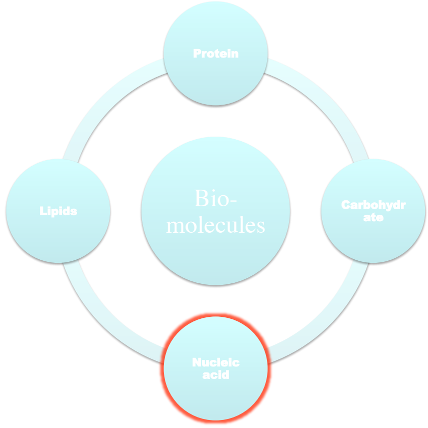
Biochemistry Part II

- Carbohydrate
- Glycobiology
- Nucleotides
- Nucleic acids
- Lipids
- Biolical membranes and transport
- Biosugnaling



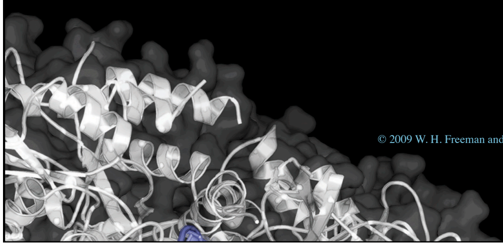
LEHNINGER
PRINCIPLES OF BIOCHEMISTRY

David L. Nelson
Michael M. Cox

FIFTH EDITION

Lecture Connections

8 | Nucleotides and Nucleic Acids



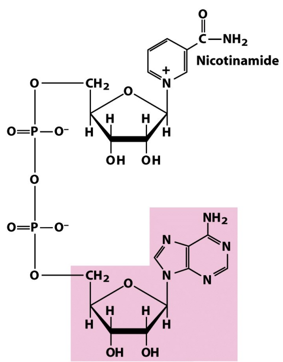
CHAPTER 8
Nucleotides and Nucleic Acids

Key topics:

– Biological function of nucleotides and nucleic acids
– Structures of common nucleotides
– Structure of double stranded DNA
– Structures of ribonucleic acids
– Denaturation and annealing of DNA
– Chemistry of nucleic acids; mutagenesis

Functions of Nucleotides and Nucleic Acids

- Nucleotide Functions:
  – Energy for metabolism (ATP)
  – Enzyme cofactors (NAD^+)
  – Signal transduction (cAMP)
- Nucleic Acid Functions:
  – Storage of genetic info (DNA)
  – Transmission of genetic info (mRNA)
  – Processing of genetic information (ribozymes)
  – Protein synthesis (tRNA and rRNA)



Nicotinamide adenine dinucleotide (NAD^+)

Figure 8-38 part 2
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Nucleotides and Nucleosides

- Nucleotide =
  - Nitrogeneous base
  - Pentose
  - Phosphate
- Nucleoside =
  - Nitrogeneous base
  - Pentose
- Nucleobase =
  - Nitrogeneous base

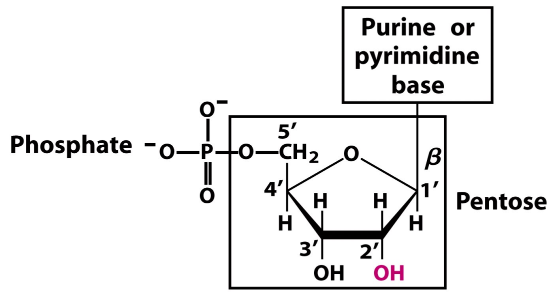

Figure 8-1a
Lehninger Principles of Biochemistry, Fifth Edition

Phosphate group

- Negatively charged at neutral pH
- Typically attached to 5' position
  - Nucleic acids are built using 5'-triphosphates
  - Nucleic acids contain one phosphate moiety per nucleotide
- May be attached to other positions:

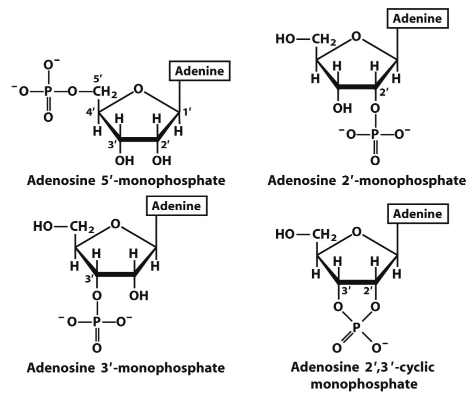

Figure 8-6
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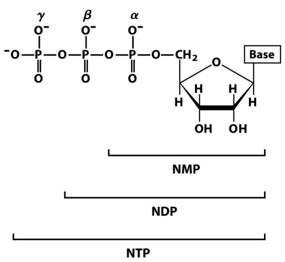

Abbreviations of ribonucleoside 5'-phosphates			
Base	Mono-	Di-	Tri-
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

