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Chapter 11

Nucleotides and Nucleic Acids

Slide 2

Nucleic Acids

- Two classes
  - DNA (Deoxyribonucleic Acid)
  - RNA (Ribonucleic Acid)
- Polymers of **nucleotides**
- DNA carries **genetic information** in the form of **nucleotide sequence**
- Central Dogma of Biochemistry
  - DNA → RNA → Protein (Figure 11.1)

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Nucleotides

- Composition
  - Heterocyclic Base
  - Pentose
  - Phosphate
- Besides being the building blocks of nucleic acids, nucleotides have many roles in metabolism

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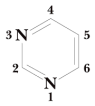
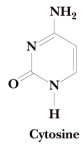
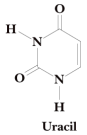
Heterocyclic Bases—Pyrimidines

The pyrimidine ring

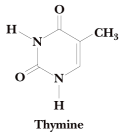




Cytosine



Uracil



Thymine

- DNA and RNA RNA only DNA only

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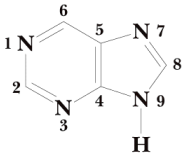
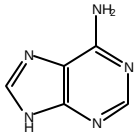
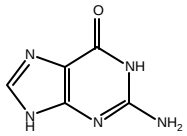
Heterocyclic Bases –Purines

The Purine Ring System:





Adenine



Guanine

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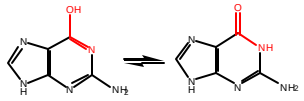
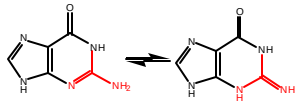
Tautomerism

- Oxygen on ring prefers keto form
- Nitrogen on ring prefers amino form



Favored Tautomer



Favored Tautomer

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UV Absorbance of Pyrimidines and Purines

- Both Pyrimidines and Purines have strong absorbance in the ultraviolet around 260 nm
  - See Figure 11.8
- This is a useful property in measuring quantities of nucleic acid in a sample

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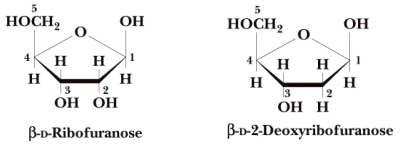
Pentoses



- **Nucleosides** are β-N-glycosides of ribose or deoxyribose and a pyrimidine or purine base

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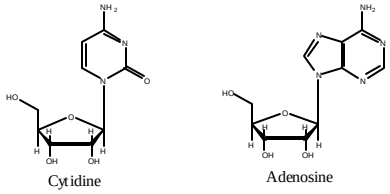
Nucleosides

- β glycosidic linkage at N-1 of pyrimidine and N-9 of purine



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Nucleosides, con't.

- Two conformations of the glycosidic bond
  - syn and anti (See Figure 11.12)
- See three dimensional models in the Course Links for Chapter 11

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Nucleoside Nomenclature

- Add **–idine** to root name of the pyrimidine
  - cytosine → cytidine
  - uracil → uridine
  - thymine → thymidine (ribothymidine)
- Add **–osine** to the root name of the purine
  - adenine → adenosine
  - guanine → guanosine
  - xanthine → xanthosine
  - **Except** hypoxanthine → inosine (See Fig. 11.11)

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Nucleoside Nomenclature, con't.

- Nucleosides of deoxyribose are deoxyribonucleosides and are prefixed by **deoxy**
  - Adenine-ribose = adenosine
  - Adenine-deoxyribose = deoxyadenosine
- Except for thymine
  - Thymine-ribose is called **ribothymidine**
  - Thymine-deoxyribose is called thymidine

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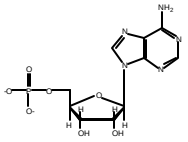
Nucleotides

- Nucleotides are phosphate esters of nucleosides
- Named as "nucleoside-X'-phosphate" where X' is the ribose position to which the phosphate is attached
- Example:
  adenosine-5'-
  monophosphate



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Nucleotides, con't.

