BCMB 3100 - Nucleic Acids - Chapter 33

- Discovery of DNA
- Nucleotides, nucleosides & bases
- Polynucleotides
- DNA as genetic material
- Structure of double-stranded DNA
- Chromatin
- RNA
- Nucleases

DNA is the genetic component of life

Central Dogma for Biological Information Flow

DNA → RNA → PROTEIN

Friedrich Miescher (1869): discovered DNA
nuclein → nucleic acid

\[ \text{C, H, O, N, P} \]

DNA and RNA are made up of nucleotides

\[ \text{base} + \text{sugar} + \text{phosphate} \]

deoxyribonucleotide (sugar = 2-deoxyribose)
ribonucleotide (sugar = ribose)

\[ \text{base} + \text{sugar} \]

of nucleotides: heterocyclic rings containing nitrogen
Two class of bases: ________ and ________

Purines and Pyrimidines

See Fig. 33.5

Pyrimidine

Purine

See Fig 33.5 Major pyrimidines and purines

Pyrimidines

thymine

adenine

Cytosine

Guanine

Uracil

2,4-Diamo-5-methylpyrimidine

2-Oxo-4-aminopyrimidine

Purines

adenine

guanine

5-Amino-4-aminopurine

2-Amino-6-oxopurine

Tautomers of adenine and cytosine

Amino versus Imino

Adenine

Cytosine

Adenine

Cytosine

Amino

Imino
Tautomers of guanine, thymine and uracil

Lactam versus Lactim

Predominant forms

Ribose and Deoxyribose

See 33.3 Figure

Nucleosides

See Fig. 33.6 Nucleoside structures

See Fig 33.6

Two conformations of nucleosides & nucleotides are possible due to rotation around the glycosidic bond: syn and anti

The _______ conformation predominates

See Fig 33.7 Chemical structure of a ________
Structures of the deoxyribonucleoside-5' monophosphates

See Fig 33.7

A. Nucleotides joined by 3'-5' phosphodiester linkages

Structure of the tetranucleotide pdApdGpdTpDC

In vivo the negatively charged phosphates on nucleotides are complexed with cations or positively charged proteins

(continued)
 Backbone of Nucleic Acids

Story of DNA as Genetic Material
Discovery of the structure of double stranded DNA, 1953
James Watson, Francis Crick, Rosalind Franklin, Maurice Wilkins

S = capsular polysaccharide = death
R = no capsule = live

Isolation & characterization of the transforming principle proved the chemical make-up of the genetic material

Evidence for transforming principle!
Evidence that DNA is the genetic material in cells!!
Both set of phage were infective

Nature (1953) 171:737
April

Nature (1953) 171:964
May
Adjacent nucleotides can hydrogen bond to each other. See Fig 33.12.

DNA is double-stranded with equal ratios of G:C and of A:T. However, the ratio of (G+C):(A+T) varies in a species specific manner.

<table>
<thead>
<tr>
<th>Source</th>
<th>A</th>
<th>G</th>
<th>C</th>
<th>T</th>
<th>(A+T)/(G+C)</th>
<th>(G+C)/(A+T)</th>
<th>Parimeter/expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria/soil</td>
<td>28.0</td>
<td>24.9</td>
<td>25.2</td>
<td>25.9</td>
<td>1.09</td>
<td>0.99</td>
<td>1.04</td>
</tr>
<tr>
<td>Mammal tissue</td>
<td>18.1</td>
<td>18.5</td>
<td>17.6</td>
<td>18.4</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Yeast</td>
<td>13.3</td>
<td>30.4</td>
<td>31.4</td>
<td>15.6</td>
<td>0.97</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Caw</td>
<td>29.0</td>
<td>21.5</td>
<td>21.2</td>
<td>28.7</td>
<td>1.01</td>
<td>1.00</td>
<td>1.01</td>
</tr>
<tr>
<td>Pig</td>
<td>29.8</td>
<td>28.7</td>
<td>28.7</td>
<td>29.1</td>
<td>1.02</td>
<td>1.00</td>
<td>1.01</td>
</tr>
<tr>
<td>Human</td>
<td>36.4</td>
<td>19.9</td>
<td>19.9</td>
<td>30.1</td>
<td>1.01</td>
<td>1.00</td>
<td>1.01</td>
</tr>
</tbody>
</table>

- Structure of B-DNA
- Sugar phosphate backbone outside
- Stacking creates two unequal grooves (major and minor)
- Hydrophobic attraction between the bases
- Van der Waals contact between bases
- H-bonds between bases
- Electrostatic repulsion between phosphates inhibited by cations (Mg++)
Watson and Crick discovered structure of B-DNA. Most common form of DNA under physiological conditions.

Double helix emphasizing the charge on the phosphate groups.