Abbreviations of deoxyribonucleoside 5'-phosphates			
Base	Mono-	Di-	Tri-
Adenine	dAMP	dADP	dATP
Guanine	dGMP	dGDP	dGTP
Cytosine	dCMP	dCDP	dCTP
Thymine	dTMP	dTDP	dTTP

Figure 8-36
Lehninger Principles of Biochemistry, Fifth Edition

Pentose in Nucleotides

- β-D-ribofuranose in RNA
- β-2'-deoxy-D-ribofuranose in DNA
- Different puckered conformations of the sugar ring are possible

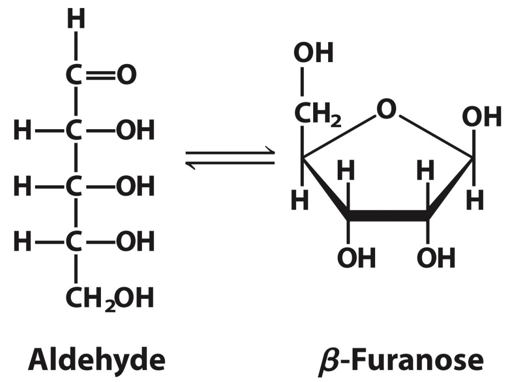

Figure 8-3a
Lehninger Principles of Biochemistry, Fifth Edition

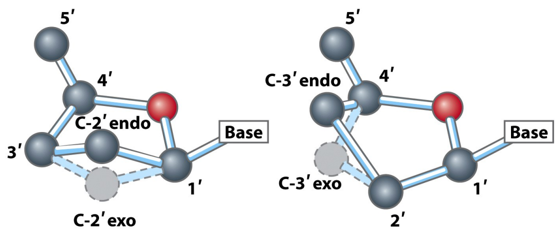

Figure 8-3b
Lehninger Principles of Biochemistry, Fifth Edition

Nucleobases

- Derivatives of pyrimidine or purine
- Nitrogen-containing heteroaromatic molecules
- Planar or almost planar structures
- Absorb UV light around 250-270 nm

Pyrimidine Bases

- Cytosine is found in both DNA and RNA
- Thymine is found only in DNA
- Uracil is found only in RNA
- All are good H-bond donors and acceptors
- Cytosine pK_a at N3 is 4.5
- Thymine pK_a at N3 is 9.5
- Neutral molecules at pH 7

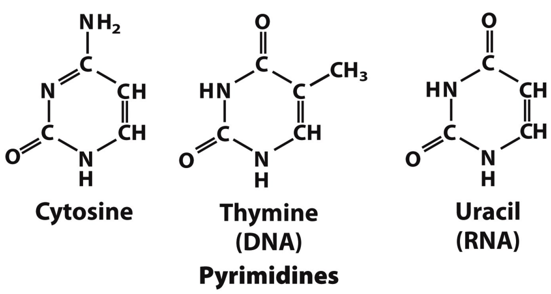

Figure 8-2 part 2
Lehninger Principles of Biochemistry, Fifth Edition

Purine Bases

- Adenine and guanine are found in in both RNA and DNA
- Also good H-bond donors and acceptors
- Adenine pK_a at N1 is 3.8
- Guanine pK_a at N7 is 2.4
- Neutral molecules at pH 7

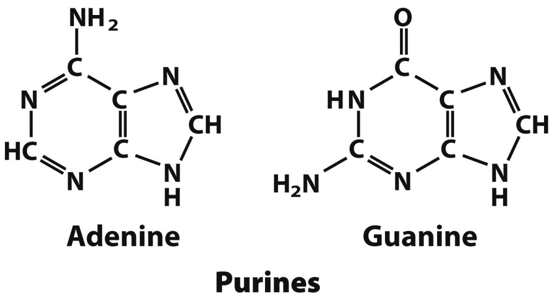

Figure 8-2 part 1
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Tautomerism of nucleobases

- Prototropic tautomers are structural isomers that differ in the location of protons
- Keto-enol tautomerism is common in ketones
- Lactam-lactim tautomerism occurs in some heterocycles
- Both tautomers exist in solution but the lactam forms are predominant at neutral pH

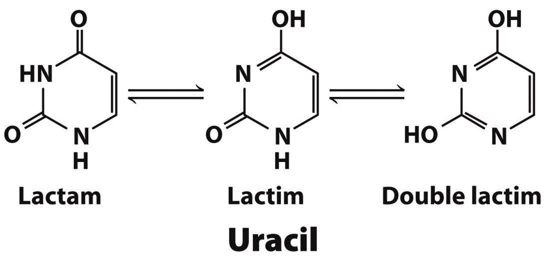

Figure 8-9
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UV Absorption of Nucleobases

- Absorption of UV light at 250-270 nm is due to $\pi \rightarrow \pi^*$ electronic transitions
- Excited states of common nucleobases decay rapidly via radiationless transitions
  - Effective photoprotection of genetic material
  - No fluorescence from nucleic acids

