Nucleotide Biosynthesis

- Read through: Horton ch 19, page 583-597

National Human Genome research Institute

http://www.genome.gov/Education/

1869: Friedrich Miescher isolates DNA for the first time.

⇩

1944: DNA is "Transforming Principle"

⇩

1952: DNA Triple Helix?

⇩

1953: DNA Double Helix

Reprinted from the Proceedings of the National Academy of Sciences,
Vol. 39, No. 2, pp. 84–97. February, 1953

A PROPOSED STRUCTURE FOR THE NUCLEIC ACIDS

By Linus Pauling and Robert B. Corey

Gates and Crellin Laboratories of Chemistry,* California Institute of Technology

Communicated December 31, 1952

The nucleic acids, as constituents of living organisms, are comparable in importance to the proteins. There is evidence that they are involved in the processes of cell division and growth, that they participate in the transmission of hereditary characters, and that they are important constituents of viruses. An understanding of the molecular structure of the nucleic acids should be of value in the effort to understand the fundamental phenomena of life.

We have now formulated a promising structure for the nucleic acids, by making use of the general principles of molecular structure and the available information about the nucleic acids themselves. The structure is not a vague one, but is precisely predicted; atomic coordinates for the principal atoms are given in table 1. This is the first precisely described structure for the nucleic acids that has been suggested by any investigator. The structure accounts for some of the features of the x-ray photographs; but detailed intensity calculations have not yet been made, and the structure cannot be considered to have been proved to be correct.

The Formulation of the Structure.—Only recently has reasonably complete information been gathered about the chemical nature of the nucleic acids. The nucleic acids are giant molecules, composed of complex units. Each unit consists of a phosphate ion, HPO_4^{--}, a sugar (ribose in the ribonucleic acids, deoxyribose in the deoxyribonucleic acids), and a purine or pyrimidine side chain (adenine, guanine, thymine, cytosine, uracil, 5-methylcytosine). The purine or pyrimidine group is attached to carbon atom 1′ of the sugar, through the ring nitrogen atom 3 in the case of the pyrimidine nucleotides,[1] and the ring nitrogen atom 9 in the case of the purine nucleotides.[2] Good

and the ring nitrogen atom 9 in the case of the purine nucleotides.[2] Good evidence has recently been obtained as to the nature of the linkage between the sugar and the phosphate, through the investigations of Todd and his collaborators;[3] it seems likely that the phosphate ester links involve carbon atoms 3′ and 5′ of the ribose or deoxyribose. New chemical evidence that the natural ribonucleosides have the β-D-ribofuranose configuration has also been reported by Todd and his collaborators,[4] and spectroscopic evidence indicating that the deoxyribonucleosides have the same configuration as the ribonucleosides has been obtained.[5] The β-D-ribofuranose configuration has been verified for cytidine by the determination of the structure of

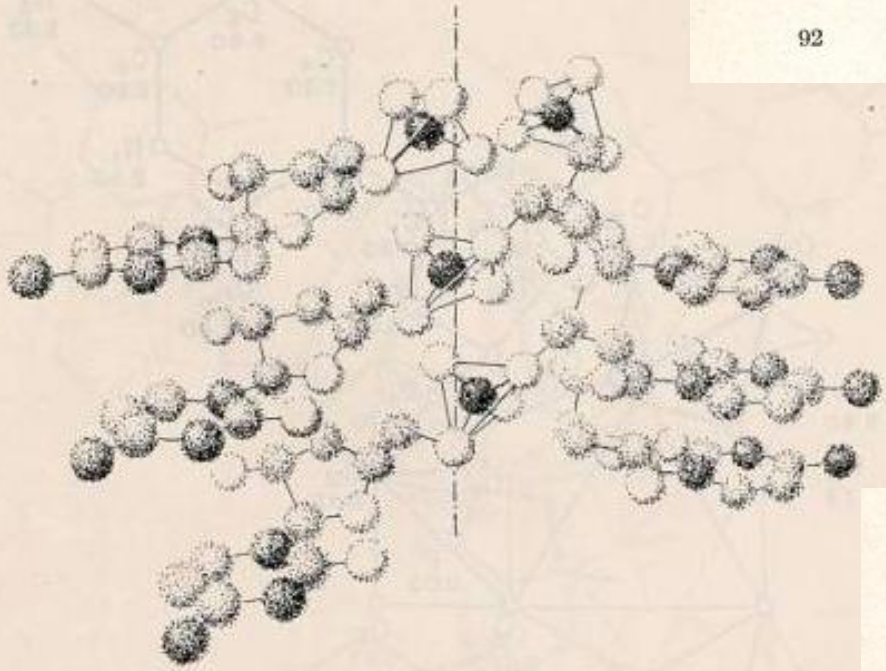


FIGURE 4

Perspective drawing of a portion of the nucleic acid structure, showing the phospha tetrahedra near the axis of the molecule, the β-D-ribofuranose rings connecting the tet hedra into chains, and the attached purine and pyrimidine rings (represented as puri rings in this drawing). The molecule is inverted with respect to the coordinates giv in table 1.

Triple helix?

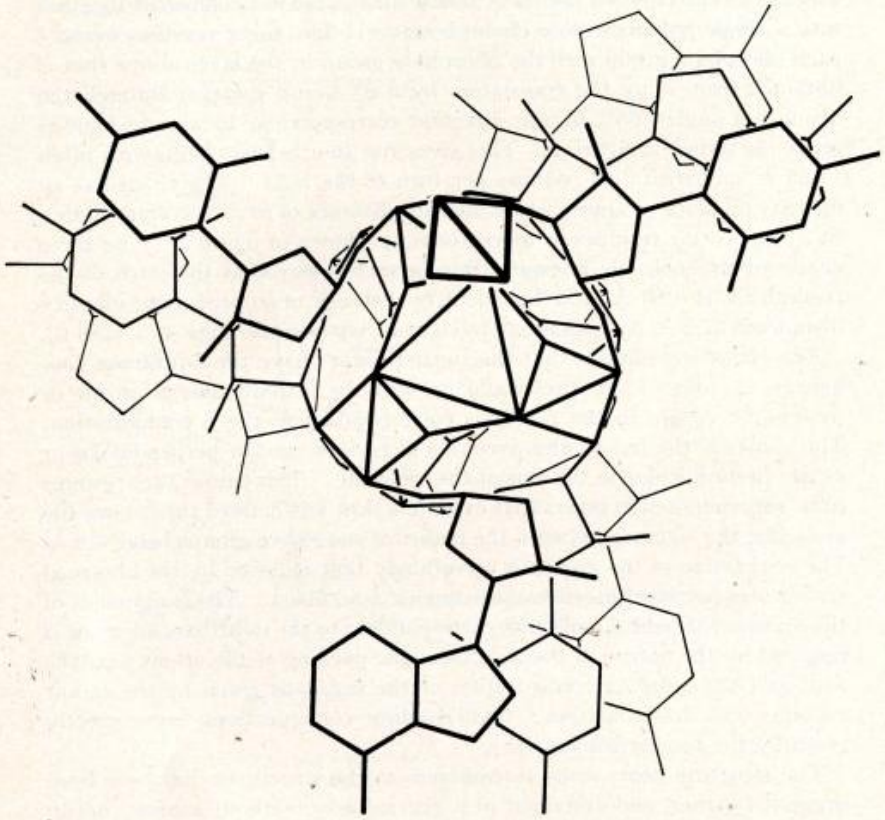


FIGURE 6

Plan of the nucleic acid structure, showing several nucleotide residues.

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion. this structure is unsatisfactory for two reasons:

(1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J.D.W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J.D. WATSON
F.H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 2.

[1] Pauling, L., and Corey, R. B. nature, 171, 346 (1953); Proc, U.S. Nat Acad. Sci., 39, 84 (1953).

[2] Furberg, S., Acta Chem. Scand., 6, 634 (1952).

[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., Biochim, et Biophys. Acta, 9 402 (1952).

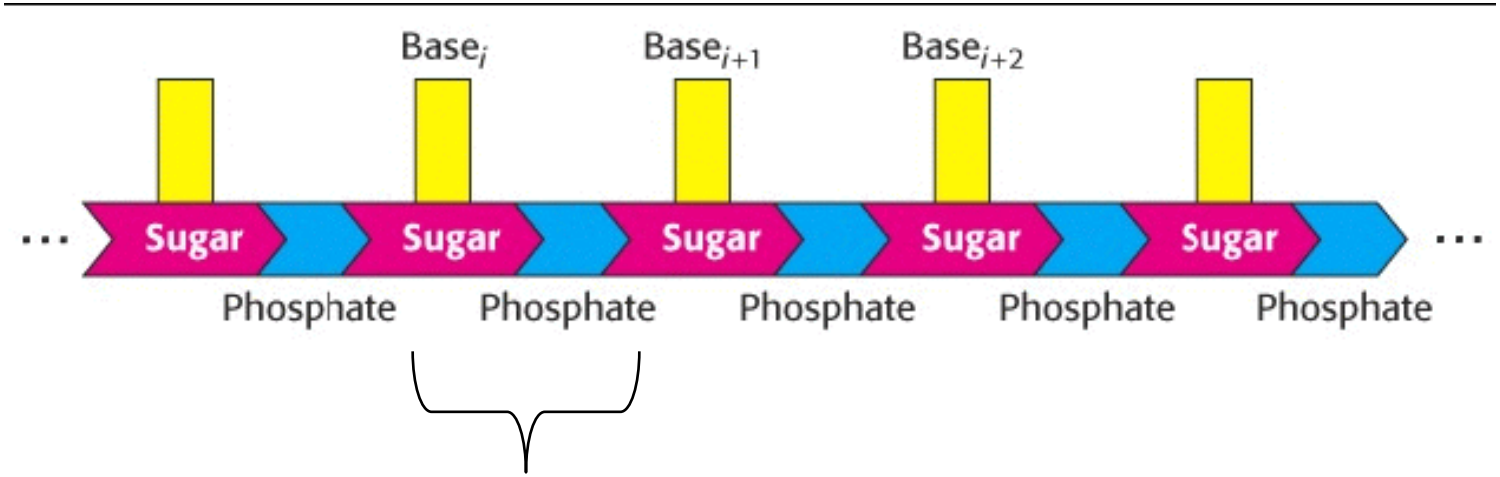
[4] Wyatt, G.R. J. Gen. Physiol., 36 201 (1952).

[5] Astbury, W.T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947)

[6] Wilkins, M. H. F. and Randall, J. T. Biochim. et. Biophys. Acta, 10, 102 (1953).

A Nucleic Acid (DNA or RNA) is a polymer that consists of:

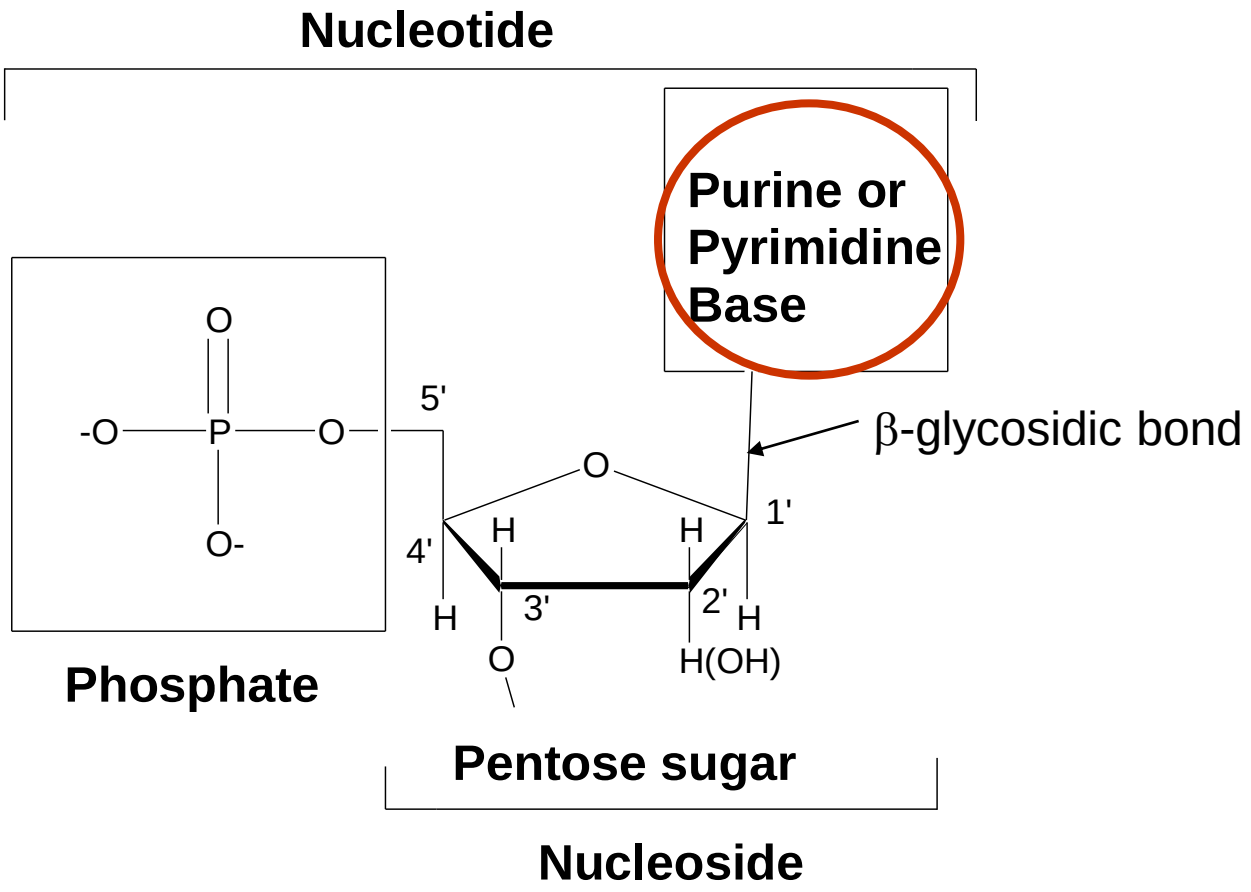
5-carbon sugars
Phosphate
Base (N-ring structure)



