MBMB, BCHM, or CHEM 451A

- This is a team taught course
  - Blaine Bartholomew: 1st section
  - Joseph Schmit: 2nd section
  - Peter Hardwicke: 3rd Section

- Text is Lehninger Principles of Biochemistry 4th edition
  - Supplementary material found in Genes VIII which is on reserve at Morris Library
Grading

• Each section is worth 100 points
• The final exam is worth 200 points
• You can drop one of the other exams for a total number of points possible being 400 points
• In my section the points are 30 points for homework and 70 point for my exam
Notes and other helps

• Notes will be posted on web at: http://web.siumed.edu/~bbartholomew/451A_Sec1.html

• Suggested that you print out the lecture outline before class to help you with your in-class note taking

• Don’t forget the suggested readings and problems
And other helps

Instructor

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Science is question driven

How does DNA encode for all the characteristics found within an organism?
Questions

• How is DNA read by the cell?
• What are the distinguishing features of DNA that accounts for its specificity?
• Are there certain chemical or mechanical properties of DNA that are vital in this process?
• What are those factors (proteins) that “read” DNA and how do they work
Foundations

One must first understand the structural parameters of DNA and its physical properties.
Next it is important to know what are the variations that can be found in nature
Chemical structure and base composition

1. Numbering system of nucleic acids
2. Phosphate linkages - phosphoester bonds
3. Nucleotide composition
4. Sugar ring - pucker
Chemical structure and base composition

1. Numbering system of nucleic acids
   a. phosphate (alpha, beta, gamma)
### Abbreviations of ribonucleoside 5'-phosphates

<table>
<thead>
<tr>
<th>Base</th>
<th>Mono-</th>
<th>Di-</th>
<th>Tri-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>AMP</td>
<td>ADP</td>
<td>ATP</td>
</tr>
<tr>
<td>Guanine</td>
<td>GMP</td>
<td>GDP</td>
<td>GTP</td>
</tr>
<tr>
<td>Cytosine</td>
<td>CMP</td>
<td>CDP</td>
<td>CTP</td>
</tr>
<tr>
<td>Uracil</td>
<td>UMP</td>
<td>UDP</td>
<td>UTP</td>
</tr>
</tbody>
</table>

### Abbreviations of deoxyribonucleoside 5'-phosphates

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<tr>
<th>Base</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>dAMP</td>
<td>dADP</td>
<td>dATP</td>
</tr>
<tr>
<td>Guanine</td>
<td>dGMP</td>
<td>dGDP</td>
<td>dGTP</td>
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<tr>
<td>Cytosine</td>
<td>dCMP</td>
<td>dCDP</td>
<td>dCTP</td>
</tr>
<tr>
<td>Thymine</td>
<td>dTMP</td>
<td>dTDP</td>
<td>dTTP</td>
</tr>
</tbody>
</table>
Chemical structure and base composition

1. Numbering system of nucleic acids
   a. phosphate (alpha, beta, gamma)
   b. base (1,2,3,...)
Pyrimididine

Purine
Chemical structure and base composition

1. Numbering system of nucleic acids
   a. phosphate (alpha, beta, gamma)
   b. base (1, 2, 3, ...)
   c. sugar (1', 2', 3', ...)
Phosphate

Purine or pyrimididine base

Pentose

1'

2'

3'

4'

5'

H

H

H

H

H

OH

OH
Chemical structure and base composition

1. Numbering system of nucleic acids
   a. phosphate (alpha, beta, gamma)
   b. base (1, 2, 3, ...)
   c. sugar (1', 2', 3', ...)
   d. shorthand notation
2. Phosphate linkages - phosphoester bonds
   a. phosphomonoester bond
   b. phosphodiester bond
   c. phosphotriester bond
Chemical structure and base composition

3. nucleotide
   a. normal base composition
Adenine

Guanine

Purines

Cytosine

Thymine (DNA)

Uracil (RNA)

Pyrimidines
Chemical structure and base composition

3. nucleotide
   a. normal base composition
   b. modified bases
Modified Bases

5-Methylcytidine

$N^6$-Methyladenosine

$N^2$-Methylguanosine

5-Hydroxymethylcytidine
Modified Bases

Inosine

Pseudouridine

7-Methylguanosine

4-Thiouridine
Chemical structure and base composition

3. nucleotide
   a. normal base composition
   b. modified bases
   c. tautomers
Tautomers

(a) Thymine (keto or lactam form) ↔ Thymine (enol or lactim form)

(b) Guanine (keto or lactam form) ↔ Guanine (enol or lactim form)
Chemical structure and base composition

4. sugar ring -
   a. nucleotide vs. nucleoside
   b. deoxyribose vs. ribose  see figure 5-1
   c. base-catalyzed hydrolysis of RNA (not DNA)
      due to 2'-OH of RNA
   d. ring pucker:
      endo vs. exo and C-3' vs C-2'
## Nucleotide versus nucleoside

