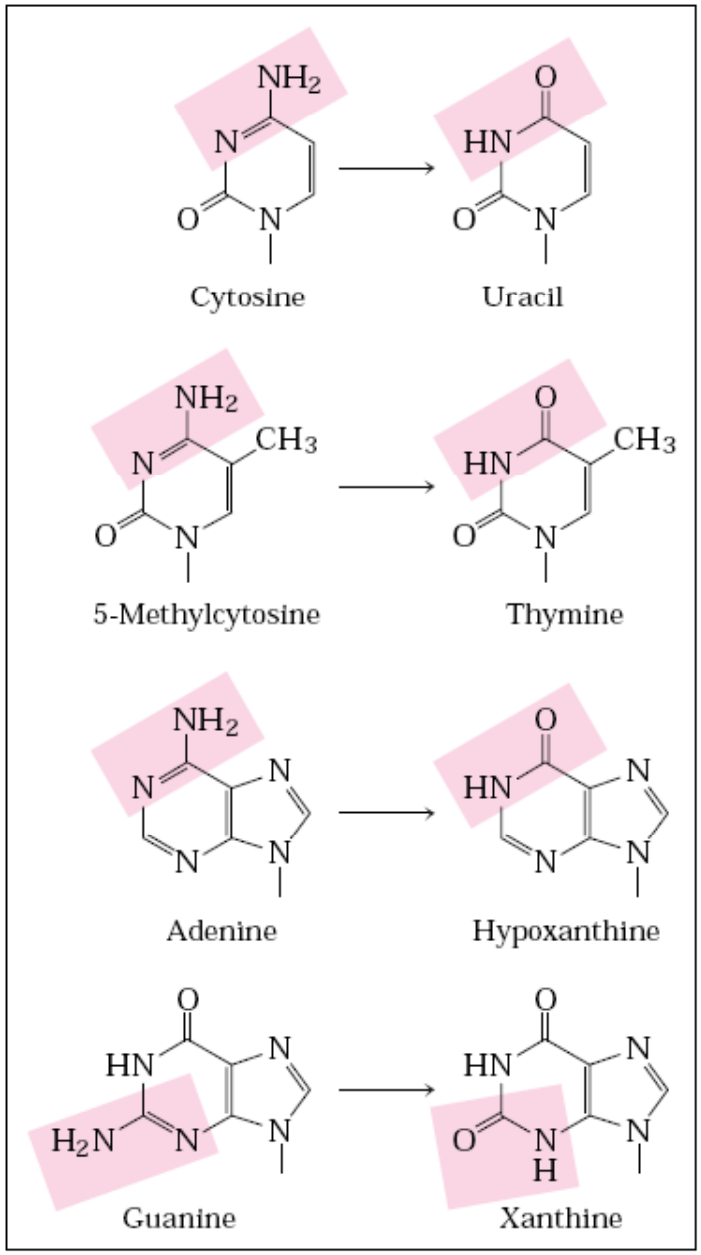


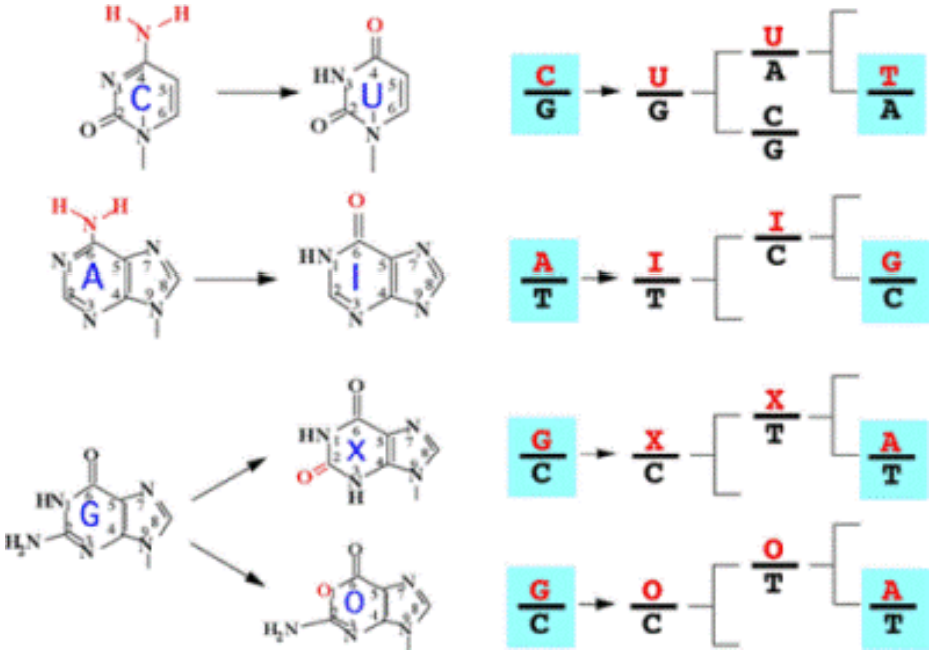

**FIGURE 10.25** Deamination of cytosine forms uracil.

*For example, under typical cellular conditions, deamination of cytosine (in DNA) to uracil occurs in about one of every 10^7 cytidine residues in 24 hours. This corresponds to about 100 spontaneous events per day, on average, in a mammalian cell. Deamination of adenine and guanine occurs at about 1/100th this rate.*