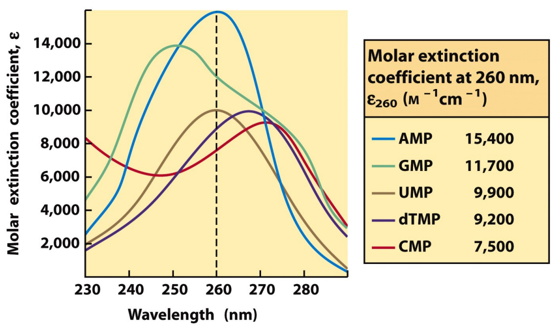

Figure 8-10
Lehninger Principles of Biochemistry, Fifth Edition

β-*N*-Glycosidic Bond

- In nucleotides the pentose ring is attached to the nucleobase via

  N-glycosidic bond.
- The bond is formed to the anomeric carbon of the sugar in *β* configuration
- The bond is formed
  - to position N1 in pyrimidines
  - to position N9 in purines
- This bond is quite stable toward hydrolysis, esp. in pyrimidines
- Bond cleavage is catalyzed by acid

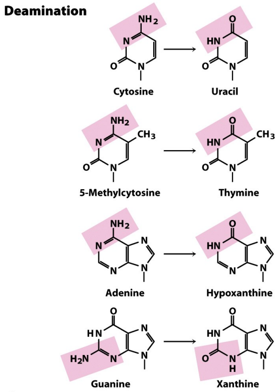

Figure 8-30a
Lehninger Principles of Biochemistry, Fifth Edition

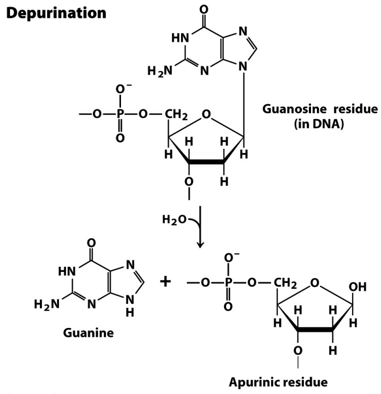

Figure 8-30b
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Conformation around *N*-Glycosidic Bond

- Relatively free rotation can occur around the N-glycosidic bond in free nucleotides
- The torsion angle about the N-glycosidic bond (N-C1') is denoted by the symbol χ
- The sequence of atoms chosen to define this angle is O4'-C1'-N9-C4 for purine,

  and O4'-C1'-N1-C2 for pyrimidine derivatives
- Angle near 0°corresponds to *syn* conformation
- Angle near 180° corresponds to *anti* conformation
- Anti conformation is found in normal B-DNA

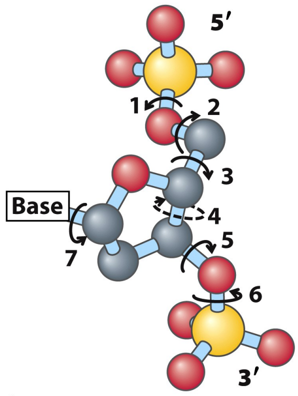

Figure 8-16a
Lehninger Principles of Biochemistry, Fifth Edition

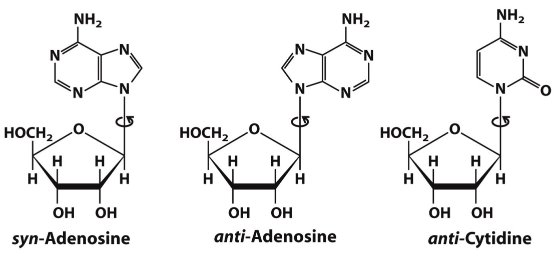

Figure 8-16b
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Nomenclature

TABLE 8–1 Nucleotide and Nucleic Acid Nomenclature

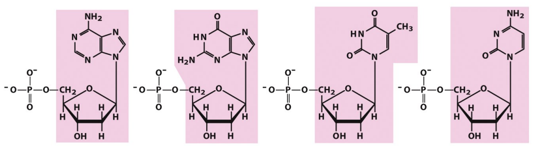
Base	Nucleoside	Nucleotide	Nucleic acid
Purines			
Adenine	Adenosine Deoxyadenosine	Adenylate Deoxyadenylate	RNA DNA
Guanine	Guanosine Deoxyguanosine	Guanylate Deoxyguanylate	RNA DNA
Pyrimidines			
Cytosine	Cytidine Deoxycytidine	Cytidylate Deoxycytidylate	RNA DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

Note: "Nucleoside" and "nucleotide" are generic terms that include both ribo- and deoxyribo- forms. Also, ribonucleosides and ribonucleotides are here designated simply as nucleosides and nucleotides (e.g., riboadenosine as adenosine), and deoxyribonucleosides and deoxyribonucleotides as deoxynucleosides and deoxynucleotides (e.g., deoxyriboadenosine as deoxyadenosine). Both forms of naming are acceptable, but the shortened names are more commonly used. Thymine is an exception; "ribothymidine" is used to describe its unusual occurrence in RNA.