<table>
<thead>
<tr>
<th>Base Formula</th>
<th>Base (X = H)</th>
<th>Nucleoside (X = ribose)</th>
<th>Nucleotide (X = ribose phosphate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₂</td>
<td>Adenine</td>
<td>Adenosine</td>
<td>Adenyllic acid</td>
</tr>
<tr>
<td></td>
<td>Ade</td>
<td>Ado</td>
<td>Adenosine monophosphate</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>A</td>
<td>AMP</td>
</tr>
<tr>
<td>H</td>
<td>Guanine</td>
<td>Guanosine</td>
<td>Guanylic acid</td>
</tr>
<tr>
<td></td>
<td>Gua</td>
<td>Guo</td>
<td>Guanosine monophosphate</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>G</td>
<td>GMP</td>
</tr>
<tr>
<td>NM</td>
<td>Cytosine</td>
<td>Cytidine</td>
<td>Cytidylic acid</td>
</tr>
<tr>
<td></td>
<td>Cyt</td>
<td>Cyd</td>
<td>Cytidine monophosphate</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>C</td>
<td>CMP</td>
</tr>
<tr>
<td>OX</td>
<td>Uracil</td>
<td>Uridine</td>
<td>Uridylic acid</td>
</tr>
<tr>
<td></td>
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<td>Urd</td>
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<td>Thymine</td>
<td>Deoxythymidine</td>
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</tr>
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<td></td>
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<td>dThd</td>
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</tr>
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<td>T</td>
<td>dT</td>
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</tr>
</tbody>
</table>
Chemical structure and base composition

4. sugar ring -
   a. nucleotide vs. nucleoside
   b. deoxyribose vs. ribose  see figure 5-1
   c. base-catalyzed hydrolysis of RNA (not DNA) due to 2'-OH of RNA
   d. ring pucker:
      endo vs. exo and C-3' vs C-2'
(a) **Ribonucleotides**

(b) **Deoxyribonucleotides**
Chemical structure and base composition

4. sugar ring -
   a. nucleotide vs. nucleoside
   b. deoxyribose vs. ribose  see figure 5-1
   c. base-catalyzed hydrolysis of RNA (not DNA) due to 2'-OH of RNA
   d. ring pucker: endo vs. exo and C-3' vs C-2'
Alkaline Hydrolysis of RNA

2',3'-Cyclic monophosphate derivative + H₂O → Mixture of 2'- and 3'-monophosphate derivatives

RNA → Shortened RNA

Alkaline Hydrolysis of RNA
Chemical structure and base composition

4. sugar ring -
   a. nucleotide vs. nucleoside
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      (not DNA)
      due to 2'-OH of RNA
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      endo vs. exo and C-3' vs C-2'
Ring Pucker

[Diagram of a molecular structure with labeled atoms: C₁', O₄', C₂', O₂', O₃', C₄', C₅', C₃ behind H (eclipsed), O₃' (eclipsed), Base, C₃']
Double Helical Structures

1. Watson Crick Structure: B-DNA
   a. antiparallel orientation, 3' vs 5' ends
   b. base pairing interactions
      i. always a purine-pyrimidine (steric constraints)
      ii. tautomeric forms of bases
   c. double helical parameters
   d. real DNA deviates from the ideal B-DNA form
Double Helical Structures

1. Watson Crick Structure: B-DNA
   c. double helical parameters
      i. helical sense: right vs left
      ii. major vs minor groove
      iii. base pairs per helical turn
      iv. helix rise per base pair or helical pitch
          - distance from one step to the next
      v. helical twist: angle between two adjacent base pairs
          - 360 deg/base pairs per turn
      vi. base tilt: slant of the step, not completely planar
      vii. glycosidic conformation: anti vs syn - figure 29-8
      viii. sugar ring pucker: 4 out of 5 ring atoms are nearly planar
           - the 5th atom is usually the C-2 or C-3 atom endo vs exo
<table>
<thead>
<tr>
<th>Property</th>
<th>A-DNA</th>
<th>B-DNA</th>
<th>Z-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helical sense</td>
<td>Right-handed</td>
<td>Right-handed</td>
<td>Left-handed</td>
</tr>
<tr>
<td>Diameter</td>
<td>~26 Å</td>
<td>~20 Å</td>
<td>~18 Å</td>
</tr>
<tr>
<td>Base pairs per helical turn</td>
<td>11.6</td>
<td>10</td>
<td>12 (6 dimers)</td>
</tr>
<tr>
<td>Helical twist per base pair</td>
<td>31°</td>
<td>36°</td>
<td>9° for pyrimidine–purine steps; 51° for purine–pyrimidine steps</td>
</tr>
<tr>
<td>Helix pitch (rise per turn)</td>
<td>34 Å</td>
<td>34 Å</td>
<td>44 Å</td>
</tr>
<tr>
<td>Helix rise per base pair</td>
<td>2.9 Å</td>
<td>3.4 Å</td>
<td>7.4 Å per dimer</td>
</tr>
<tr>
<td>Base tilt normal to the helix axis</td>
<td>20°</td>
<td>6°</td>
<td>7°</td>
</tr>
<tr>
<td>Major groove</td>
<td>Narrow and deep</td>
<td>Wide and deep</td>
<td>Flat</td>
</tr>
<tr>
<td>Minor groove</td>
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</tr>
<tr>
<td>Sugar pucker</td>
<td>C3'-endo</td>
<td>C2'-endo</td>
<td>C2'-endo for pyrimidines; C3'-endo for purines</td>
</tr>
<tr>
<td>Glycosidic bond</td>
<td>Anti</td>
<td>Anti</td>
<td>Anti for pyrimidines; syn for purines</td>
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Double Helical Structures