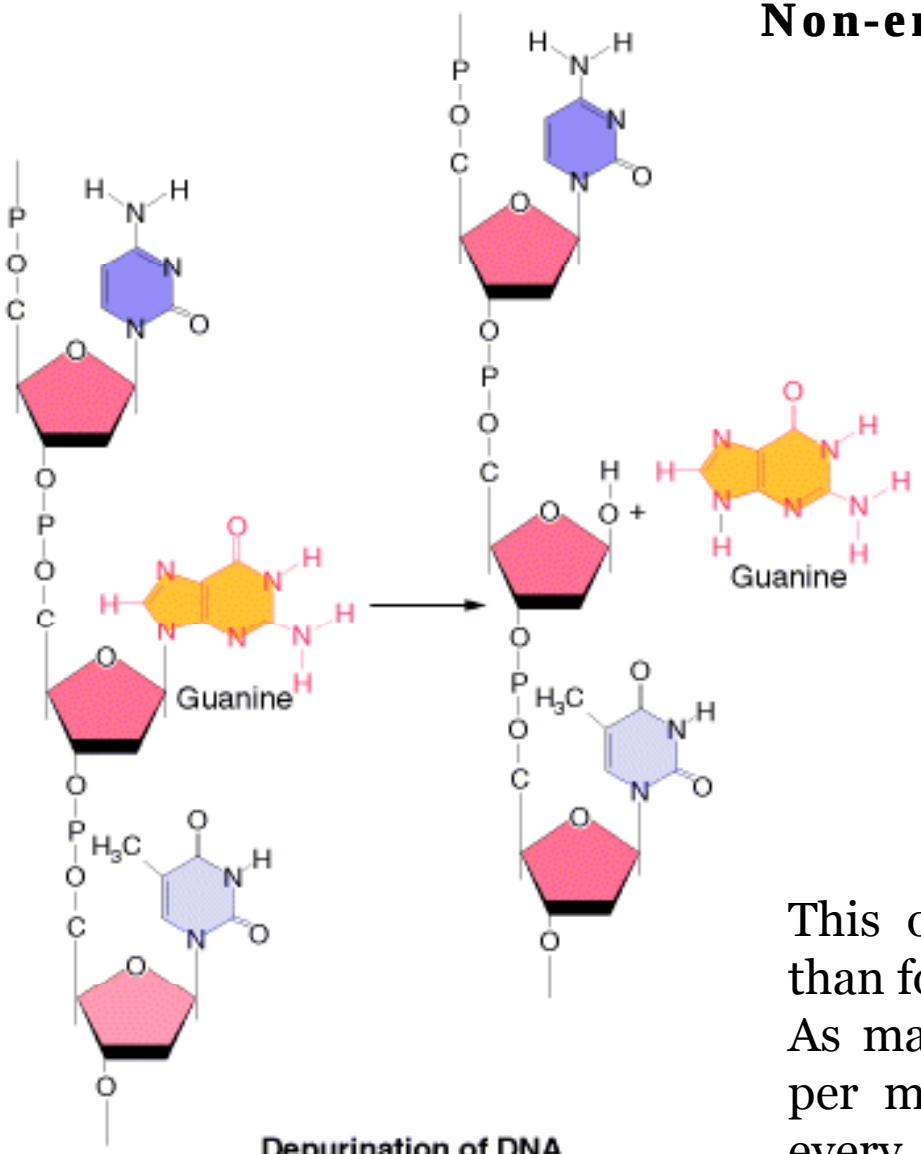

(a) Deamination

## Non-enzymatic reactions of nucleotides

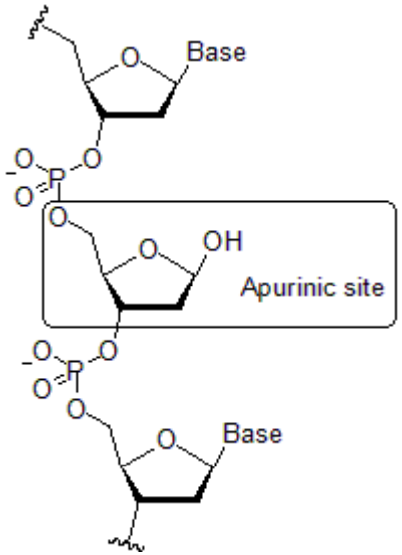


O = oxanine
X = Xanthine
I = Inosine
(Hypoxanthine)

# Non-enzymatic reactions of nucleotides



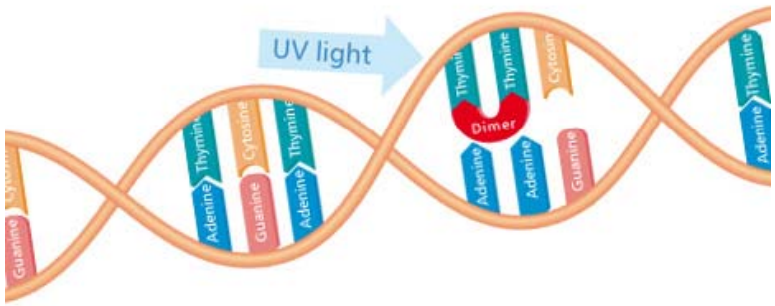

Depurination of DNA



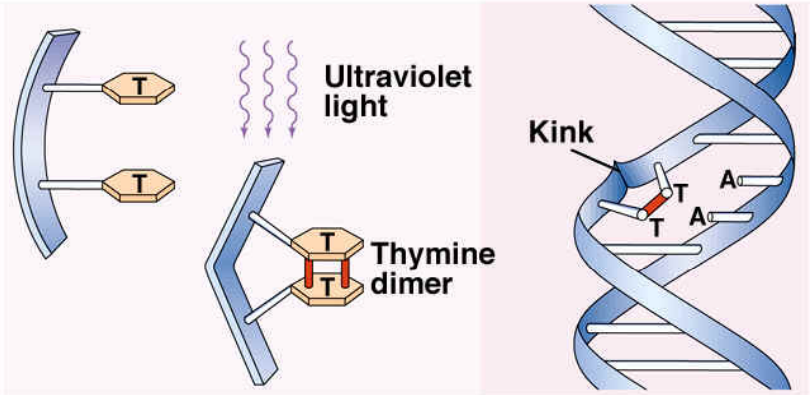

This occurs at a higher rate for purines than for pyrimidines.

As many as one in 10^5 purines (10,000 per mammalian cell) are lost from DNA every 24 hours under typical cellular conditions.

## Transformation by UV light




**Pyrimidine Dimer**




➢UV light induces the condensation of two ethylene groups to form a cyclobutane ring.

➢In the cell, the same reaction between adjacent pyrimidine bases in nucleic acids forms cyclobutane pyrimidine dimers. This happens most frequently between adjacent thymidine residues on the same DNA strand.

➢A second type of pyrimidine dimer, called a 6-4 photoproduct, is alsoformed during UV irradiation.