Nucleotide = Base + pentose sugar + phosphate

Nucleotides linked by phosphodiester bonds (covalent)

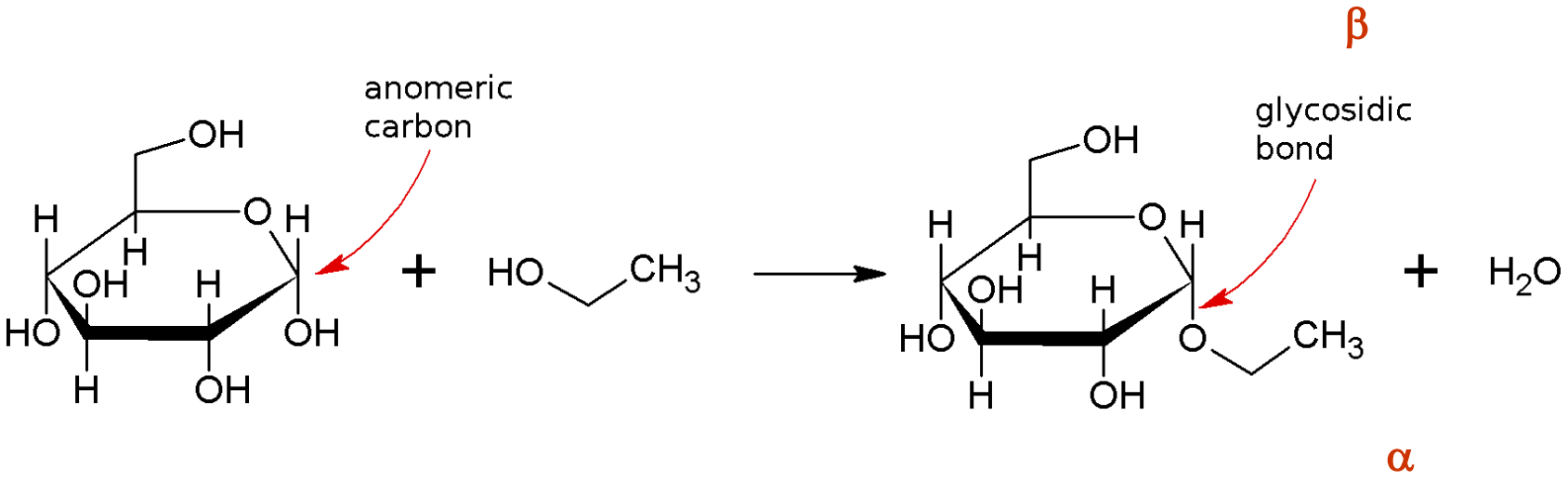
Nucleotides



Phosphate binds to
5’ or 3’ of pentose sugar

RNA- ribose (R)
DNA – deoxyribose (dR)

GLYCOSIDIC BOND: A bond between a sugar and another organic molecule by way of an intervening nitrogen or oxygen atom.

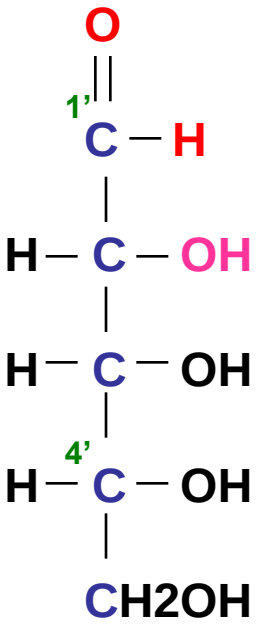


Sugars in Nucleic Acids

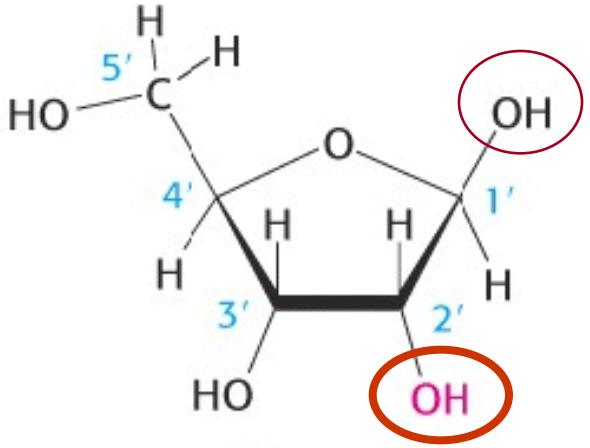
aldehyde

β-furanose

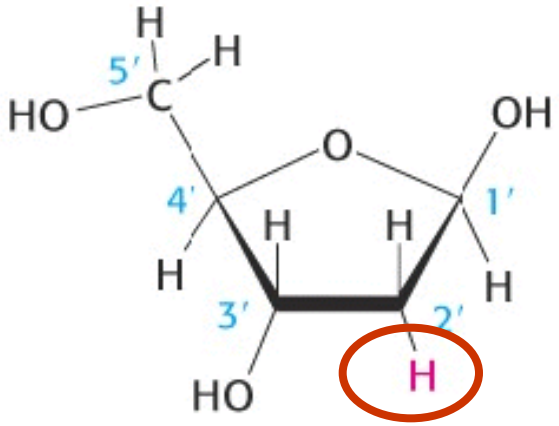
RNA



DNA



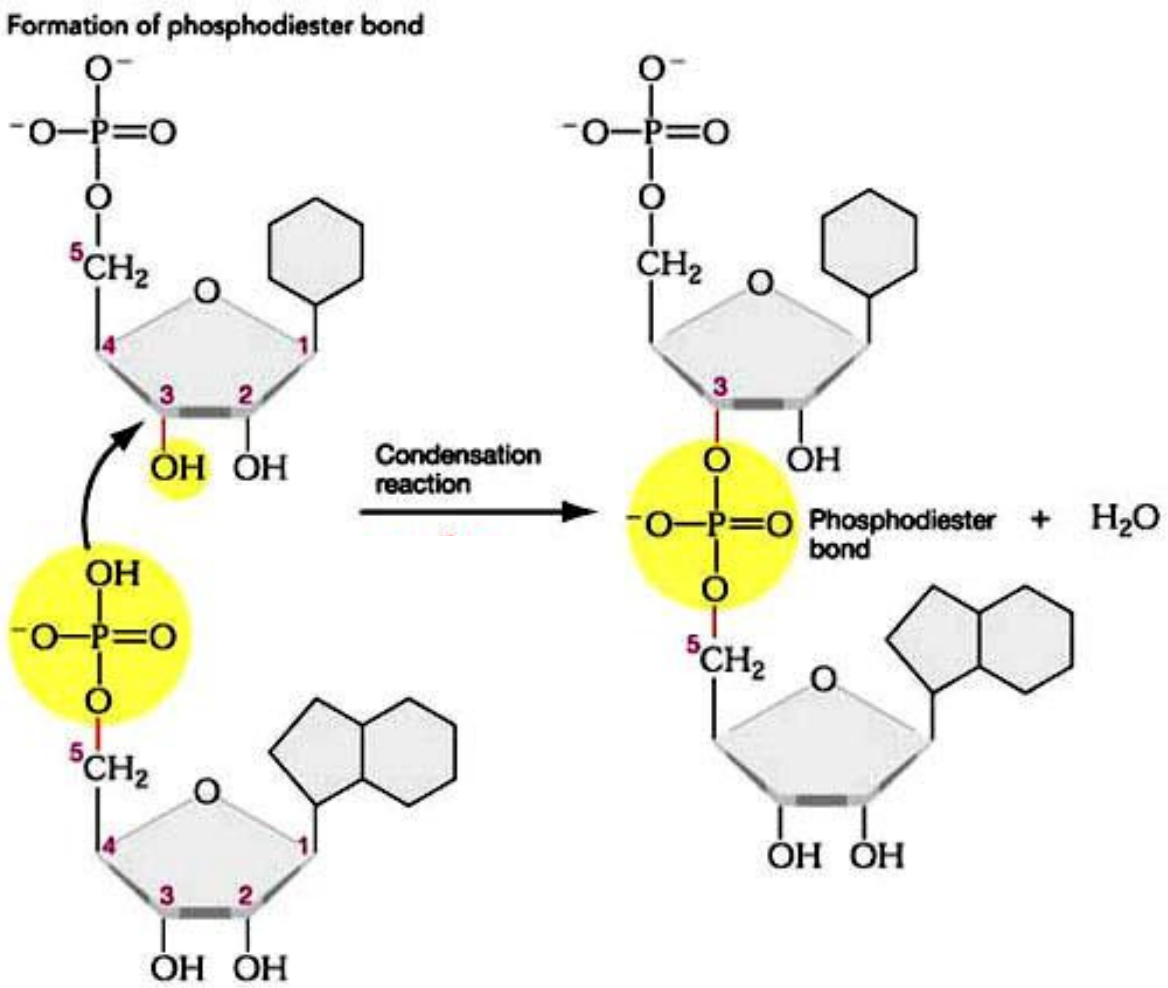
Ribose



Deoxyribose

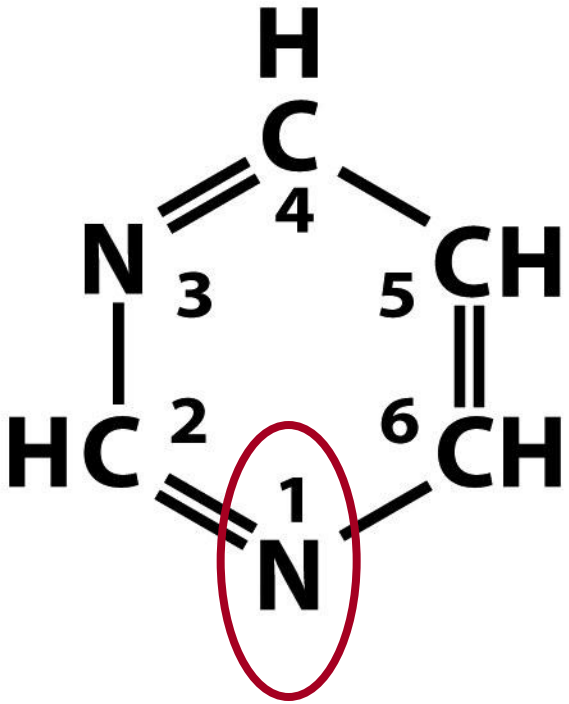
Both in β-furanose form (ring closure of aldehyde between C1 and C4)

ESTER: condensation product of an alcohol and an acid (water eliminated)

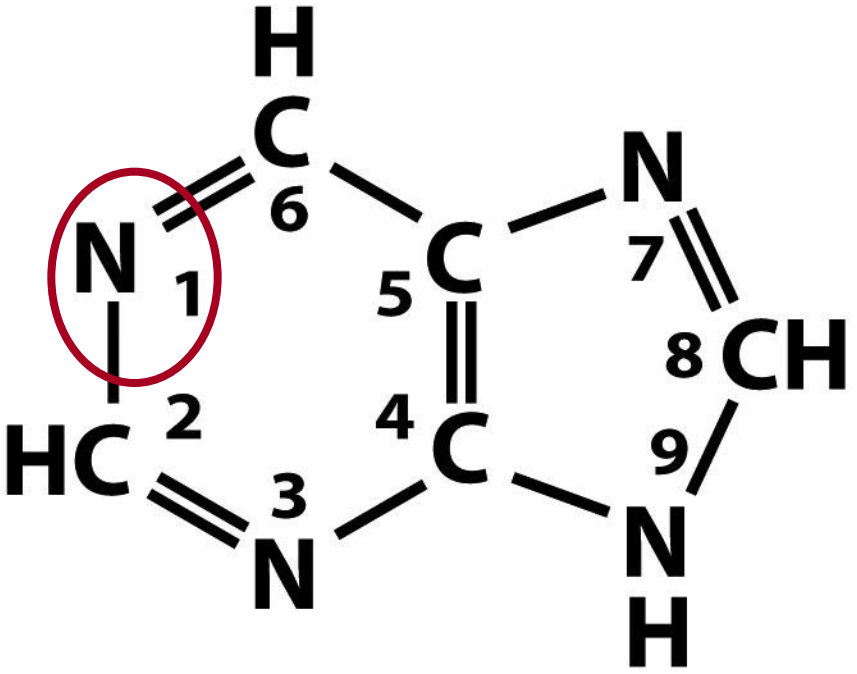


PHOSPHODIESTER: A bond between two sugar groups via a phosphate group.