Table 8-1
Lehninger Principles of Biochemistry, Fifth Edition

Nomenclature: Deoxyribonucleotides

You need to know structures, names and symbols (both two-letter (dA) and four-letter (dAMP) codes)



Deoxyribonucleotides

Figure 8-4a
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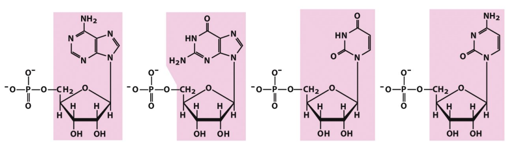
Nomenclature: Ribonucleotides

You need to know structures, names, and symbols (both one letter and three-letter codes)



Ribonucleotides

Figure 8-4b
Lehninger Principles of Biochemistry, Fifth Edition

Minor Nucleosides in DNA

- Modification is done after DNA synthesis
- 5-Methylcytosine is common in eukaryotes, also found in bacteria
- N^6-Methyladenosine is common in bacteria, not found in eukaryotes
- Epigenetic marker:
  - Way to mark own DNA so that cells can degrade foreign DNA (prokaryotes)
  - Way to mark which genes should be active (eukaryotes)
  - Could the environment turn genes on and off in an inheritable manner?

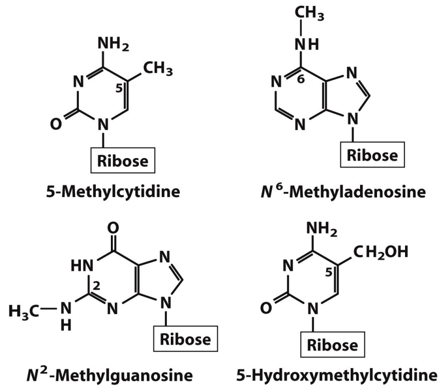


Figure 8-5a
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Minor Nucleosides in RNA

- Inosine sometimes found in the "wobble position" of the anticodon in tRNA
  - Made by de-aminating adenosine
  - Provides richer genetic code
- Pseudouridine (Ψ) found widely in tRNA and rRNA
  - More common in eukaryotes but found also in eubacteria
  - Made from uridine by enzymatic isomerization after RNA synthesis
  - May stabilize the structure of tRNA
  - May help in folding of rRNA

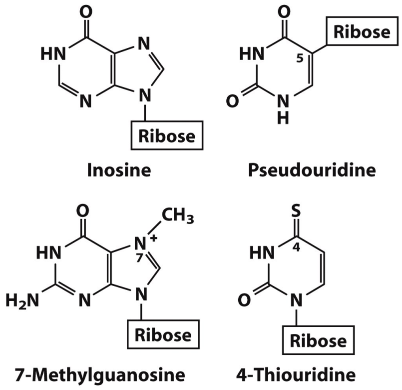

Figure 8-5b
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Polynucleotides

- Covalent bonds formed via phosphodiester linkages
  - negatively charged backbone
- DNA backbone is fairly stable
  - DNA from mammoths?
  - Hydrolysis accelerated by enzymes (DNAse)
- RNA backbone is unstable
  - In water, RNA lasts for a few years
  - In cells, mRNA is degraded in few hours
- Linear polymers
  - No branching or cross-links
- Directionality
  - 5' end is different from 3' end
  - We read the sequence from 5' to 3'

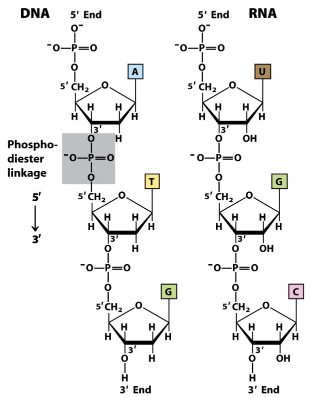

Figure 8-7
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Hydrolysis of RNA

- RNA is unstable under alkaline conditions
- Hydrolysis is also catalyzed by enzymes (RNase)
- RNase enzymes are abundant around us:
  - S-RNase in plants prevents inbreeding
  - RNase P is a ribozyme (enzyme made of RNA) that processes tRNA precursors
  - Dicer is an enzyme that cleaves double-stranded RNA into oligonucleotides
    - protection from viral genomes
    - RNA interference technology

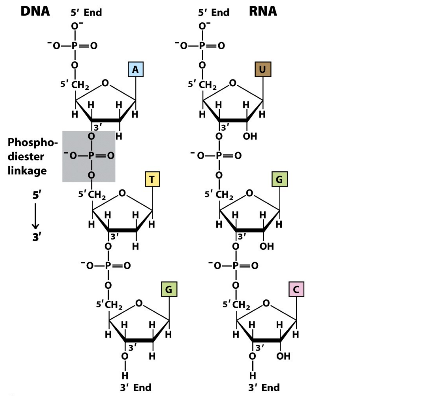

Figure 8-7
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Mechanism of Base-catalyzed RNA Hydrolysis