➢Ionizing radiation (x rays and gamma rays) can cause ring opening and fragmentation of bases as well as breaks in the covalent backbone of nucleic acids.

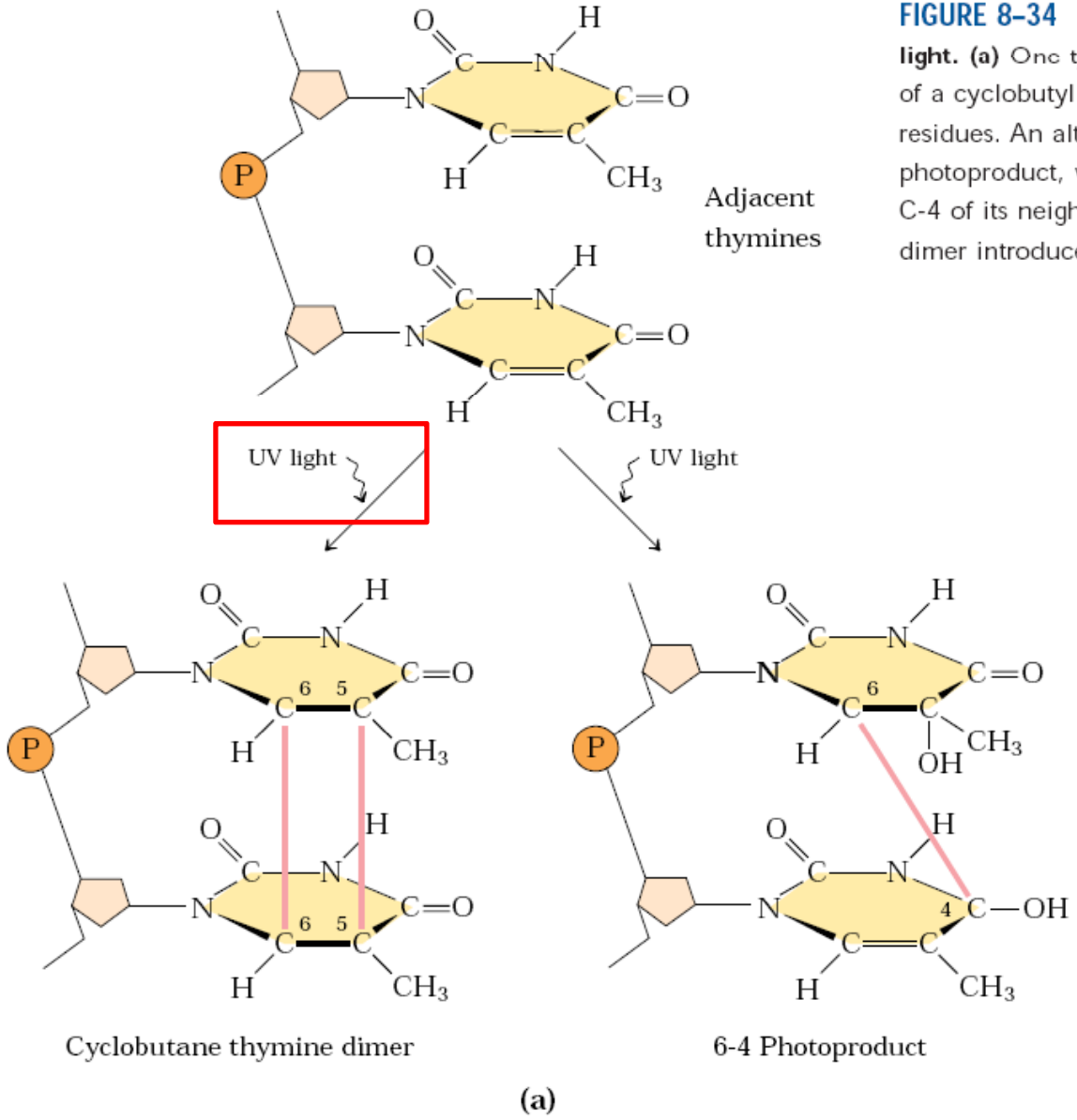
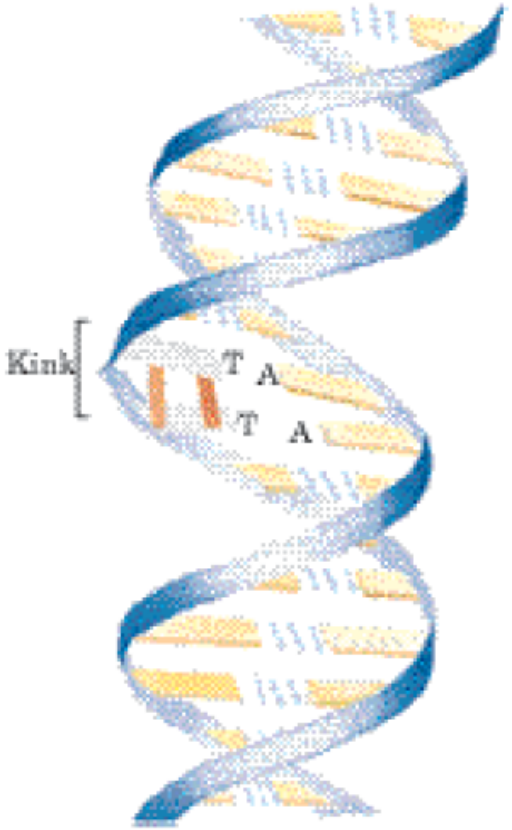