Generic structure and numbering



Pyrimidine



Purine

Figure 8-1b
Lehninger Principles of Biochemistry, Fifth Edition

N1 joins to pentose

N9 joins to pentose

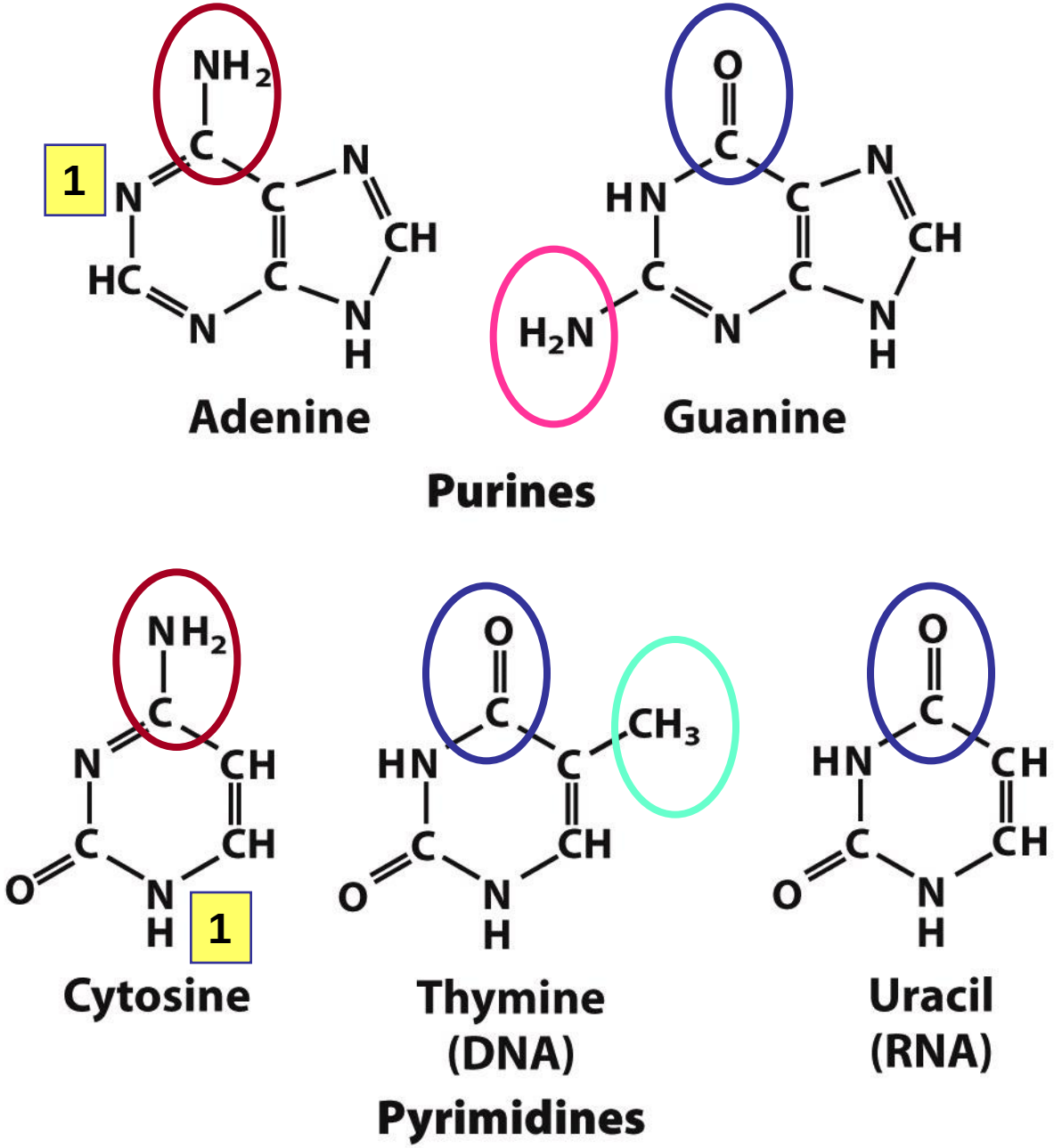
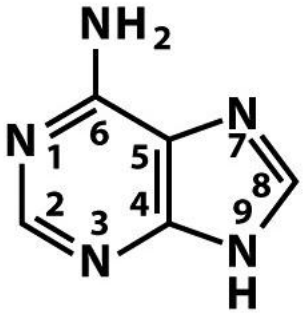


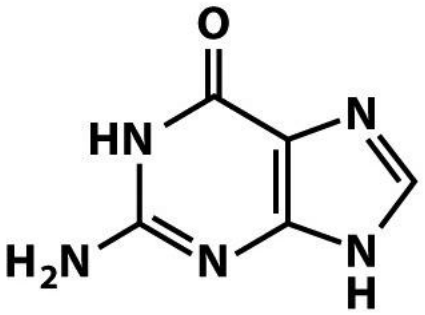
Figure 8-2
Lehninger Principles of Biochemistry, Fifth Edition

PURINES



Common name

Adenine
(6-Aminopurine)

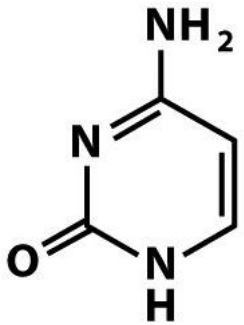


Guanine
(2-Amino-6-oxopurine)

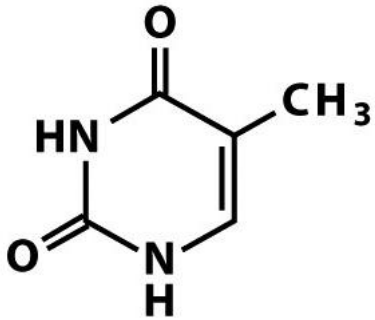
Chemical name

Figure 19-4 Principles of Biochemistry, 4/e

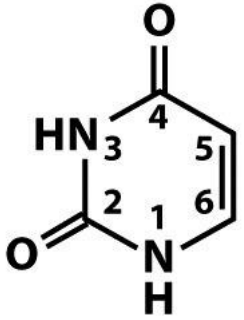
PYRIMIDINES



Cytosine
(2-Oxo-4-aminopyrimidine)

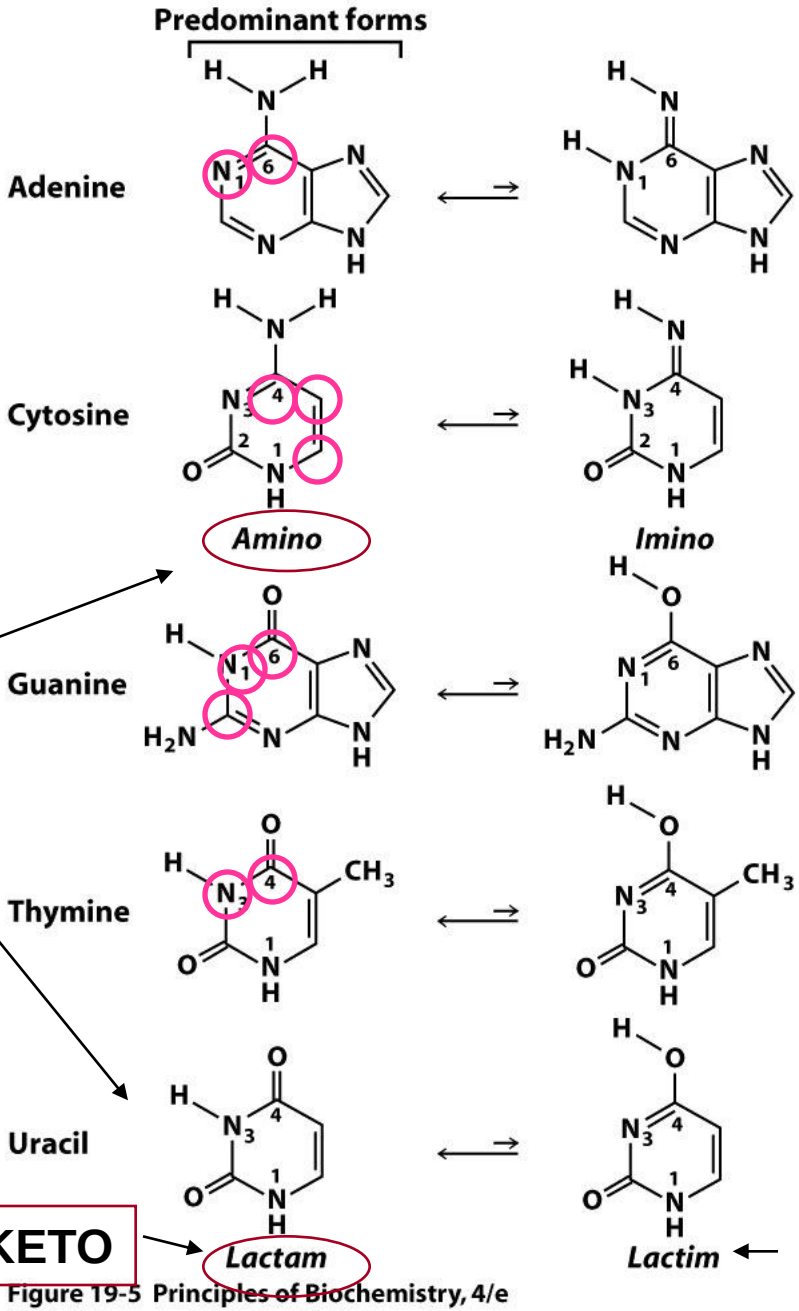


Thymine
(2,4-Dioxo-5-methylpyrimidine)



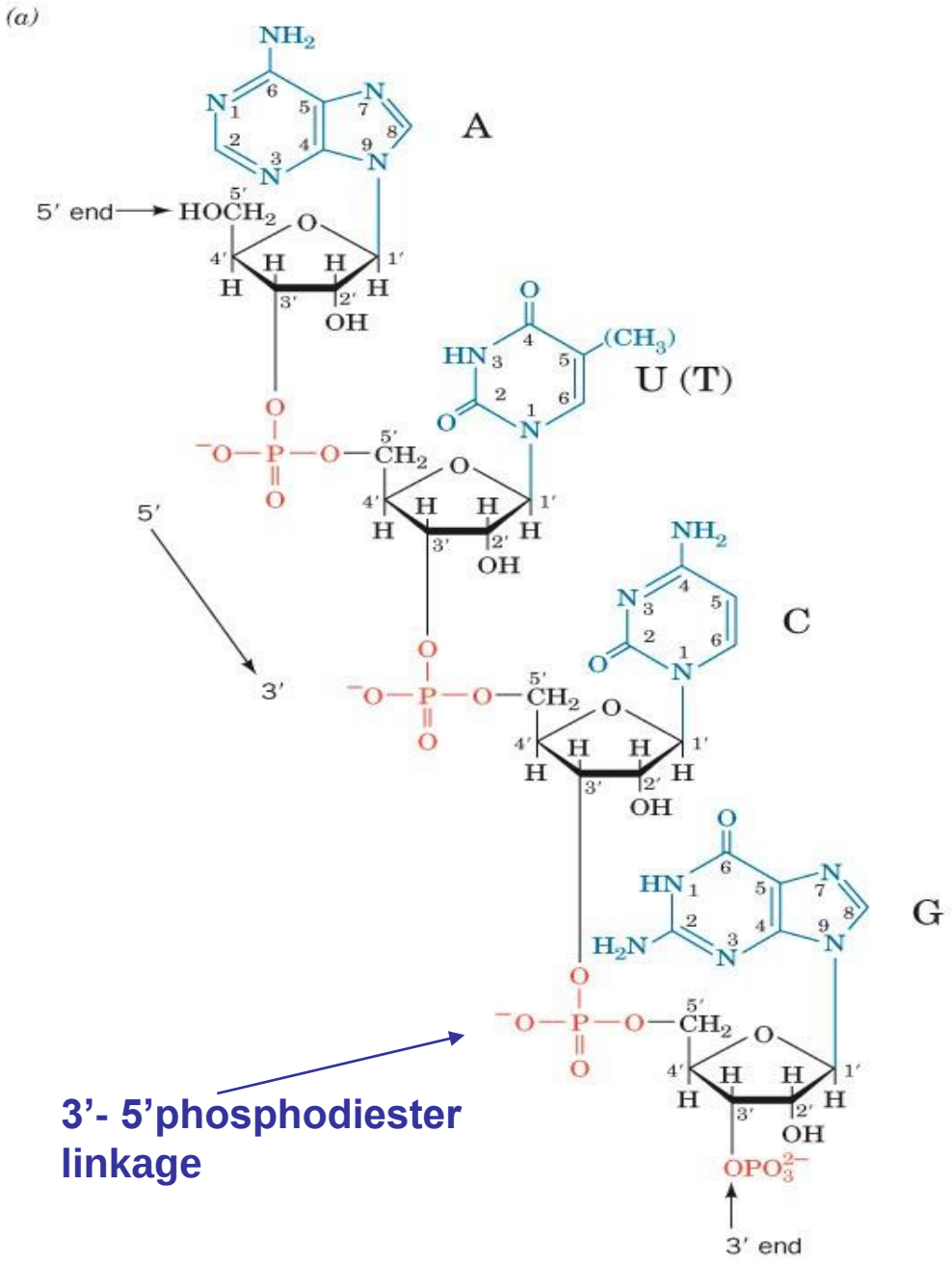
Uracil
(2,4-Dioxopyrimidine)

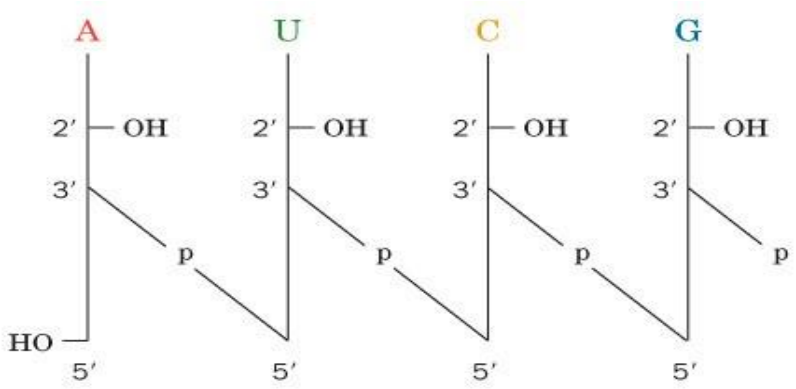
Each base can exist in two tautomeric forms;