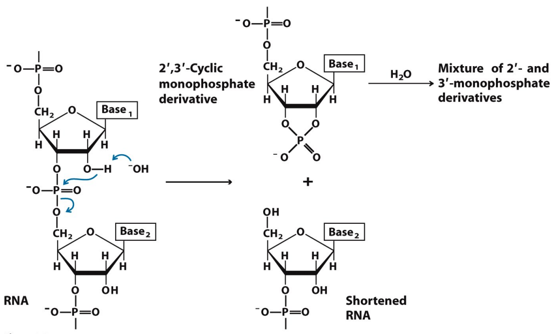

Figure 8-8
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Hydrogen Bonding Interactions

- Two bases can hydrogen bond to form a base pair
- For monomers, large number of base pairs is possible
- In polynucleotide, only few possibilities exist
- Watson-Crick base pairs predominate in double-stranded DNA
- A pairs with T
- C pairs with G
- Purine pairs with pyrimidine

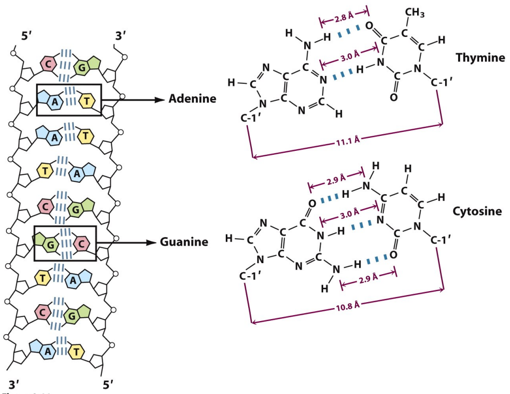

Figure 8-11
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AT and GC Base Pairs

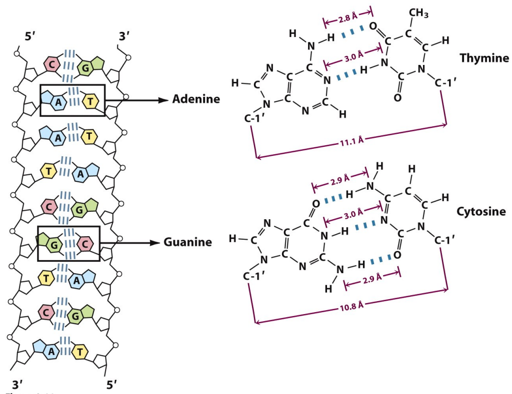

Figure 8-11
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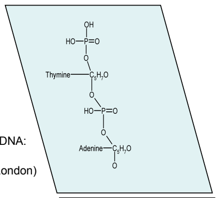
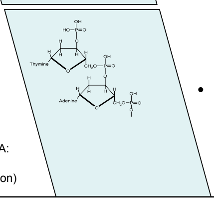
Discovery of DNA Structure

- One of the most important discoveries in biology
- Why is this important
  - "*This structure has novel features which are of considerable biological interest*"
    --- Watson and Crick, Nature, 1953
- Good illustration of science in action:
  - Missteps in the path to a discovery
  - Value of knowledge
  - Value of collaboration
  - Cost of sharing your data too early

Covalent Structure of DNA (1868-1935)

Structure of DNA:
1929
(Levene and London)





Structure of DNA:
1935
(Levene and Tipson)

- Friedrich Miescher isolates "nuclein" from cell nuclei
- Hydrolysis of nuclein:
  - phosphate
  - pentose
  - and a nucleobase
- Chemical analysis:
  - phosphodiester linkages
  - pentose is ribofuranoside

Road to the Double Helix

- Watson and Crick:
  - Missing layer means alternating pattern (major & minor groove)
  - Hydrogen bonding:
    A pairs with T
    G pairs with C

Double helix fits the data!

- Franklin and Wilkins:
  - "Cross" means helix
  - "Diamonds" mean that the phosphate-sugar backbone is outside
  - Calculated helical parameters

Watson, Crick, and Wilkins shared
1962 Nobel Prize
Franklin died in 1958

James D. Watson

Francis Crick, 1916–2004

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Rosalind Franklin, 1920–1958

Maurice Wilkins, 1916–2004

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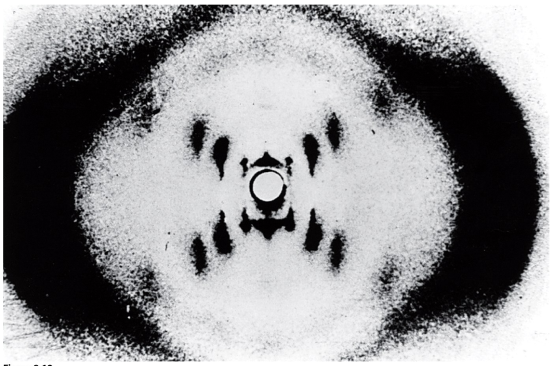