1. Watson Crick Structure: B-DNA
   d. real DNA deviates from the ideal B-DNA form
      i. local deviations are common
      ii. some DNA is naturally bent
      iii. deviations are sequence dependent
Double Helical Structures

1. Watson Crick Structure: B-DNA
d. real DNA deviates from the ideal B-DNA form
   i. local deviations are common
   ii. some DNA is naturally bent
   iii. deviations are sequence dependent
Double Helical Structures

2. A-DNA
   a. wider and flatter than B-DNA
      i. very shallow minor groove
      ii. deeper major groove
   b. tilt is 20 deg (most tilted)
   c. dried out DNA, 75% vs 92% humidity
   d. flat ribbon wound around a 6 angstrom hole
   e. found in spores because of close packaging and RNA-RNA/RNA-DNA hybrids assume an A-DNA like structure
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<td>Anti for pyrimidines; syn for purines</td>
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</tbody>
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3. **Z-DNA**
   a. Characteristics of
      i. occurs in alternating purine-pyrimidine tracts
      ii. favored in high salt; helps eliminate electrostatic repulsion of phosphate groups (8 vs 12 angstrom distance)
      iii. methylation of deoxycytidine helps formation of Z-DNA
      iv. phosphate backbone forms a zig-zag conformation
3. Z-DNA
   b. double helical parameters
      i. syn vs. anti conformation
         purines flip to assume syn
      ii. helical sense is left handed
      iii. deep minor groove, no major groove
   c. biological role of Z-DNA
      i. unclear at this point
      ii. anti Z-DNA antibody detection, artifactual
      iii. methylation proved to be less artificial
Double Helical Structures

3. Z-DNA  figure 29-3 a & b
   b. double helical parameters
      i. syn vs. anti conformation
         purines flip to assume syn
      ii. helical sense is left handed
      iii. deep minor groove, no major groove
   c. biological role of Z-DNA
      i. unclear at this point
      ii. anti Z-DNA antibody detection, artifactual
      iii. methylation proved to be less artificial
4. Unusual DNA structures
   a. palindrome vs. mirror repeat
      i. example - placement of invert repeats
      ii. hairpin
      iii. cruciform
   b. Hoogsteen base pairing
      i. triplex formation - figure a and b
      ii. G tetraplex - found at telomeres
   c. Triple helix
Palindrome

T T A G C A C G T G C T A A
A A T C G T G C A C G A T T

Mirror repeat

T T A G C A C C A C G A T T
A A T C G T G G T G C T A A
Double Helical Structures

4. Unusual DNA structures
   a. palindrome vs. mirror repeat
      i. example - placement of invert repeats
      ii. hairpin
      iii. cruciform
   b. Hoogsteen base pairing
      i. triplex formation
      ii. G tetraplex - found at telomeres
   c. Triple helix
Guanosine tetraplex
(c)
Double Helical Structures

4. Unusual DNA structures
   a. palindrome vs. mirror repeat
      i. example - placement of invert repeats
      ii. hairpin
      iii. cruciform
   b. Hoogsteen base pairing
      i. triplex formation - figure a and b
      ii. G tetraplex - found at telomeres
   c. Triple helix
Forces that help to form the DNA double helix

1. Rigid phosphate backbone
2. Stacking interactions - electronic interactions between planar bases
3. Hydrophobic interactions - highly negative phosphate backbone vs. nonpolar bases
4. Hydrogen bonding is not the most energetically significant component
   note: maintenance of distance from the two phosphate backbone requires Pur-Pyr
5. Ionic interactions - salt stabilizes the duplex form of DNA
   shielding of phosphate backbone
Denaturation and Renaturation

1. Tm: (melting temperature) temperature at which half of the DNA is melted

   Marmur-Doty equation for Tm correlated to G+C percent and salt
   \[ Tm = 41.1 \times G+C + 16.6 \log[Na+] + 81.5 \]

2. Denaturation is a cooperative process - caused by: heat, change in pH, organic solvents (urea, formamide)

3. Hyperchromic shift - increase of absorbance of DNA when it goes from being double- to single- stranded

   40% increase in absorbance

4. Annealing: Hybridization
$0.15M$ NaCl + $0.15M$ Na citrate

G + C (mol %) vs. $T_m$ (°C)