Amino and Lactam forms are more stable; therefore predominate in the cell

Figure 19-5 Principles of Biochemistry, 4/e





ApUpCpGp

AUCGp

Polynucleotide chains have 5’-3’directionality

Task 1

- **Draw the following nucleotide sequence in full. Show the structure and numbering of each base and pentose sugar**
  - **pGpCpA**
- **Draw the shorthand of the above structure showing orientation of the phosphodiester bond, pentose sugar and base**

Chemical and Physical Properties of Nucleic Acids

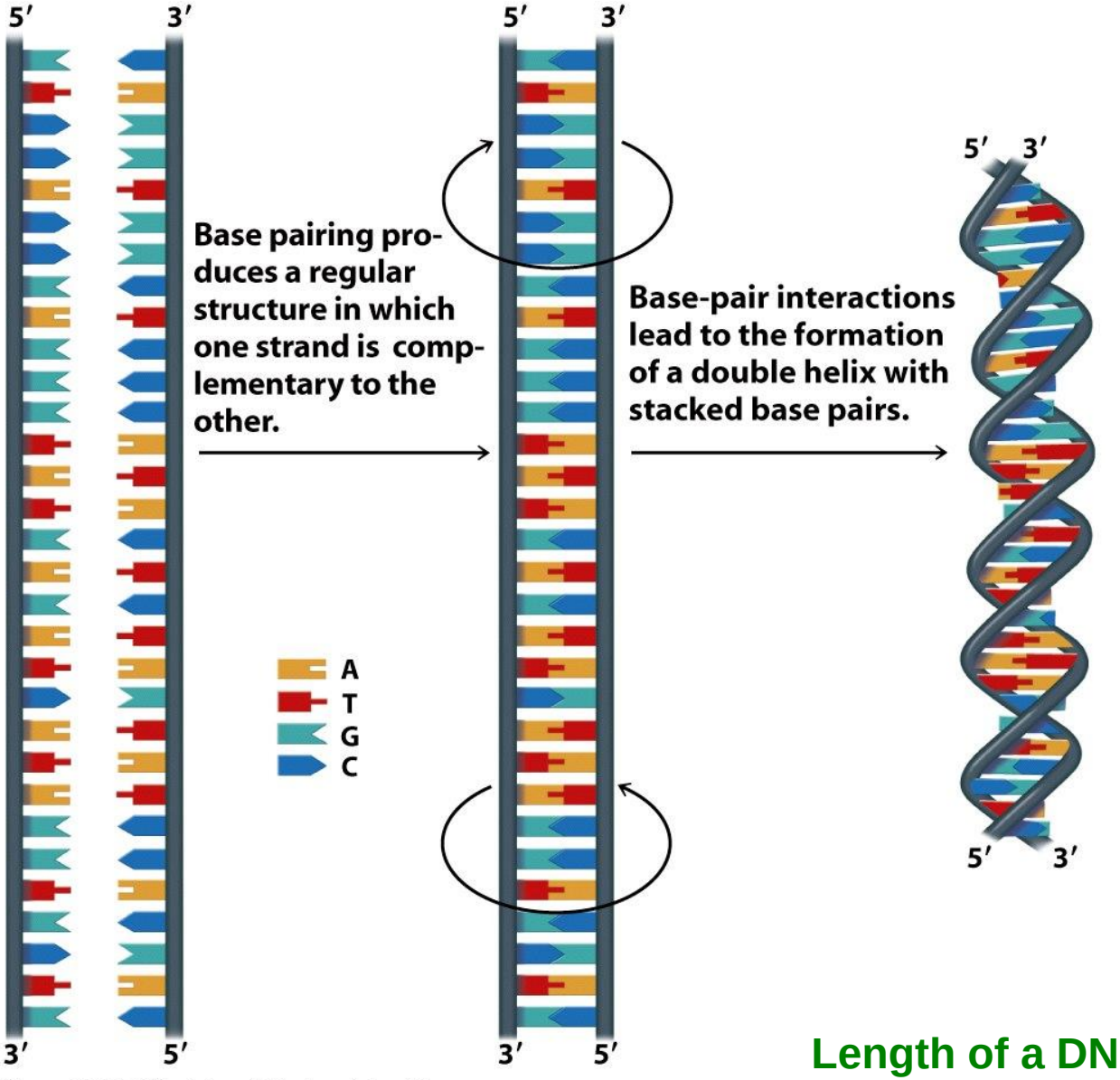


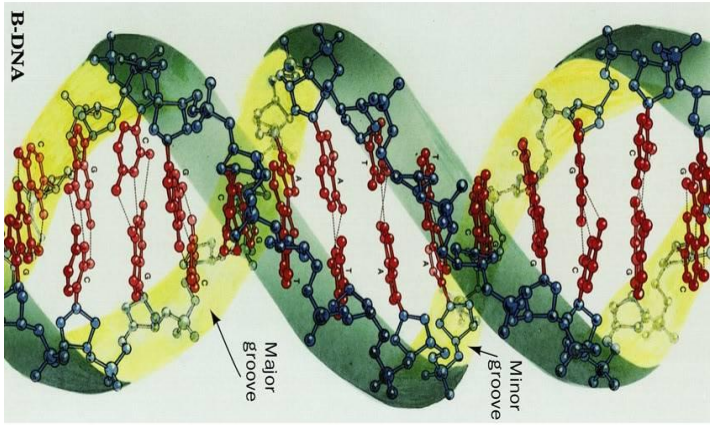
Figure 19-13 Principles of Biochemistry, 4/e

Hydrophilic on the outside (sugar phosphates)

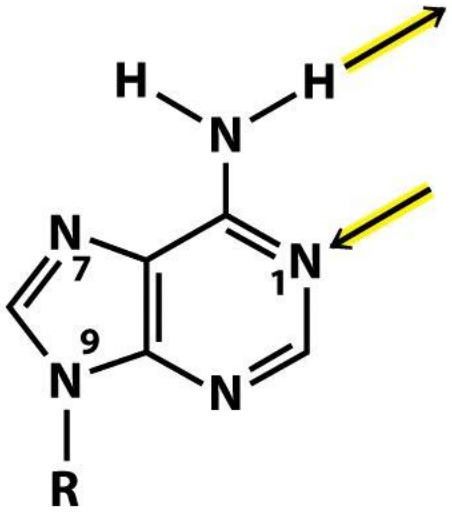
Hydrophobic on the inside (bases)

Length of a DNA molecule is expressed in base pairs (bp's) or kilo bp's

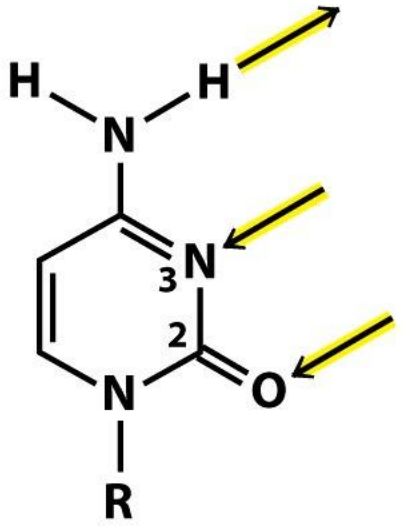
Structure and Stability



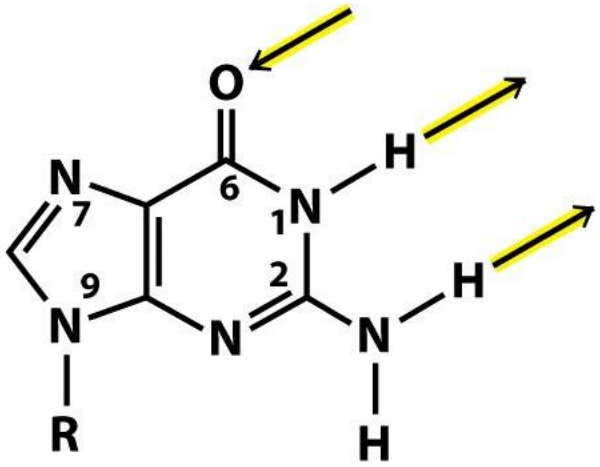
- Two anti-parallel strands form a double helix
- Hydrogen bonding responsible for specificity (i.e. A-T/U or G-C)
- Hydrophobic interactions and van der Waals forces between the stacked bases – helix and stability
- Phosphodiester bond (backbone) – covalent
- DNA more chemically stable than RNA (2'-OH group)



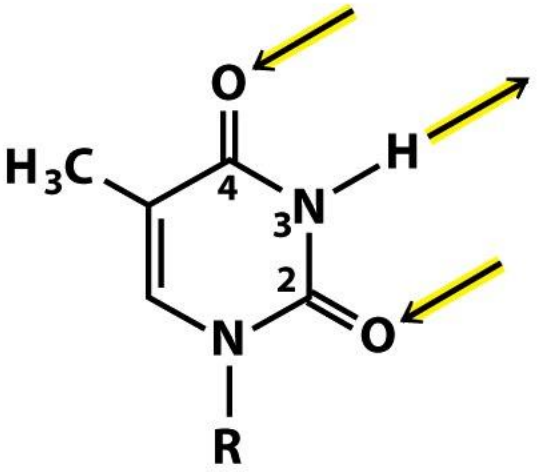
(Deoxy)Adenosine



(Deoxy)Cytidine



(Deoxy)Guanosine



(Deoxy)Thymidine

Figure 19-6 Principles of Biochemistry, 4/e

purines pyrimidines

TABLE 19.2 Base composition of DNA (mole %) and ratios of bases

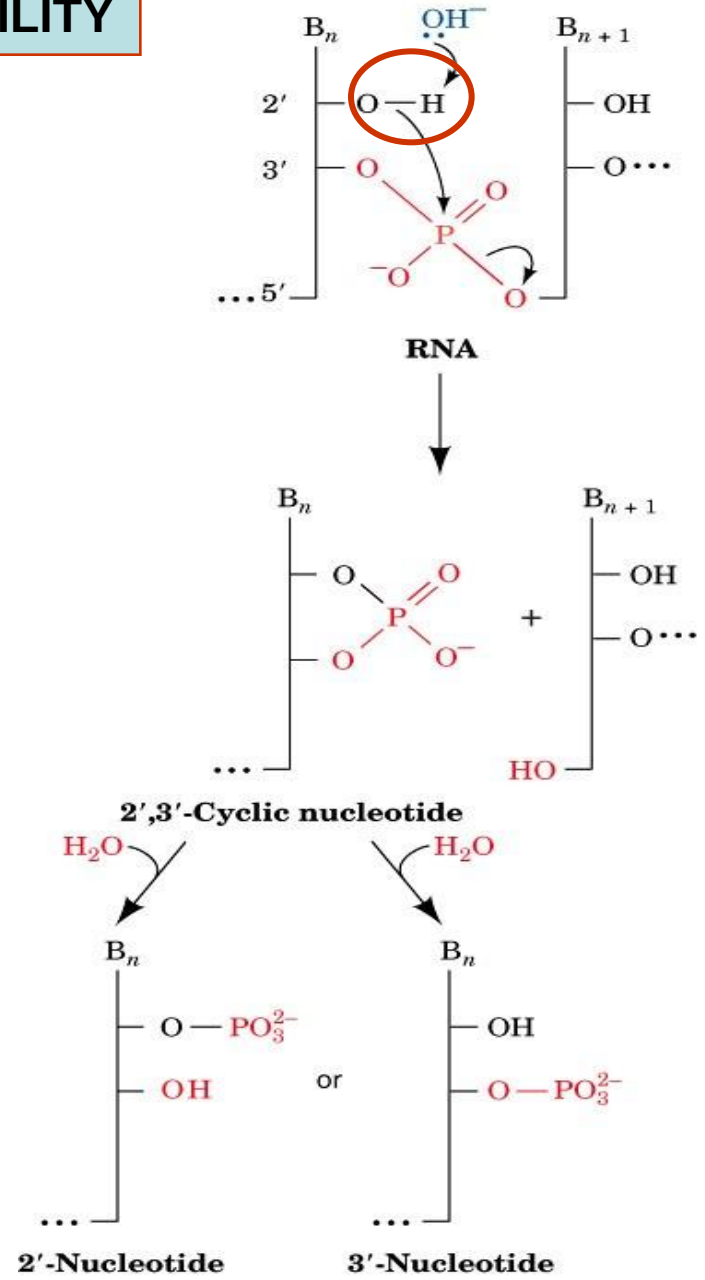
Source	A	G	C	T	A/T[a]	G/C[a]	(G + C)	Purine/ pyrimidine[a]
Escherichia coli	26.0	24.9	25.2	23.9	1.09	0.99	50.1	1.04
Mycobacterium tuberculosis	15.1	34.9	35.4	14.6	1.03	0.99	70.3	1.00
Yeast	31.7	18.3	17.4	32.6	0.97	1.05	35.7	1.00
Cow	29.0	21.2	21.2	28.7	1.01	1.00	42.4	1.01
Pig	29.8	20.7	20.7	29.1	1.02	1.00	41.4	1.01
Human	30.4	19.9	19.9	30.1	1.01	1.00	39.8	1.01

[a] Deviations from a 1:1 ratio are due to experimental variations.