- See Figure 11.13 and 11.14 for other examples
- Abbreviations
  - Add –**ylic acid** to base stem
    - adenylic acid, cytidylic acid, etc.
  - 3-letter abbreviation
    - AMP or 5'-AMP, ADP, GDP, CMP, etc

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Nucleotide Functions

- Building blocks of nucleic acids
- Triphosphates are energy intermediates
  - ATP major energy currency
  - GTP involved in driving protein synthesis
- "Carriers" of metabolic intermediates
  - UDP intermediates in sugar metabolism
  - CDP intermediates in lipid metabolism
  - NAD and CoA are ADP intermediates
- Chemical signaling "second messengers"
  - cyclic AMP and cyclic GMP

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Nucleic Acid Structure

- Linear polymer of nucleotides
- Phosphodiester linkage between 3’ and 5’ positions
- See Figure 11.17

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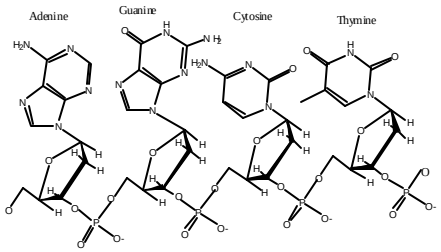
Nucleic Acid Sequence Abbreviations

- Sequence normally written in 5’-3’ direction, for example:



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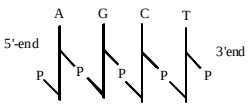
Sequence Abbreviations, con’t.

Let Letter stand for **base:**



Let Letter stand for **nucleoside**

5'-end pApGpCpTp 3'-end

Let Letter stand for **nucleotide**

5'-end AGCT 3'-end

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Biological Roles of Nucleic Acids

- DNA carries genetic information
  - 1 copy (haploid) or 2 copies (diploid) per cell
  - See "History of Search for Genetic Material" in Course Links for Chapter 12
- RNA at least four types and functions
  - messenger RNA—structural gene information
  - transfer RNA—translation "dictionary"
  - ribosomal RNA—translation "factory"
  - small nuclear RNA—RNA processing

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DNA Structure

- Watson-Crick Double Helix
  - Clues from Chargaff's Rules
    - A=T, C=G, purines=pyrimidines
  - Helical dimensions from Franklin and Wilkins X-ray diffraction studies
  - Recognition of complementary base pairing possibility given correct tautomeric structure (See Figure 11.20)

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Nature of DNA Helix

- Antiparallel strands
- Ribose phosphate chain on outside
- Bases stacked in middle like stairs in a spiral staircase
  - Figure 11.19—schematic representation
- Complementary strands provide possible mechanism for replication
  - Figure 11.12 representation of replication process

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Size of DNA Molecules

- 2 nm diameter, about 0.35 nm per base pair in length
- Very long, millions of base pairs

Organism	Base Pairs	MW	Length
• SV 40 virus	5.1 Kb	3.4×10^6	1.7 µm
• λ phage	48 Kb	32×10^6	17 µm
• E. coli	4,600 Kb	2.7×10^9	1.6 mm
• Yeast	13,500 Kb	9×10^9	4.6 mm
• Human	2.9×10^6 Kb	1.9×10^{12}	0.99 m

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Packaging of DNA

- Very compact and folded
  - E. coli DNA is 1.6 mm long, but the E. coli cell is only 0.002 mm long
    - See Figure 11.22
  - Eukaryotic cells have DNA packaged in chromosomes, with DNA wrapped around an octameric complex of **histone** proteins
    - See Figure 11.23

Histones are rich in the basic amino acids lysine and arginine, which have positive charges. These positively charged residues provide binding for the negatively charged ribose-phosphate chain of DNA.

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Messenger RNA

- "Transcription" product of DNA
- Carries sequence information for proteins
  - Prokaryote mRNA may code for multiple proteins
  - Eukaryote mRNA codes for single protein, but code ("exon") might be separated by non-coding sequence ("introns")
    - See Figure 11.24

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Ribosomal RNA

- "Scaffold" for proteins involved in protein synthesis
- RNA has catalytic activity as the "peptidyl transferase" which forms the peptide bond
- Prokaryotes and Eukaryotes have slightly different ribosomal structures (See Figure 11.25)
- Ribosomal RNA contains some modified nucleosides (See Figure 11.26)

Remember that the sedimentation rate is related to molecular weight, but is not directly proportional to it because it depends both on molecular weight (which influences the sedimentation force) and the shape of the molecule (which influences the frictional force).