Figure 8-12
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Watson-Crick Model of B-DNA

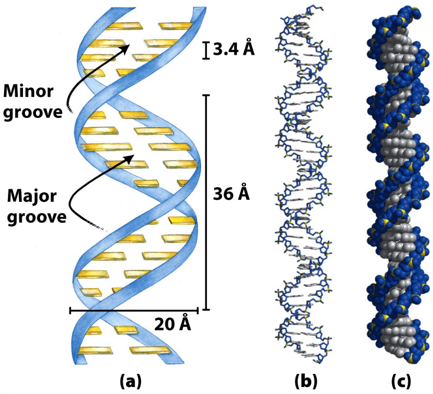

Figure 8-13
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Complementarity of DNA strands

- Two chains differ in sequence
  (sequence is read from 5' to 3')
- Two chains are complementary
- Two chains run antiparallel

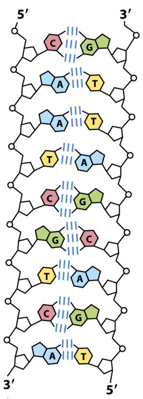

Figure 8-14
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Replication of Genetic Code

- Strand separation occurs first
- Each strand serves as a template for the synthesis of a new strand
- Synthesis is catalyzed by enzymes known as DNA polymerases
- Newly made DNA molecule has one daughter strand and one parent strand.

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

Watson and Crick, in their Nature paper,1953

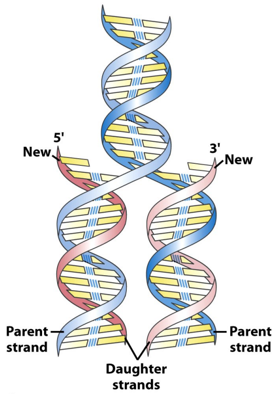

Figure 8-15
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Palindromic Sequences can form Hairpins and Cruciforms

Palindromes: words or phases that are the same when read backward or forward:

Saippuakuppinippukauppias: (Finnish word for "soap cup batch trader“)

'Nipson anomémata mé monan opsin' (Ancient greek fountain text: 'Wash the sin as well as the face’

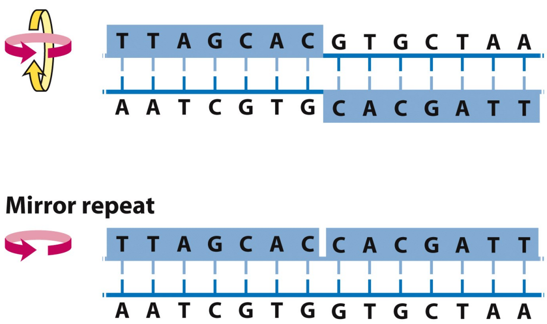

Figure 8-18
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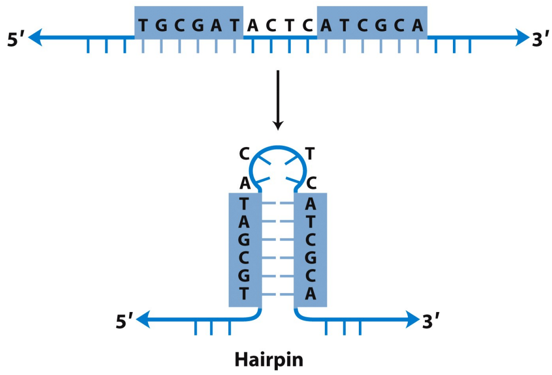

Figure 8-19a
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Other Forms of DNA

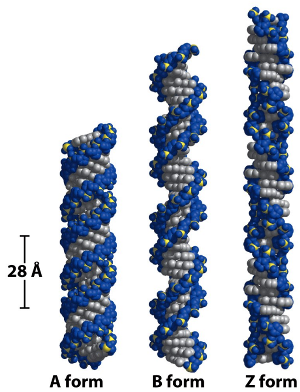

Figure 8-17 part 1
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	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar 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 8-17 part 2
Lehninger Principles of Biochemistry, Fifth Edition

Messenger RNA: Code Carrier for the Sequence of Proteins

- Is synthesized using DNA template
- Contains ribose instead of deoxyribose
- Contains uracil instead of thymine
- One mRNA may code for more than one protein

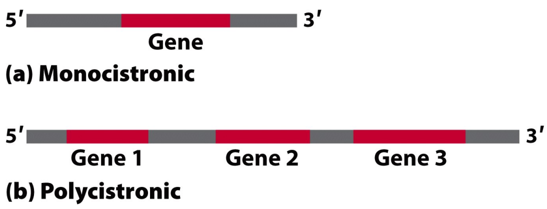

Figure 8-21
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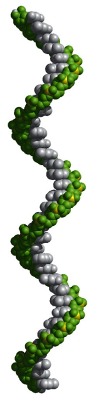