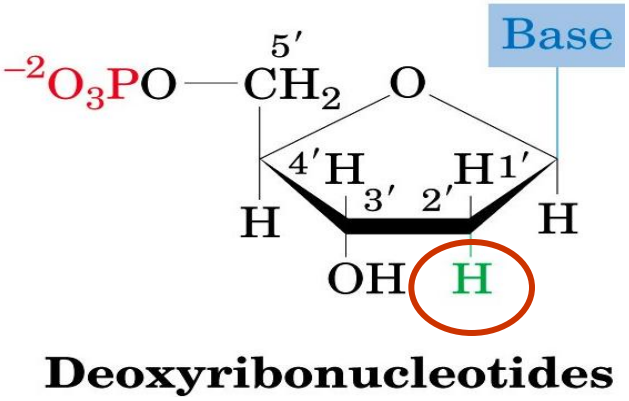
Table 19-2 Principles of Biochemistry, 4/e

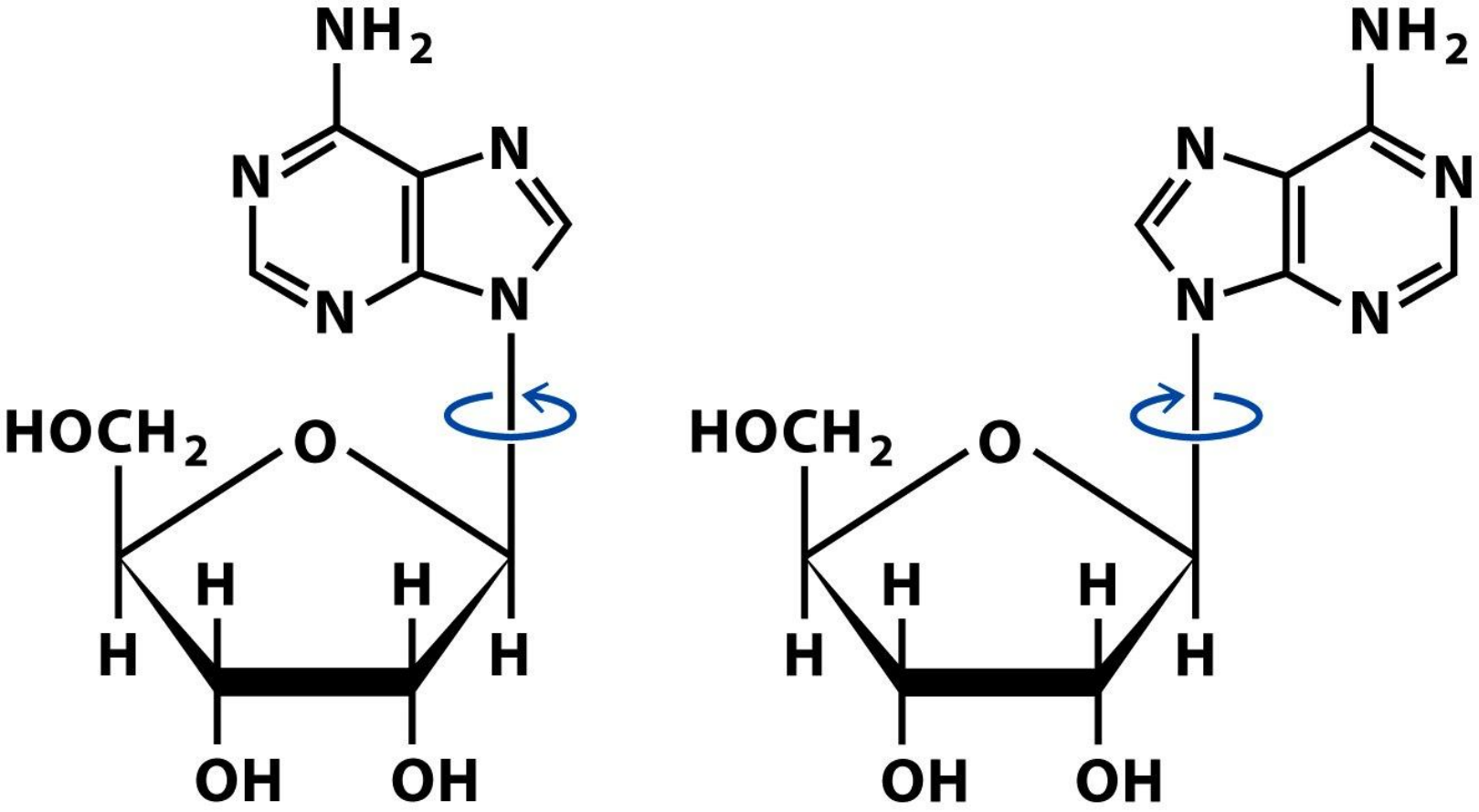
Chargaff's rule

STABILITY



Base-catalyzed RNA hydrolysis.





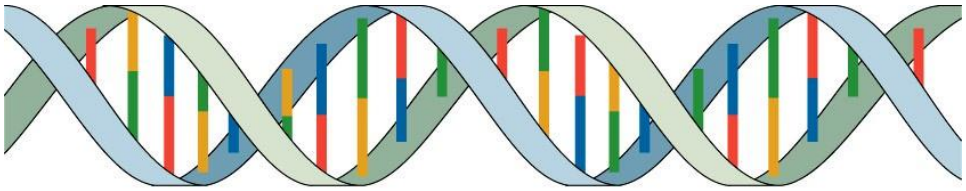
***syn* Adenosine** ***anti* Adenosine**

Figure 19-8 Principles of Biochemistry, 4/e

More common

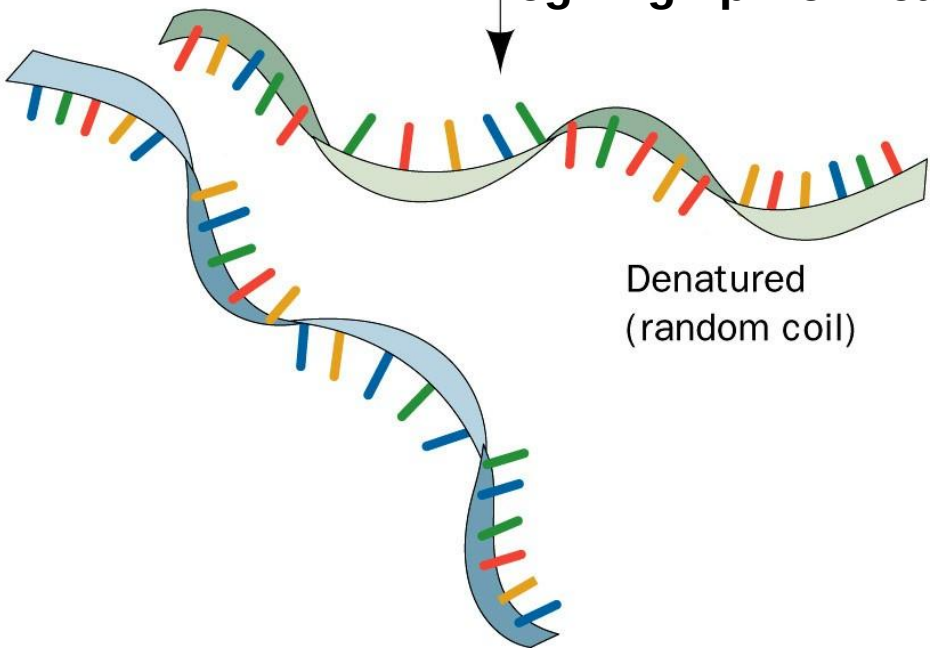
DNA Denaturation

More stable

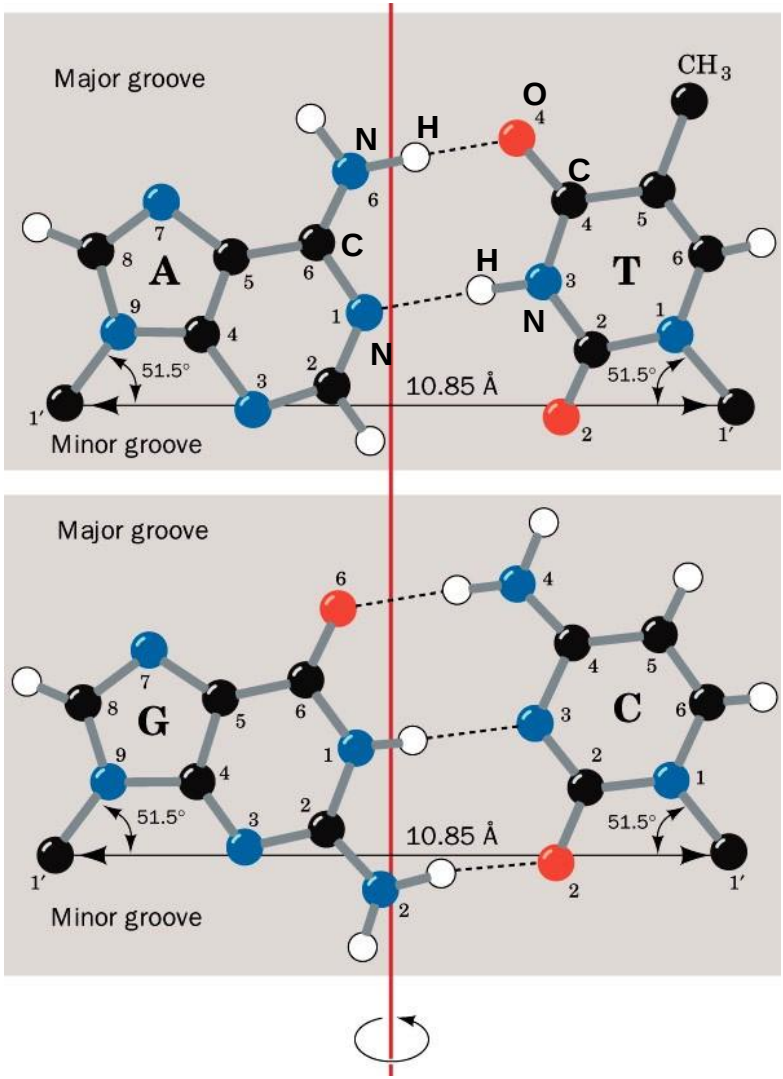


Native (double helix)

eg. High pH or heat



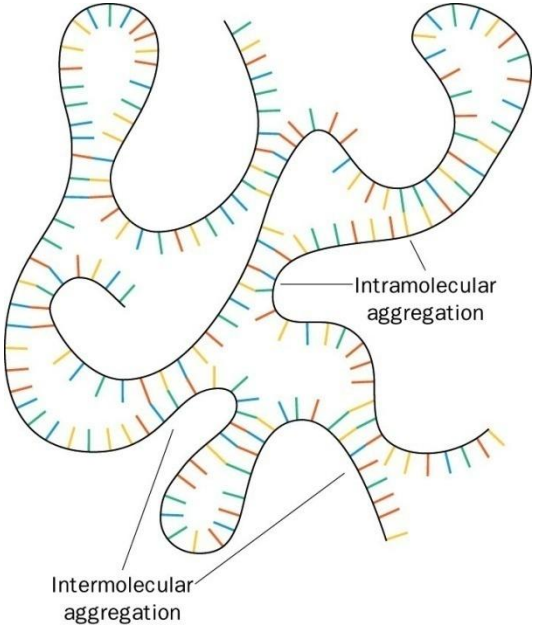
Denatured
(random coil)



Covalent bonds are not affected

Renaturation

- Complementary bases pair as temperature/pH returns to normal
- Two strands anneal/hybridize



Denaturation/Renaturation required for

- Replication and transcription of DNA
- PCR (polymerase chain reaction)
- Sequencing
- Southern and Northern Blotting