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Transfer RNA

- Small molecules—73-94 residues
- Carries an amino acid for protein synthesis
- One or more t-RNA's for each amino acid
- "Anti-codon" in t-RNA recognizes the nucleotide "code word" in m-RNA
- 3'-Terminal sequence always CCA
- Amino acid attached to 2' or 3' of 3'-terminal A
- Many modified bases (Also Figure 11.26)

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Small Nuclear RNA's

- Found in Eukaryotic cells, principally in the nucleus
- Similar in size to t-RNA
- Complexed with proteins in **small nuclear ribonucleoprotein particles** or **snRNPs**
- Involved in processing Eukaryotic transcripts into m-RNA

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Chemical Differences Between DNA and RNA

- Base Hydrolysis
  - DNA stable to base hydrolysis
  - RNA hydrolyzed by base because of the 2'-OH group. Mixture of 2' and 3' nucleotides produced
    - See Figure 11.29
  - DNA more susceptible to mild (1 N) acid
    - Hydrolyzes purine glycosidic bond, forming **apurinic acid**

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Enzymatic Hydrolysis of Nucleic Acids

- Many different kinds of **nucleases** in nature
- Hydrolysis of **phosphodiester** bond
- **Exonucleases** hydrolyze terminal nucleotides
- **Endonucleases** hydrolyze in middle of chain. Some have specificity as to the base at which hydrolysis occurs

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Enzymatic Hydrolysis, con't.

- Specificity as to the bond which is cleaved
  - *a* type cleaves the 3' phosphate bond
    - Produces 5'-phosphate products
  - *b* type cleaves the 5' phosphate bond
    - See Figure 11.30
- Examples: (Also see Table 11.4)
  - Snake venom phosphodiesterase
    - "*a*" specific exonuclease
  - Spleen phosphodiesterase
    - "*b*' specific exonuclease
    - See Figure 11.31

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Restriction Endonucleases

- Enzymes of bacteria that hydrolyze "foreign" DNA
- Name based on "restricted growth" of bacterial viruses
- Enzymes specific for a short sequence of nucleotides (4-8 bases in length)
- Methylation of the same sequence protects "self" DNA from hydrolysis

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Restriction Endonucleases, con't.

- Discovery of the phenomenon has provided a powerful tool for analysis of DNA
- Allows specific "cutting" of DNA into small fragments, similar to proteolytic digestion of proteins
- Average length of fragments depends on number of bases recognized

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Specificity of Restriction Endonucleases

- 4-base sequence occurs randomly every 4^4 bases, or every 256 bases
- 6-base sequence occurs randomly every 4^6 bases, or every 4096 bases
- 8-base sequence occurs randomly every 4^8 bases, or every 65,536 bases

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Specificity of Restriction Endonucleases, con't.

- Type II most commonly studied (Don't worry about types I and III)
- Many target sequences, called "restriction sites" are **palindromes**
- Cleavage of palindromic sites leave single stranded "sticky ends", either 5' or 3'
- Some create "blunt" ends
- Most are type *a* phosphodiesterases, leaving a 5' phosphate and a free 3'-OH

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Restriction Endonucleases, con't.

- Nomenclature based on bacterial strain
  - 1st letter genus, 2nd, 3rd species,
  - 4th letter strain, number is order of discovery
- See Table 11.5 for about 40 of the thousand known enzymes

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Restriction Mapping

- Samples of DNA cut with a particular restriction enzyme yield a set of characteristic polynucleotides, separable electrophoresis according to size.
- Each enzyme produces its own characteristic set of sized fragments
- Fragments can be reassembled as in a jigsaw puzzle to produce a "restriction map"
  - See Figure 11.33

It is difficult to isolate large fragments of DNA without random shearing of the molecules. Treatment of the sheared pieces with restriction enzymes produces the same set of restriction fragments as with intact DNA, except for a little loss of material at the points where the shearing occurred.

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DNA Cloning

- We will skip Chapter 13 which discusses cloning in some detail
- Nevertheless, the principle is important to understand
  - Restriction fragments can be re-combined by association of "sticky ends", or enzymatic ligation of blunt ends
  - Insertion of DNA fragments into "vectors" such as viruses can lead to replication of the fragment

The fragments replicated in such "cloning" experiments can be used in a variety of ways. One is to select "clones" carrying a fragment with a particular characteristic, having it multiply, then isolating it to determine the sequence. Another is to "clone" in such a way that the DNA will be expressed in the form of a protein, which can be isolated and used for studies (or therapy).