**FIGURE 8–34** **Formation of pyrimidine dimers induced by UV light.** **(a)** One type of reaction (on the left) results in the formation of a cyclobutyl ring involving C-5 and C-6 of adjacent pyrimidine residues. An alternative reaction (on the right) results in a 6-4 photoproduct, with a linkage between C-6 of one pyrimidine and C-4 of its neighbor. **(b)** Formation of a cyclobutane pyrimidine dimer introduces a bend or kink into the DNA.



## Chemical agents that cause DNA damage

❖DNA also may be damaged by reactive chemicals introduced into the environment as products of industrial activity. Such products may not be injurious but may be metabolized by cells into forms that are.

❖Two prominent classes of such agents are (1) **deaminating agents**, particularly nitrous acid ($HNO_2$) or compounds that can be metabolized to nitrous acid or nitrites, and (2) **alkylating agents**.

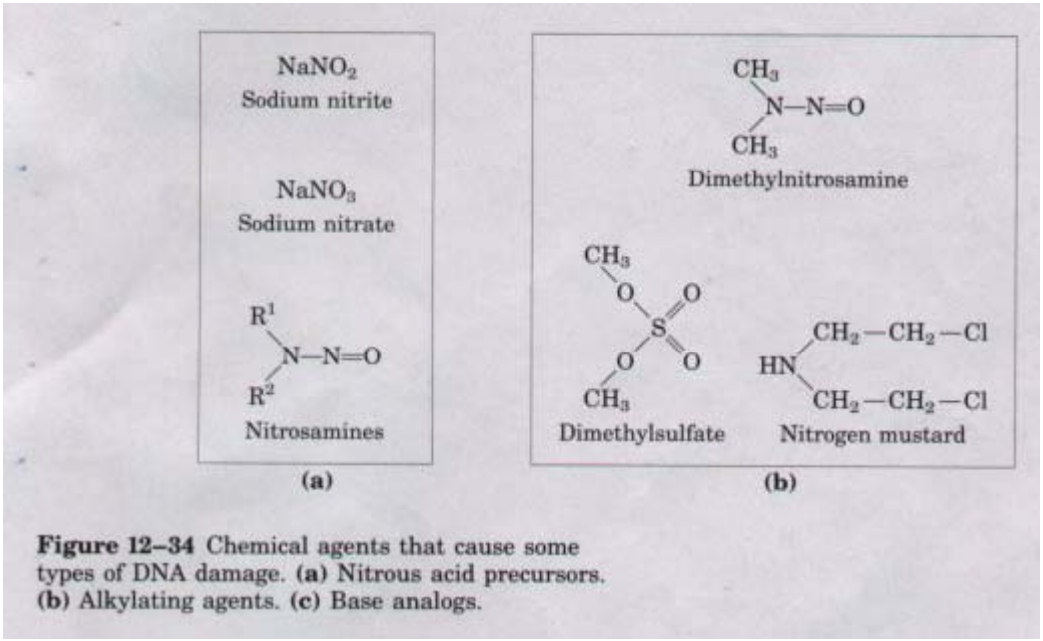
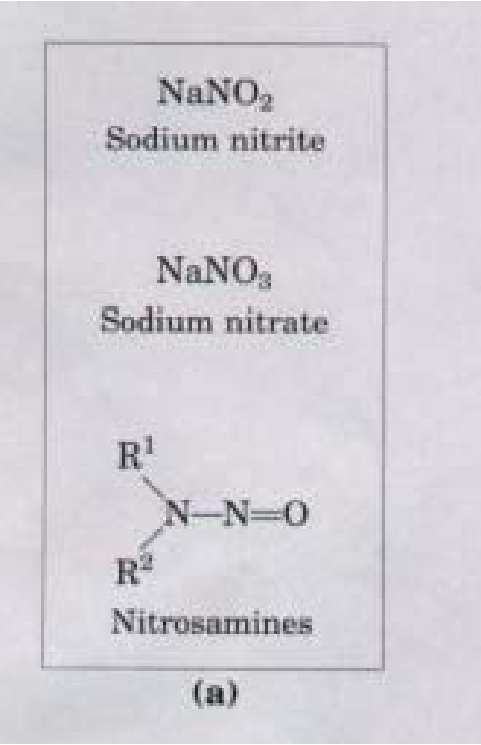