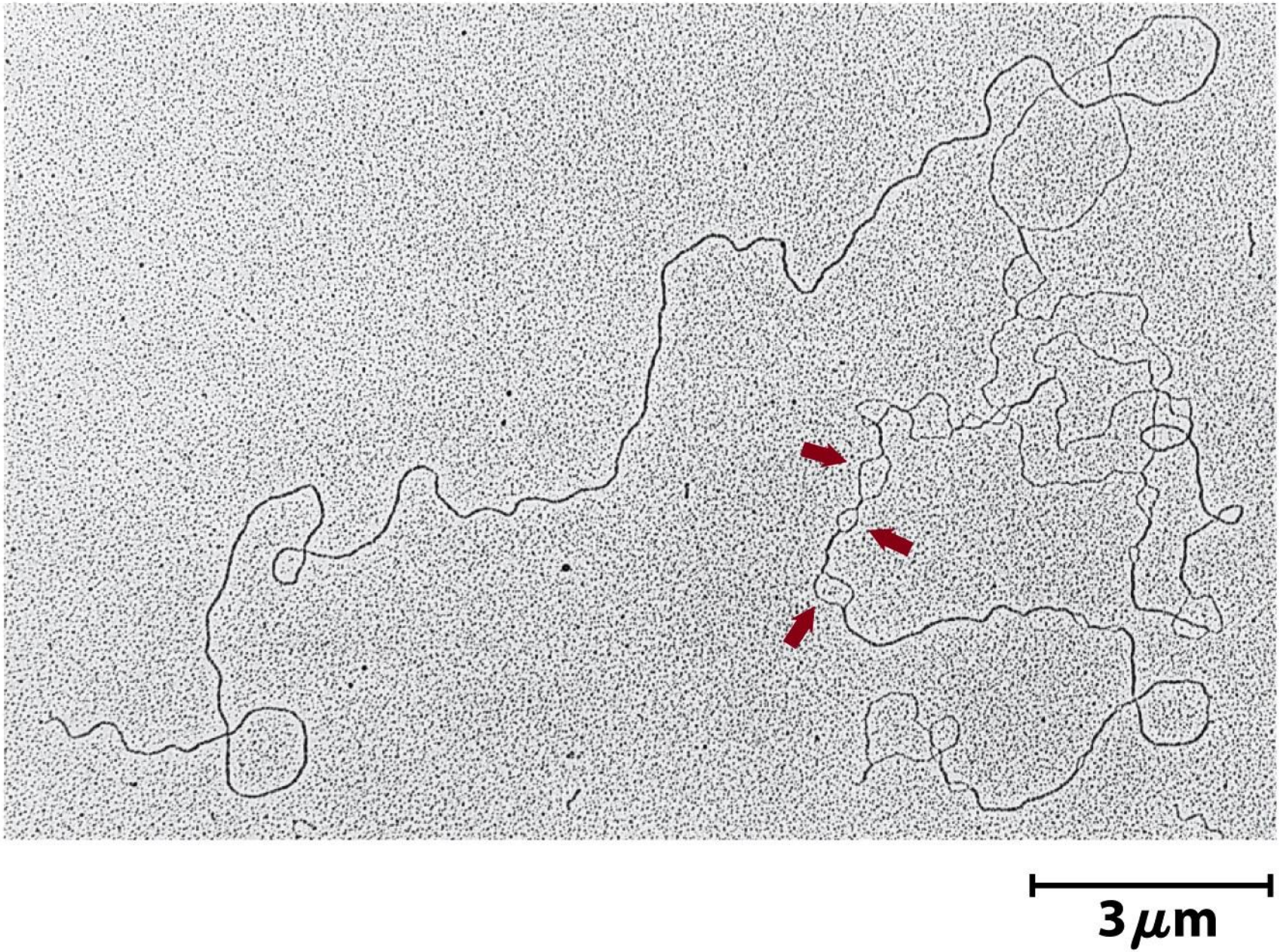
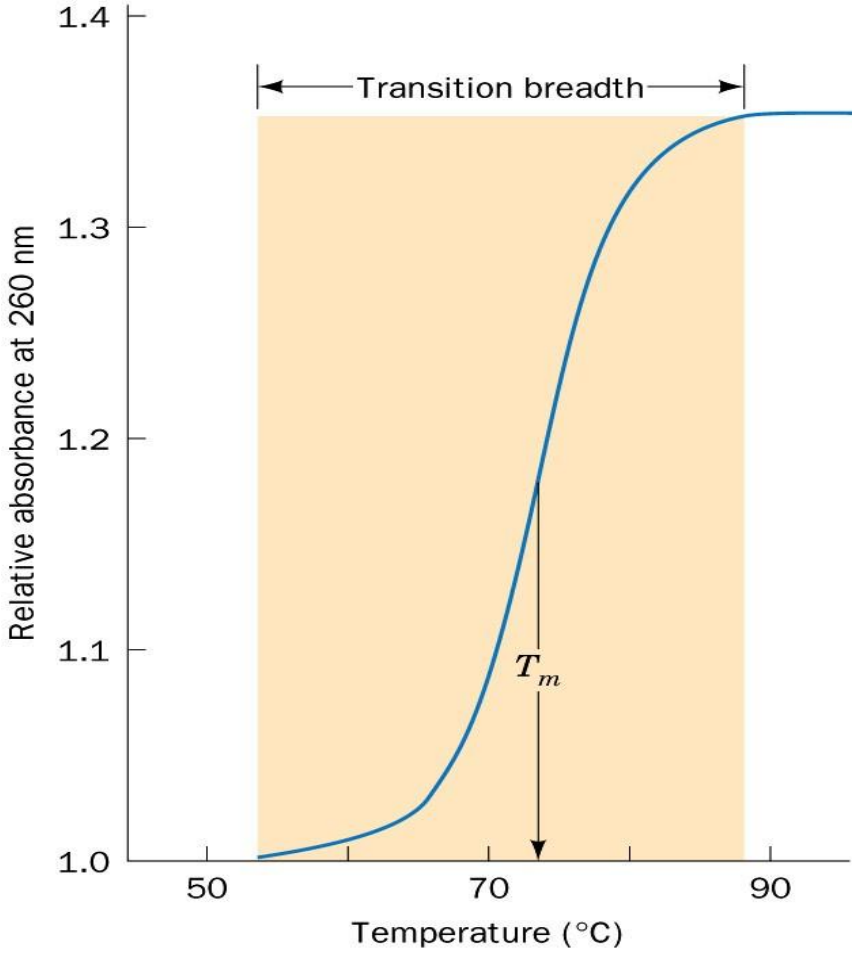


Figure 8-28
Lehninger Principles of Biochemistry, Fifth Edition

DNA melting curve

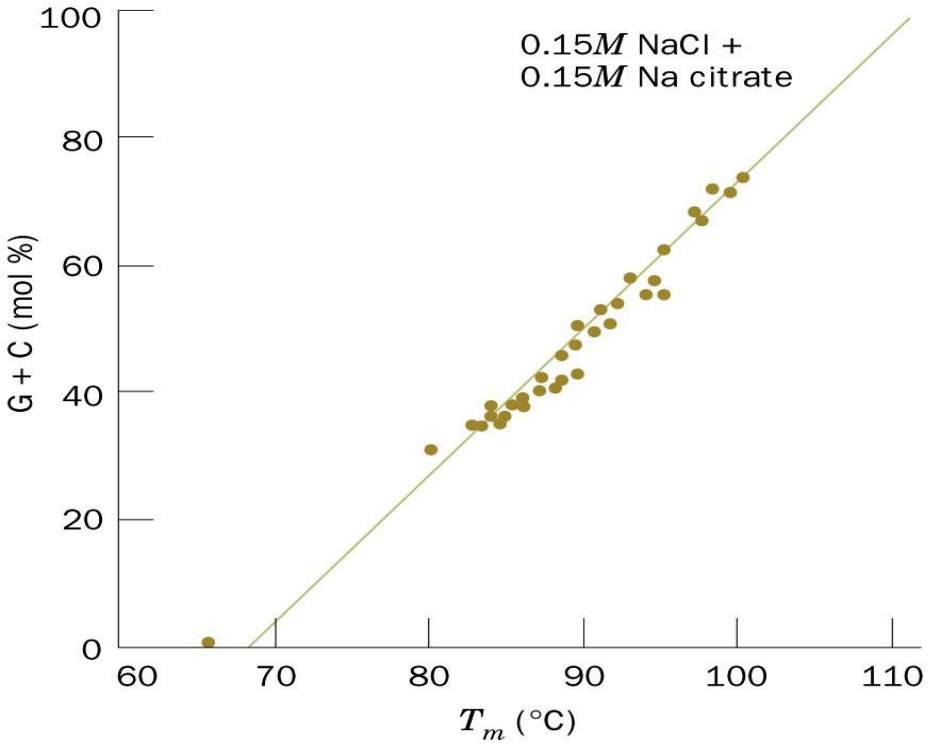
Temp at which DNA is 50% melted/denatured = Tm (melting point)



Cooperative process
Collapse of one part destabilizes rest of duplex – narrow temp. range

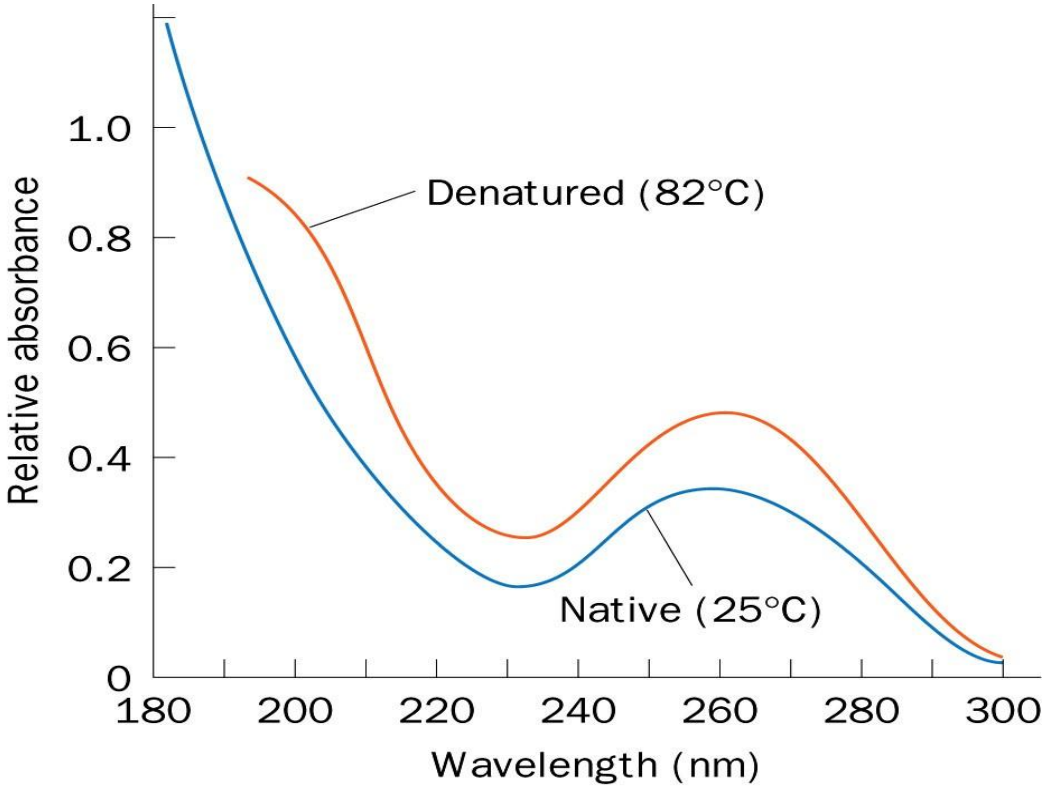
Tm is important for procedures where annealing/hybridization is required
If temp is << Tm then non-specific annealing
If temp is >> Tm then denaturation

The T_m depends on base pairs in DNA



…….as well as solvent, pH and ionic strength

Hyperchromicity

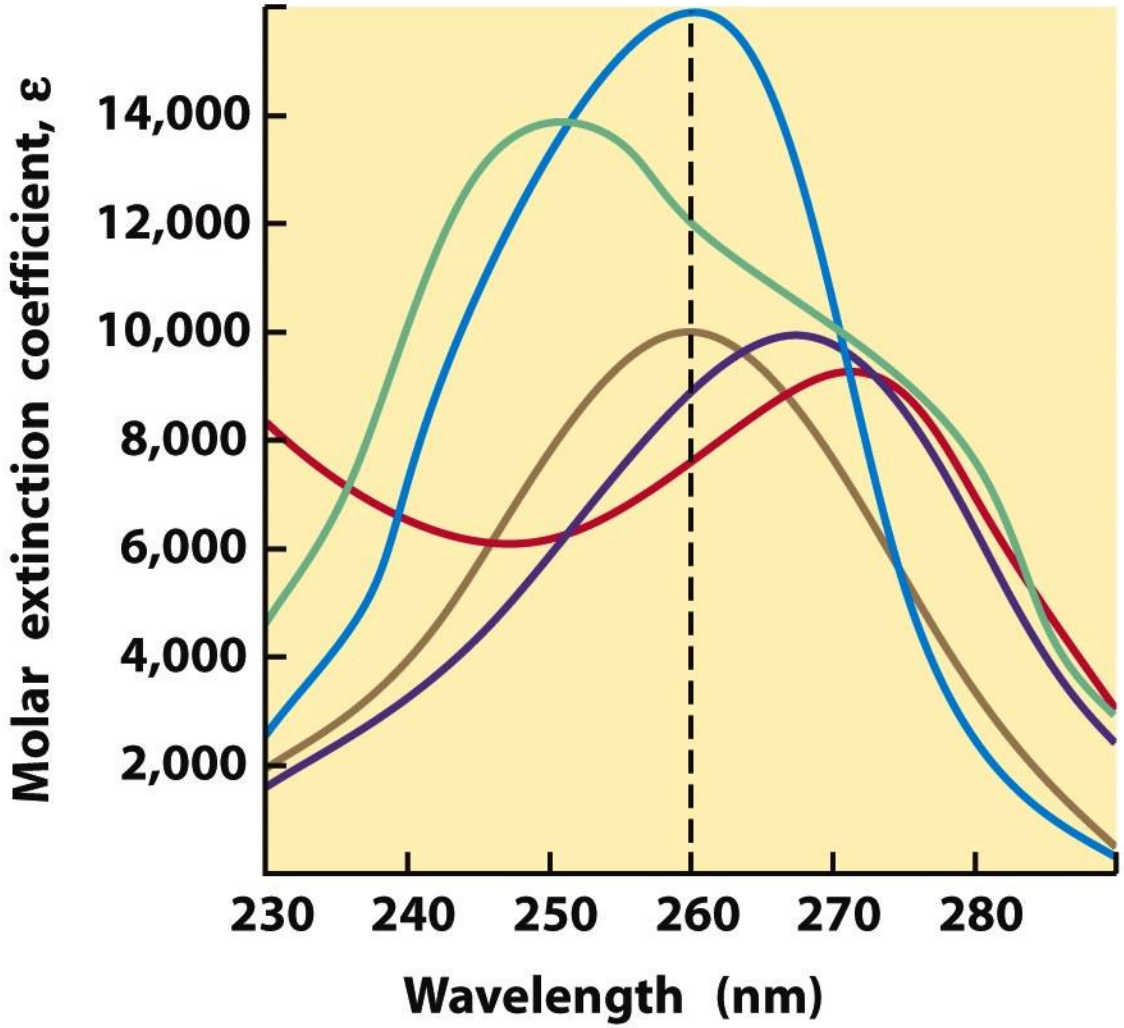


Hyperchromic effect: Absorbance at 260nm increases as DNA becomes denatured because bases are free to absorb light

Hyperchromicity: A_{260} : nucleotides>>RNA>>ssDNA>>dsDNA

i.e. ds DNA is less coloured/hyperchromic compared to ssDNA

Identification of nucleotides at 260nm



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

Figure 8-10
Lehninger Principles of Biochemistry, Fifth Edition

Purity of DNA

- Can be estimated by determination of the ratio of absorbance at 260nm (DNA/RNA) and 280nm (Protein)
- 260/280 ratio:
  - Pure dsDNA: 1.8
  - Pure RNA : 2.0
  - Pure protein: 0.5

A solution is measured at:
260nm – 1.2 , and
280nm – 0.8
What does it contain?