Figure 8-22
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Transfer RNA: Matching Amino Acids with the mRNA Code

tRNA molecules have quite complex structures, this is an actively studied field

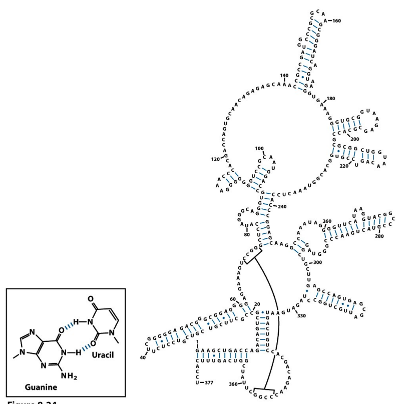

Figure 8-24
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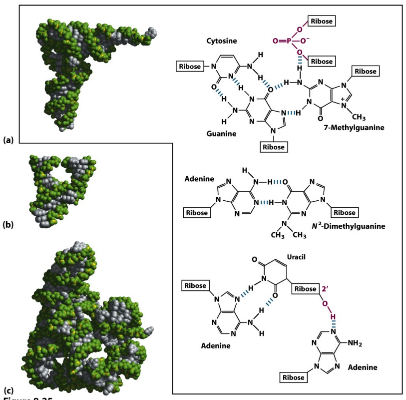

Figure 8-25
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DNA Denaturation

- Covalent bonds remain intact
  - Genetic code remains intact
- Hydrogen bonds are broken
  - Two strands separate
- Base stacking is lost
  - UV absorbance increases

Denaturation can be induced by high temperature, or change in pH

Denaturation may be reversible:annealing

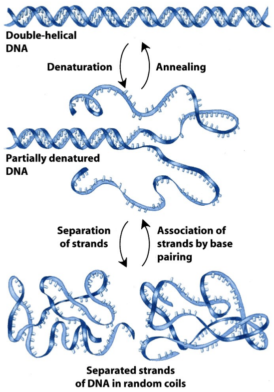

Figure 8-26
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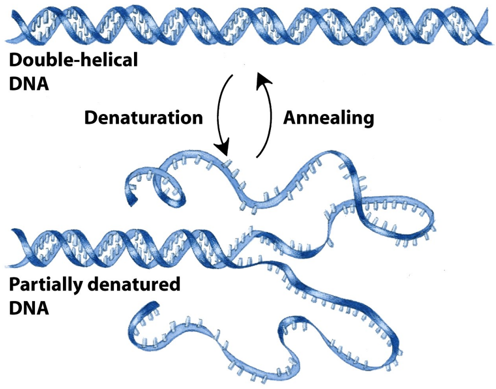

Figure 8-26 part 1
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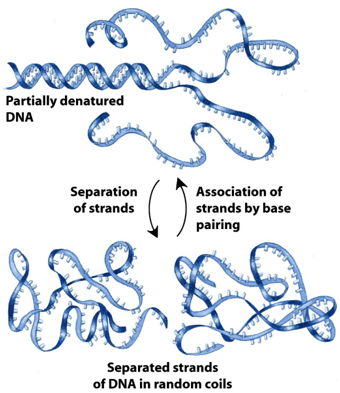

Figure 8-26 part 2
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Thermal DNA Denaturation (Melting)

- DNA exists as double helix at normal temperatures
- Two DNA strands dissociate at elevated temperatures
- Two strands re-anneal when temperature is lowered
- The reversible thermal denaturation and annealing form basis for the polymerase chain reaction
- DNA denaturation is commonly monitored by UV spectrophotometry at 260 nm

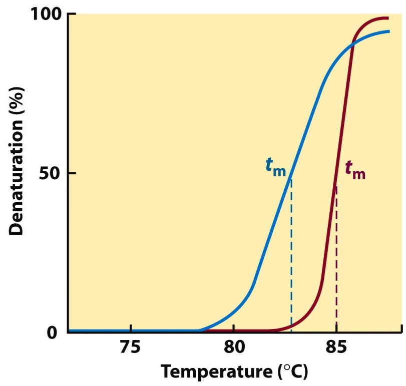

Figure 8-27a
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Factors Affecting DNA Denaturation

- The midpoint of melting (T_m) depends on base composition
  – high CG increases T_m
- T_m depends on DNA length
  – Longer DNA has higher T_m
  – Important for short DNA
- T_m depends on pH and ionic strength
  – High salt increases T_m