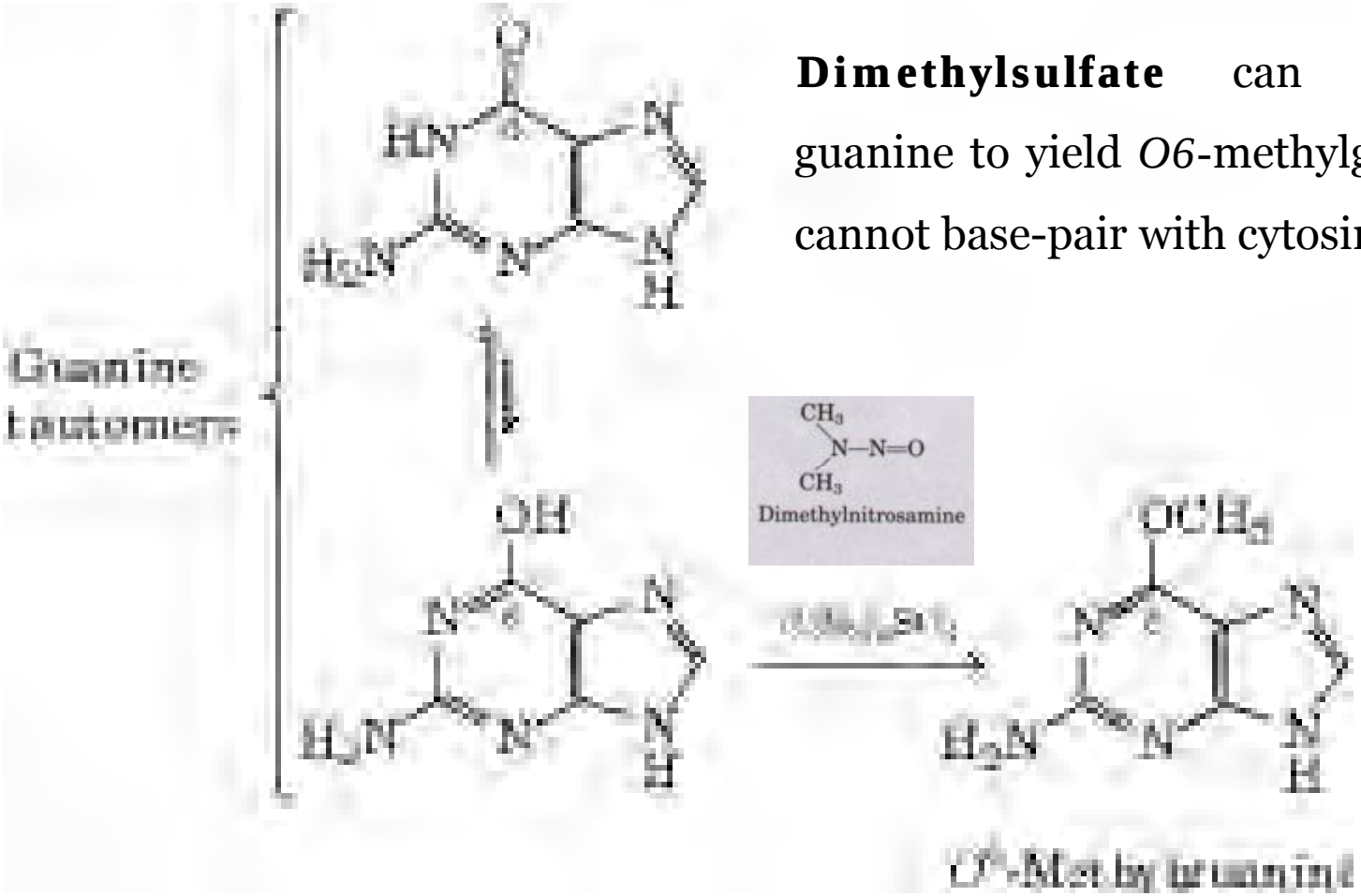

**Figure 12–34** Chemical agents that cause some types of DNA damage. **(a)** Nitrous acid precursors. **(b)** Alkylating agents. **(c)** Base analogs.




**Nitrous acid and Bisulfites**

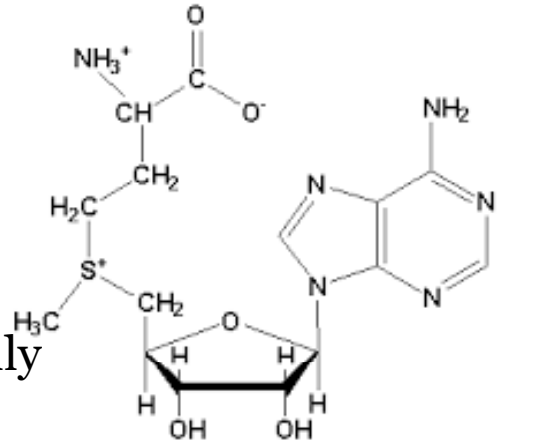
Are used as preservatives in processed foods to **prevent the growth of toxic** bacteria. They do not appear to increase cancer risks significantly when used in this way, perhaps because they are used in small amounts and make only a minor contribution to the overall levels of DNA damage.

## Alkylating agents can alter certain bases of DNA




**Dimethylsulfate** can methylate a guanine to yield *O6*-methylguanine, which cannot base-pair with cytosine.

**Some Bases of DNA Are Methylated.**



S-Adenosylmethionine (S-AdoMet)