215-230 nm	**Minimum absorbance for nucleic acids Peptide bonds in proteins absorb light**	Measurements are generally not performed at this wavelength because commonly used buffers and solvents, such as Tris, also absorb at these wavelengths.
260 nm	**Nucleic acids have maximum absorbance**	Purines absorbance maximum is slightly below 260; pyrimidines maximum. is slightly above 260. Proteins have little absorbance at this wavelength.
270 nm	**Phenol absorbs strongly**	Phenol may be a contaminant in nucleic acid preparations.
280 nm	**Aromatic amino acids absorb light**	Nucleic acids also have some absorbance at this wavelength.

Nucleases

- Hydrolysis of phosphodiester linkages are catalysed by nucleases
  - RNA (ribonuclease) and DNA (deoxyribonuclease)
  - Exonuclease: Either 5'→3' or 3'→ 5'
  - Endonuclease: various sites within the polynucleotide chain

Exonucleases

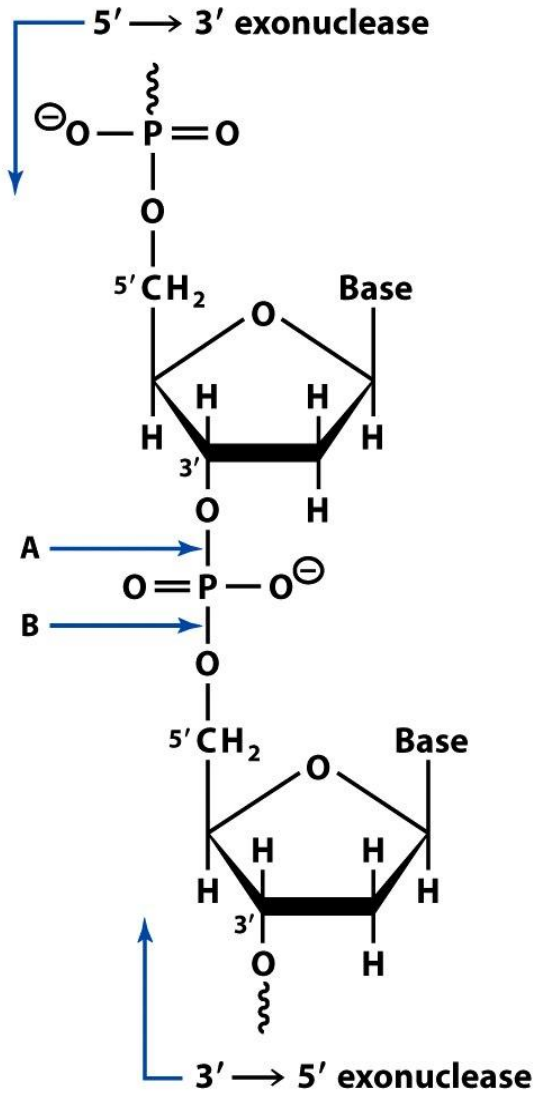


Figure 19-27 Principles of Biochemistry, 4/e

Some enzyme have both exonuclease and endonuclease activity

Endonucleases

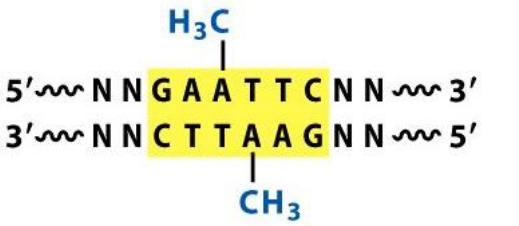
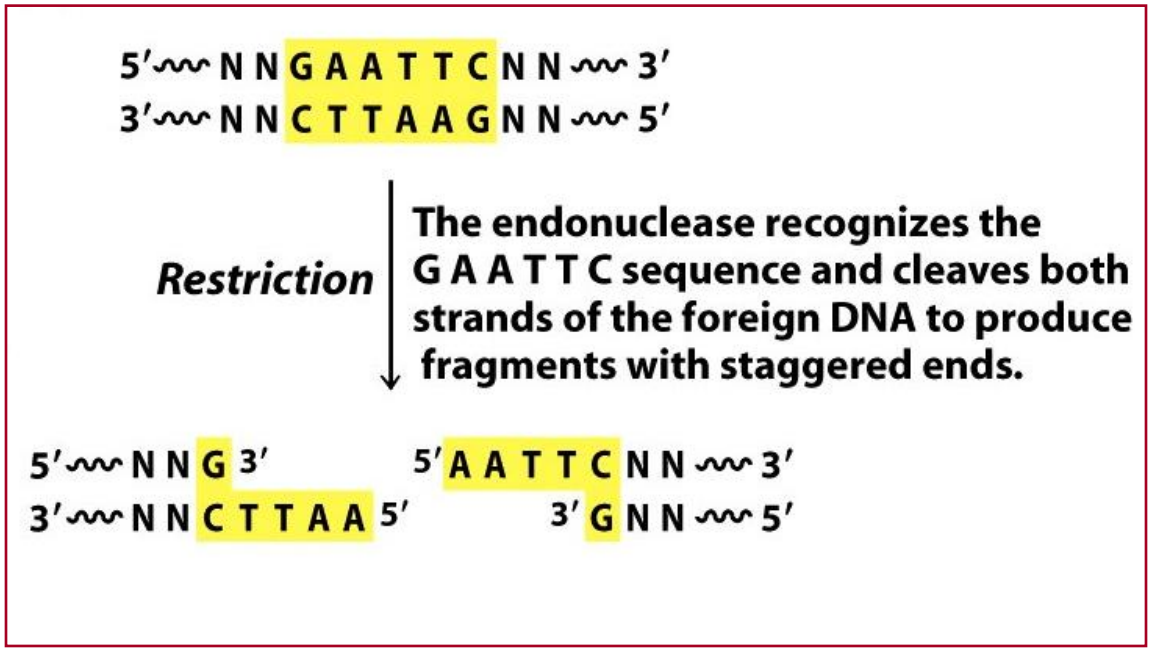


Figure 19-30 Principles of Biochemistry, 4/e

- Restriction endonucleases (also known as restriction enzymes)
  - Recognize specific DNA sequences (mostly palindrome) and cut both strands
  - Strands must be unmethylated
  - Methylation of host DNA protects own DNA

Type 1
Methylase and endonuclease activity

Type 2
Only endonuclease activity



Klebsiella pneumoniae

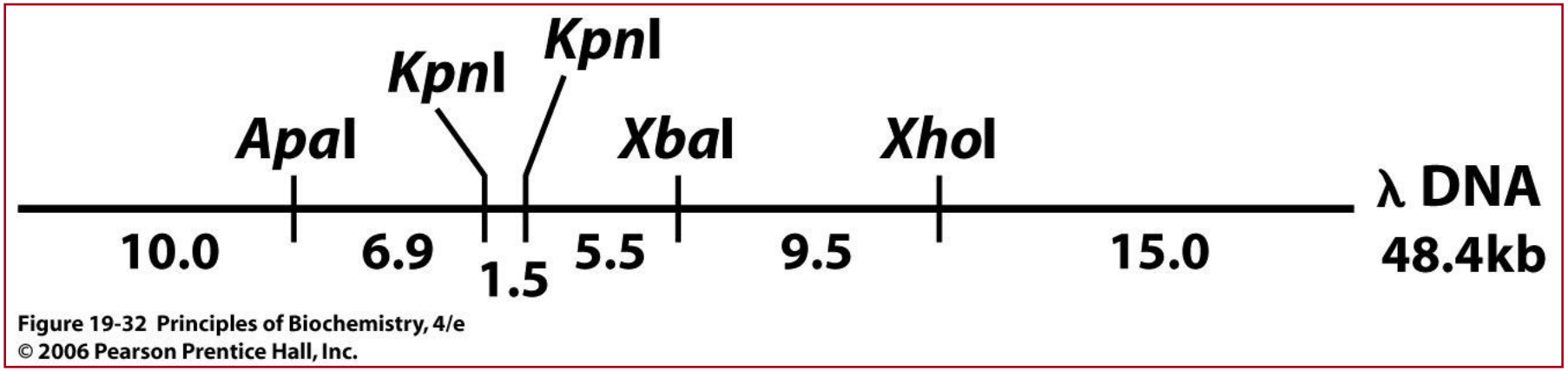


Figure 19-32 Principles of Biochemistry, 4/e

TABLE 19.4 Specificities of some common restriction endonucleases

Source	Enzyme[a]	Recognition sequence[b]
Acetobacter pasteurianus	*Apa*I	GGGCC↓C
Bacillus amyloliquefaciens H	*Bam*HI	G↓GATCC
Escherichia coli RY13	*Eco*RI	G↓AA*TTC
Escherichia coli R245	*Eco*RII	↓CC*TGG
Haemophilus aegyptius	*Hae*III	GG↓CC
Haemophilus influenzae R_d	*Hin*dIII	A*↓AGCTT
Haemophilus parainfluenzae	*Hpa*II	C↓CGG
Klebsiella pneumoniae	*Kpn*I	GGTAC↓C
Nocardia otitidis-caviarum	*Not*I	GC↓GGCCGC
Providencia stuartii 164	*Pst*I	CTGCA↓G
Serratia marcescens S_b	*Sma*I	CCC↓GGG
Xanthomonas badrii	*Xba*I	T↓CTAGA
Xanthomonas holcicola	*Xho*I	C↓TCGAG

[a] The names of restriction endonucleases are abbreviations of the names of the organisms that produce them. Some abbreviated names are followed by a letter denoting the strain. Roman numerals indicate the order of discovery of the enzyme in that strain.

[b] Recognition sequences are written 5′ to 3′. Only one strand is represented. The arrows indicate cleavage sites. Asterisks represent known positions where bases can be methylated.

Table 19-4 Principles of Biochemistry, 4/e

In humans, certain regions of the genome are very different from one individual to the next – you can therefore generate restriction fragments which are unique (like fingerprints)

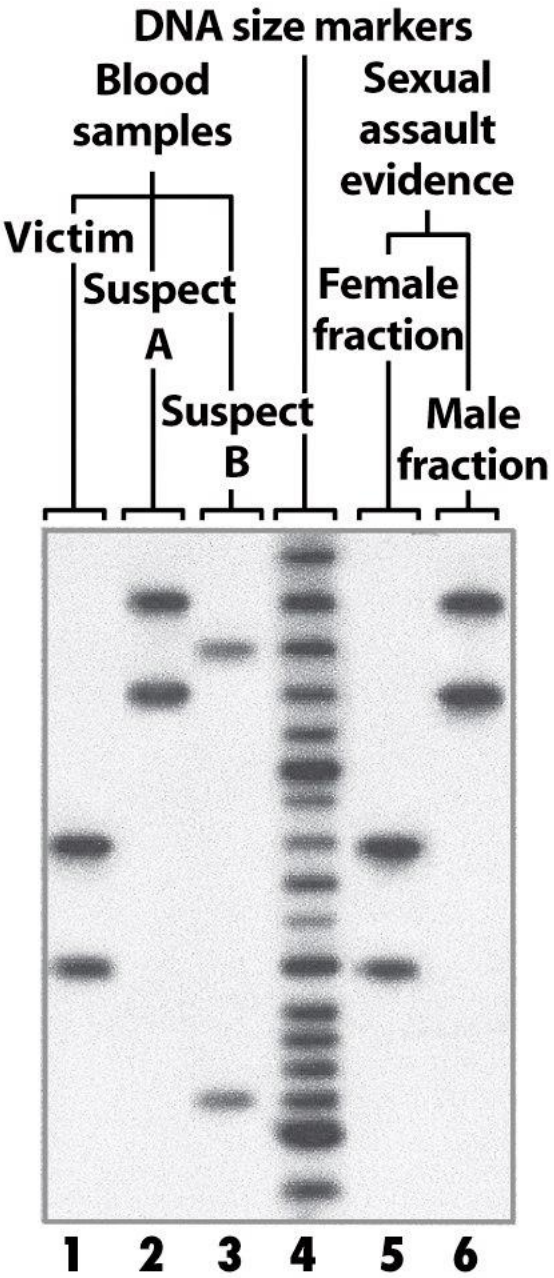


Figure 19-34 Principles of Biochemistry, 4/e

Spontaneous alterations can lead to mutations in DNA

- Nucleotides undergo non-enzymatic changes which could produce permanent changes in the genetic information (i.e. mutations)
- Link to aging and cancer