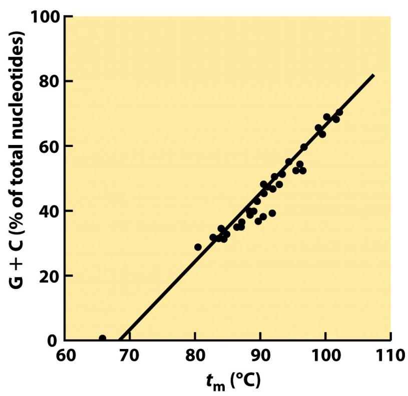

Figure 8-27b
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Denaturation of Large DNA Molecules is not Uniform

- AT rich regions melt at a lower temperature than GC-rich regions

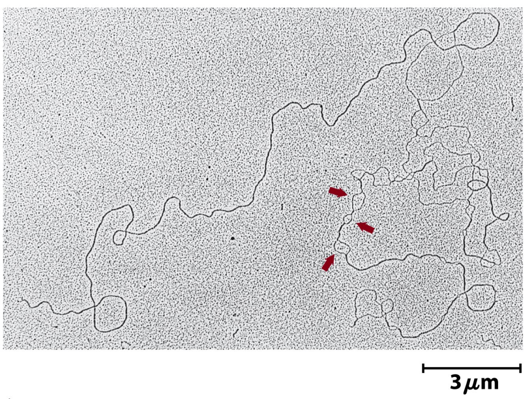

Figure 8-28
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Two Near-Complementary DNA Strands Can Hybridize

- Detection of a specific DNA molecule in complex mixture
  - radioactive detection
  - fluorescent DNA chips
- Amplification of specific DNA
  - polymerase chain reaction
  - site-directed mutagenesis
- Evolutionary relationships
- Antisense therapy

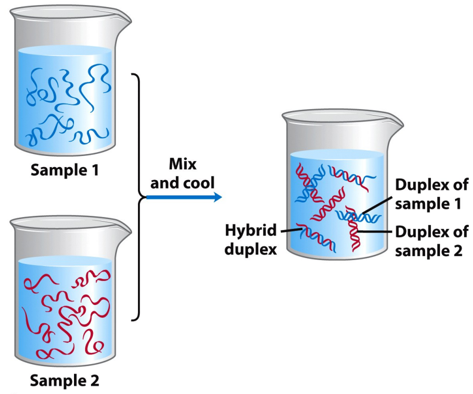

Figure 8-29
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Molecular Mechanisms of Spontaneous Mutagenesis

- Deamination
  - Very slow reactions
  - Large number of residues
  - The net effect is significant: 100 C → U events /day in a mammalian cell
- Depurination
  - N-glycosidic bond is hydrolyzed
  - Significant for purines: 10,000 purines lost/day in a mammalian cell
- Cells have mechanisms to correct most of these modifications.

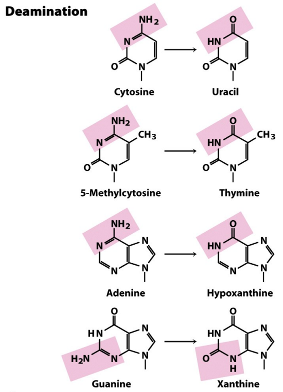

Figure 8-30a
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Molecular Mechanisms of Oxidative and Chemical Mutagenesis

- Oxidative damage
  - Hydroxylation of guanine
  - Mitochondrial DNA is most susceptible
- Chemical alkylation
  - Methylation of guanine
- Cells have mechanisms to correct most of these modifications

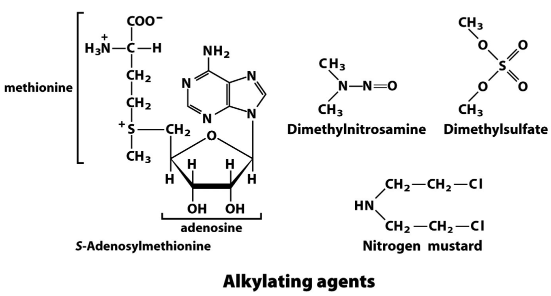

Figure 8-32b
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Molecular Mechanisms of Radiation-Induced Mutagenesis

- UV light induces dimerization of pyrimidines, this may be the main mechanism for skin cancers
- Ionizing radiation (X-rays and γ-rays) causes ring opening and strand breaking. These are difficult to fix
- Cells can repair some of these modifications, but others cause mutations. Accumulation of mutations is linked to aging and carcinogenesis

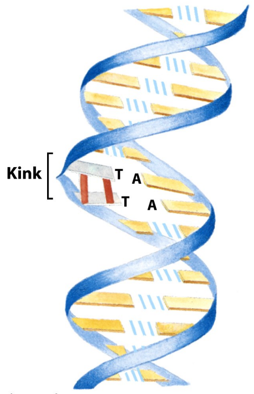

Figure 8-31b
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Chapter 8: Summary

In this chapter, we learned about:

- Function of nucleotides and nucleic acids
- Names and structures of common nucleotides
- Structural basis of DNA function
- Reversible denaturation of nucleic acids
- Chemical basis of mutagenesis