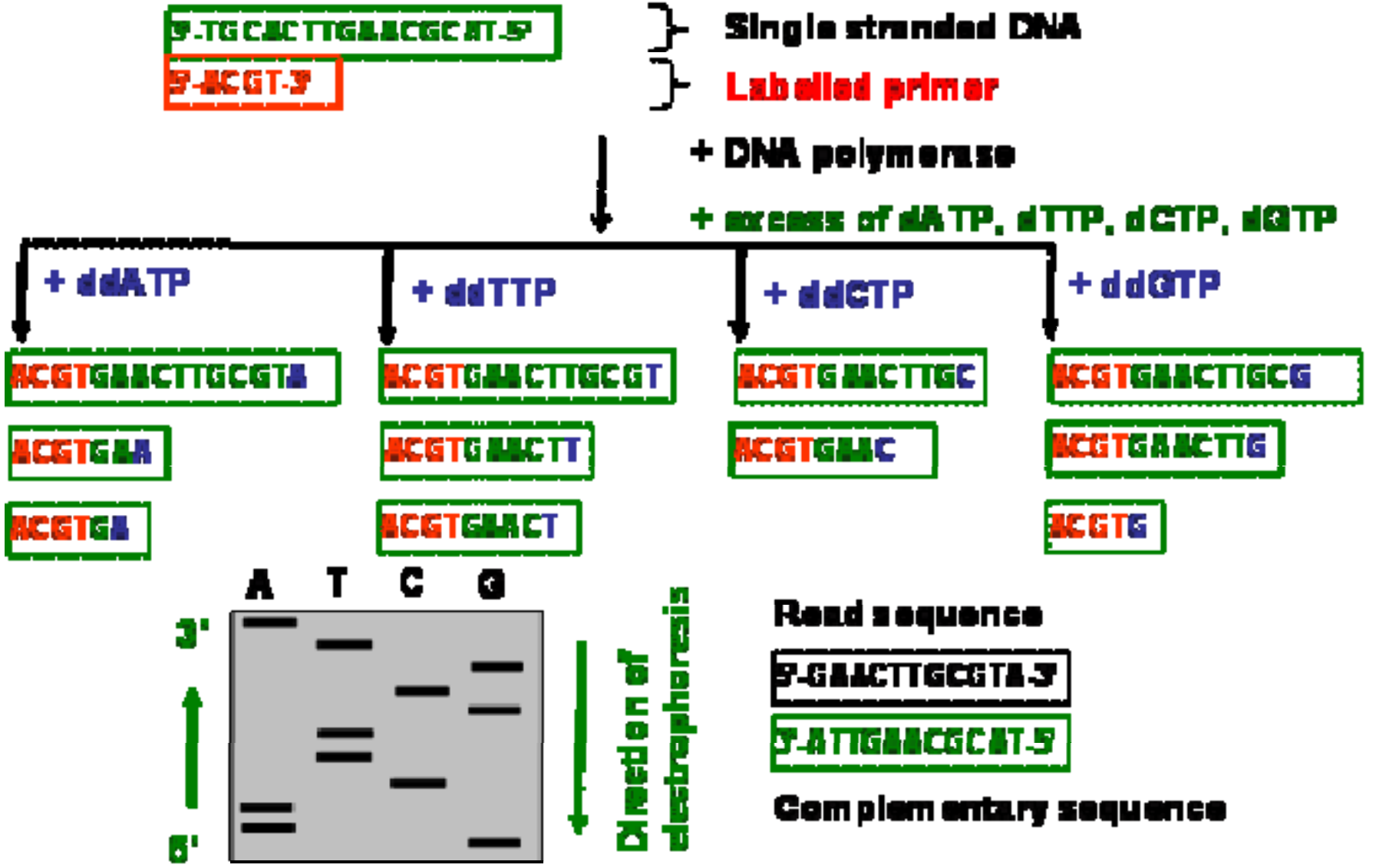
❖ Certain nucleotide bases in DNA molecules are enzymatically methylated.

❖ E. coli has two prominent methylation systems.

❖ One serves as part of a defense mechanism that helps the cell to distinguish its DNA from foreign DNA by marking its own DNA with methyl groups and destroying (foreign) DNA without the methyl groups.

❖ The other system methylates adenosine residues within the sequence (5)GATC(3) to N6-methyladenosine. This is mediated by the Dam (DNA adenine methylation) methylase, a component of a system that repairs mismatched base pairs formed occasionally during DNA replication

# The Sequences of Long DNA Strands Can Be Determined


5'-TGCACTTGAACGCAT-5'
3'-ACGT-5'
Single stranded DNA
Labelled primer
+ DNA polymerase
+ excess of dATP, dTTP, dCTP, dGTP
+ ddATP
+ ddTTP
+ ddCTP
+ ddGTP
ACGTGAACTTGCGTA
ACGTGAA
ACGTGA
ACGTGAACTTGCGT
ACGTGAACTT
ACGTGAACT
ACGTGAACTTGC
ACGTGAAC
ACGTGAACTTGCG
ACGTGAACTTG
ACGTG
A T C G
3'
5'
Direction of electrophoresis
Read sequence
5'-GAACTTGCGTA-3'
3'-ATTGAACGCAT-5'
Complementary sequence