  - Deamination
  - Depurination
  - UV-induced pyrimidine dimers

Deamination

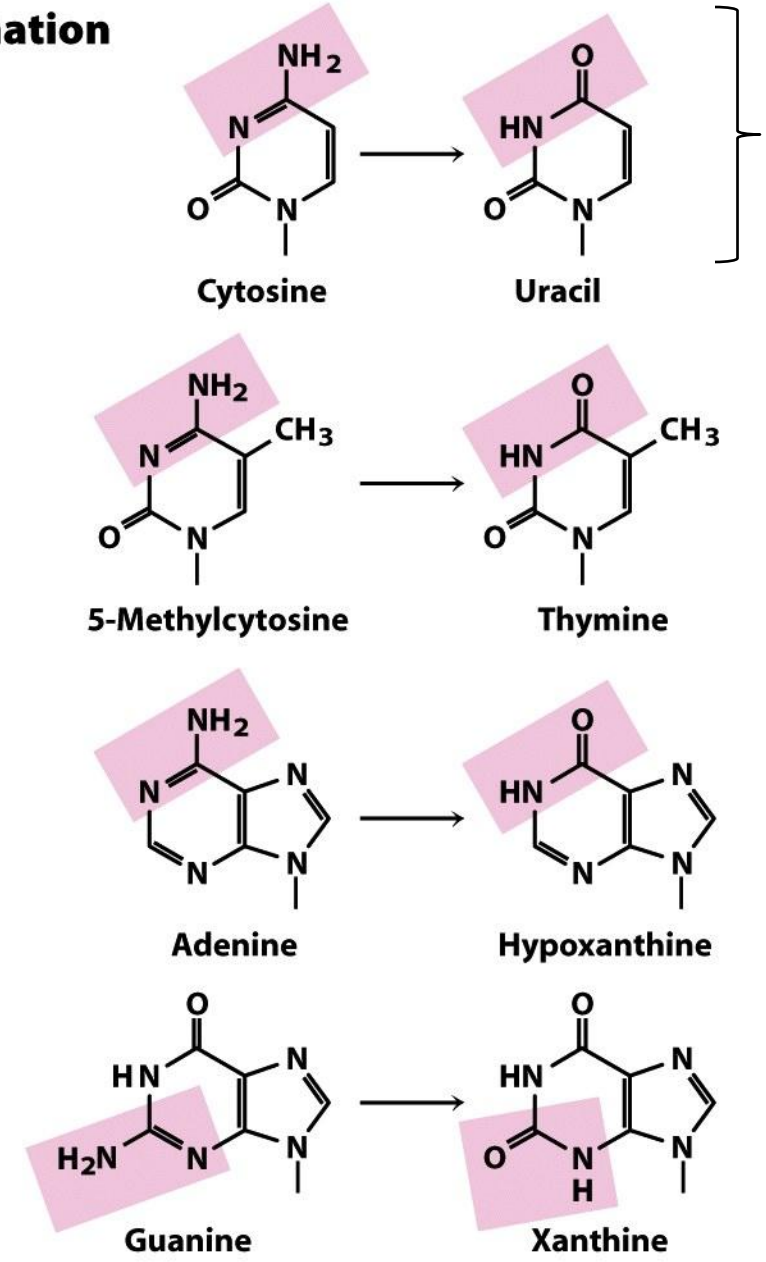


Figure 8-30a
Lehninger Principles of Biochemistry, Fifth Edition

Doesn't affect long-term storage of genetic material in form of DNA

↓

Because uracil seen as foreign and will be removed by DNA repair mechanism (Base excision repair)

Sodium nitrate and nitrite (food preservatives) promote deamination

Depurination

Base excision repair

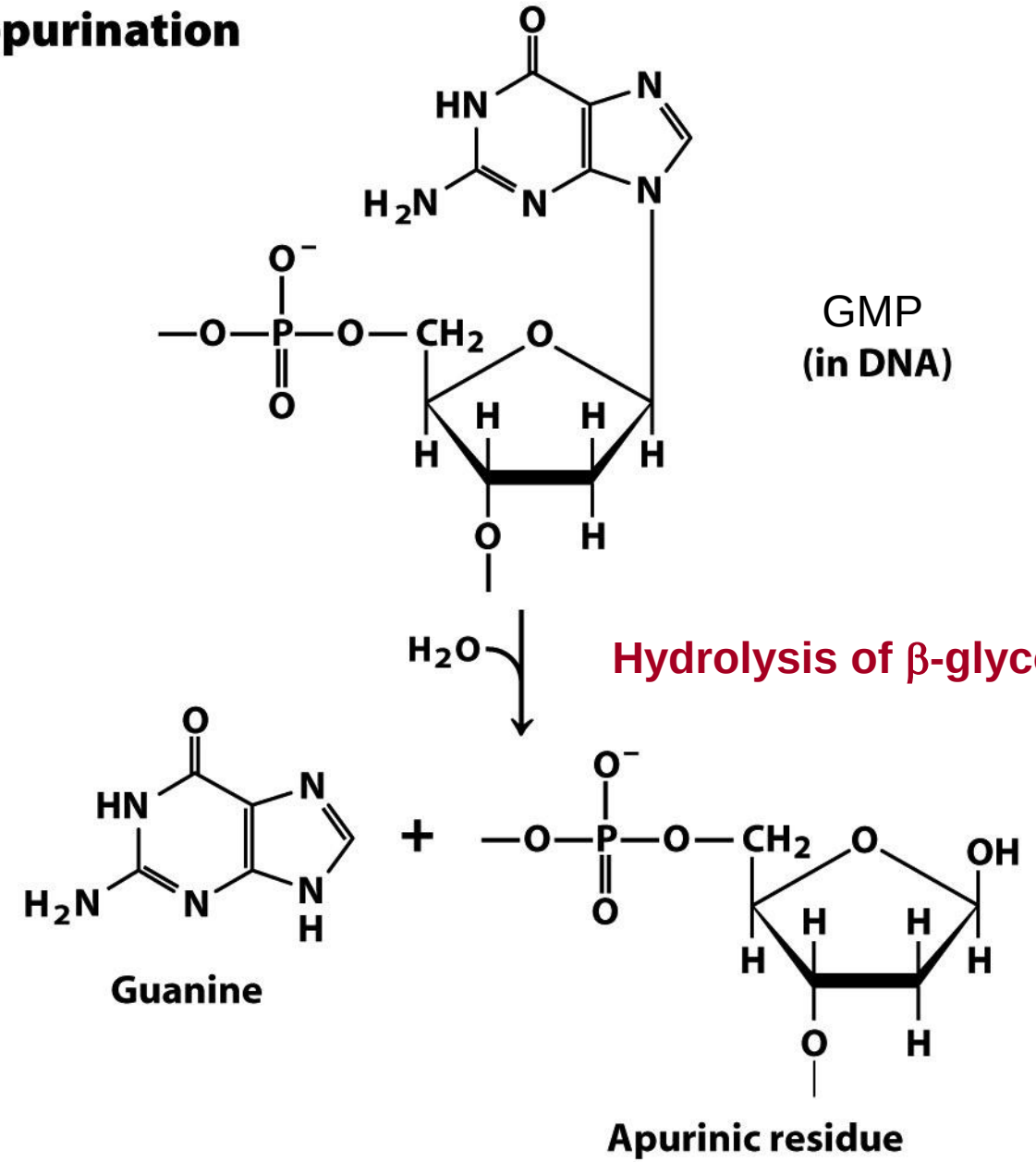


Figure 8-30b
Lehninger Principles of Biochemistry, Fifth Edition

UV-induced pyrimidine dimers

Repaired by nucleotide-excision repair

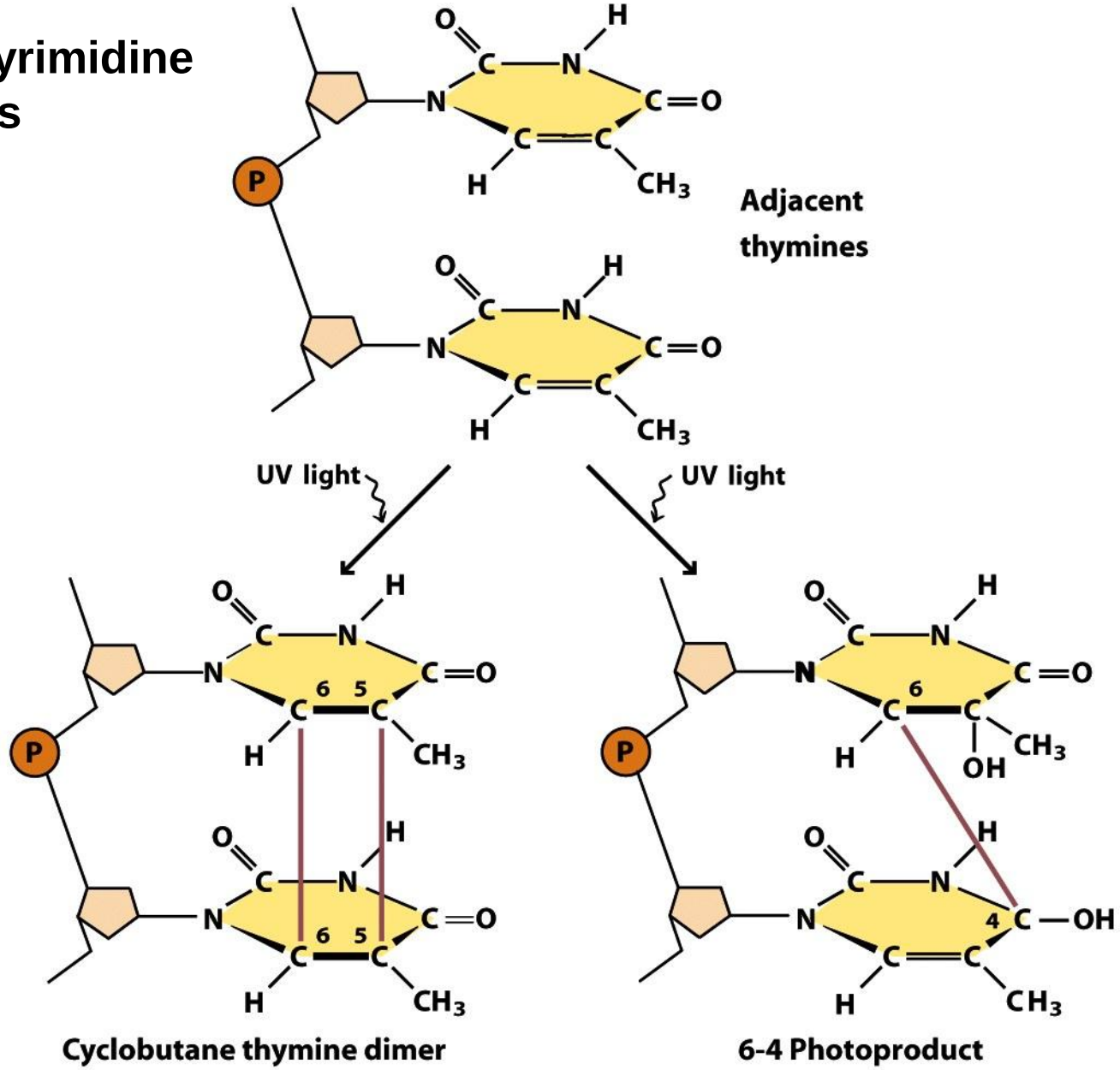


Figure 8-31a
Lehninger Principles of Biochemistry, Fifth Edition

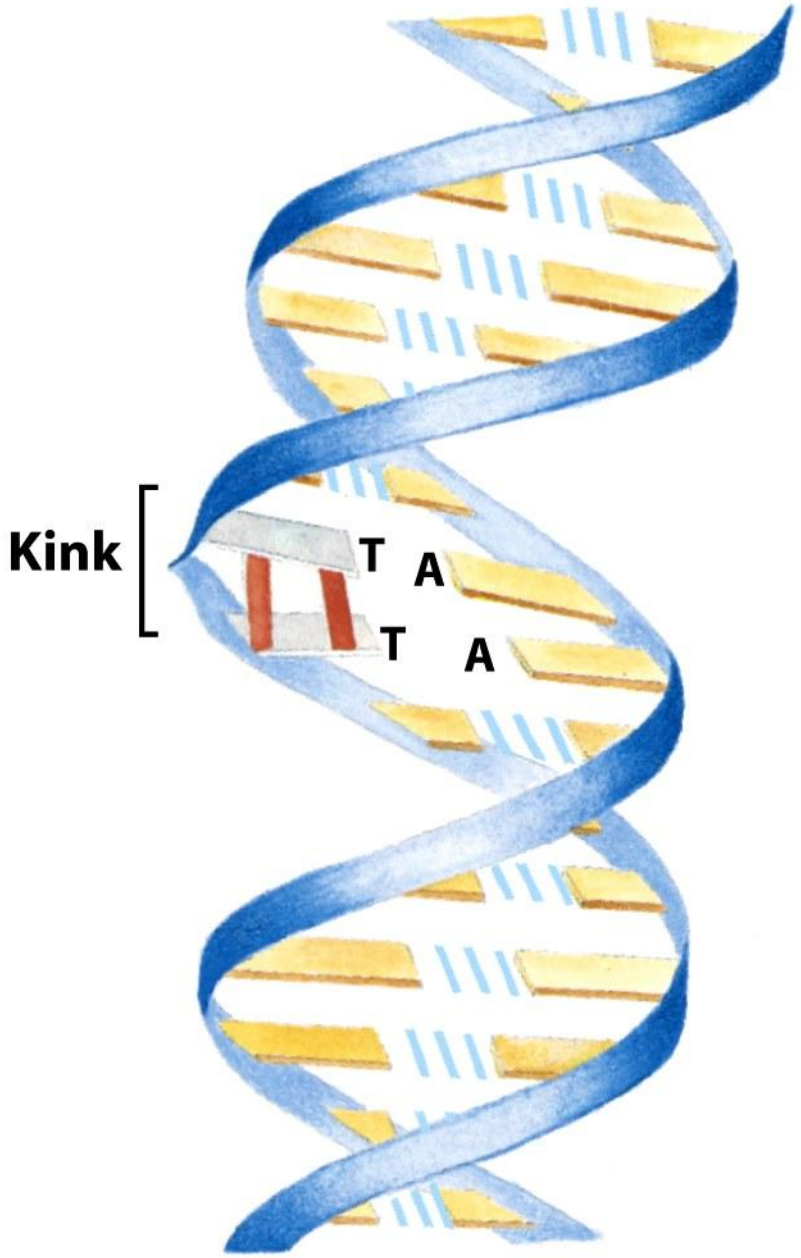


Figure 8-31b
Lehninger Principles of Biochemistry, Fifth Edition