Forms of DNA

- B-DNA - Ball-and-stick model
- B-DNA - Space-filling model

- Dehydrated DNA
- RNA:DNA hybrid
- ds RNA

- DNA in vivo

- RNA:DNA hybrid
ds RNA

DNA in vivo

Dehydrated DNA

DNA in vivo

Dehydrated DNA

RNA:DNA hybrid
ds RNA

Z-DNA

Zigzag backbones
Major groove: wider - 12Å; deeper – 8.5Å

Minor groove: 6Å wide; 7.5Å deep

Table 33.1 Comparison of A, B-, and Z-DNA

<table>
<thead>
<tr>
<th>Shape</th>
<th>A</th>
<th>B-</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rise per base pair</td>
<td>3.4 Å</td>
<td>3.8 Å</td>
<td>2.8 Å</td>
</tr>
<tr>
<td>Helix diameter</td>
<td>~26 Å</td>
<td>~20 Å</td>
<td>~18 Å</td>
</tr>
<tr>
<td>Screw sense</td>
<td>Right-handed</td>
<td>Right-handed</td>
<td>Left-handed</td>
</tr>
<tr>
<td>Glycosidic bond*</td>
<td>anti</td>
<td>anti</td>
<td>Alternating anti and syn</td>
</tr>
<tr>
<td>Base pairs per turn of helix</td>
<td>11</td>
<td>10.4</td>
<td>12</td>
</tr>
<tr>
<td>Pitch per turn of helix</td>
<td>25.3 Å</td>
<td>25.4 Å</td>
<td>45.6 Å</td>
</tr>
<tr>
<td>Tilt of base pairs from perpendicular to helix axis</td>
<td>19 degrees</td>
<td>1 degree</td>
<td>9 degrees</td>
</tr>
</tbody>
</table>

*anti and syn refer to the orientation of the glycosidic bond between the base and deoxyribose. In the anti orientation, the base is above the deoxyribose. Pyrimidines can be in anti or syn orientations only, whereas purines can be anti or syn.

DNA molecules vary greatly in length depending upon the organism and organelle

<table>
<thead>
<tr>
<th>Species</th>
<th>Length</th>
<th>Genome size</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>4.2 x 10⁶ bp</td>
<td>same</td>
</tr>
<tr>
<td>fruit fly</td>
<td>62 x 10⁶ bp</td>
<td>130 x 10⁶ bp</td>
</tr>
<tr>
<td>mitochondria</td>
<td>0.015 x 10⁶ bp</td>
<td>same (from mammals; can be up to 2.5 x 10⁸ in plants)</td>
</tr>
<tr>
<td>mitochondria</td>
<td>0.015 x 10⁶ bp</td>
<td>same (circular in mammals; can be linear or circular in plants)</td>
</tr>
<tr>
<td>Human</td>
<td>240 x 10⁶ bp</td>
<td>3200 x 10⁶ bp (46 chromosomes)</td>
</tr>
</tbody>
</table>

How can you detect DNA in solution?

Absorption spectra of double-stranded and single-stranded DNA

- Double-stranded (ds) DNA absorbance max 260 nm
- ___________ absorbs more than ds DNA
- dsDNA can be denatured by heat and chaotropic agents
- Extent of denaturation can be measured by OD₂₆₀

Melting curve for DNA

Temperature at which amount of dsDNA = ssDNA is Tm

Tm for poly GC is greater than Tm for poly AT
Stem-loop structures in RNA

- ssRNA can also have ds regions
- _________ or _________ can form from short regions of complementary base pairs
- Stem: base-paired nucleotides
- Loop: noncomplementary nucleotides

Four Classes of RNA in living organisms

- __________ (rRNA) - ~80% of total RNA, part of ribosomes (translation machinery)
- __________ (tRNA) - ~15% of total RNA, 73-95 nucleotides long, carry activated amino acids to ribosomes during translation
- __________ (mRNA) - linear “copies” of DNA that encode genetic information. Encode primary structure of protein. ~1-3% of total RNA, relatively unstable
- __________ - may have catalytic activity and/or associate with proteins to enhance activity, some involved with RNA processing in the nucleus

Alternative Classification of RNA

- RNAs involved in protein synthesis
  - rRNA, tRNA, mRNA, others
- RNAs involved in post-transcriptional modification or DNA replication
  - modification or DNA replication
  - snRNA, snoRNA, SmY, RNase P, others
- Regulatory RNAs
  - sRNA (antisense RNA), miRNA (microRNA), siRNA (small interfering RNA), others
- Parasitic RNAs
- Other RNAs

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Structure of supercoiled DNA. Circular B-DNA has 10.4 bases/turn of helix. If DNA is underwound (or overwound), it is supercoiled to restore 10.4 bases/turn. Supercoiling is done by topoisomerases.

Human topoisomerase I bound to DNA

- Topoisomerases can add or remove supercoils in DNA
- Cleave one or both DNA strands, unwind or overwind by rotating cleaved ends, then rejoin ends

In the nucleus DNA is found as ______________

Chromatin: an association of DNA with proteins (mostly histones) → compact & manageable packing. Chromatin looks like long threads of 30 nm diameter.

Histones - the major proteins of chromatin

Eukaryotes contain five small, basic histone proteins containing many lysines and arginines: H1, H2A, H2B, H3, and H4

Positively charged histones bind to negatively-charged sugar-phosphates of DNA

A structural unit in chromatin is the ______________

Nucleosome: a ~200 bp DNA strand wound around a histone core.

Chromatin treated with a low salt solution extends into a “beads on a string” structure. Beads are the nucleosomes; the string is DNA.
Histone octamer

Nucleosome core particle Fig. 33.26

Histone octamer

Nucleosome core particle Fig. 33.26

Histone octamer

Nucleosome core particle Fig. 33.26

Histone octamer

Nucleosome core particle Fig. 33.26

Histone octamer

Nucleosome core particle Fig. 33.26

Histone octamer

Nucleosome core particle Fig. 33.26

Nucleosome gives 10-fold packing

Solenoid: a higher level of chromatin structure in which adjacent nucleosome associate via histone H1

Histone-depleted chromosome scaffold. Attachment of DNA to RNA-protein scaffold gives further 200-fold packing

Solenoid give further 4-fold packing

Histone-depleted chromosome scaffold. Attachment of DNA to RNA-protein scaffold gives further 200-fold packing
Fig. 33.28

Final chromosome is 1/8000 of length of B-DNA. This allows DNA to be packaged into cells. For example, the largest human chromosome is 2.4 x 10^8 bp.

This chromosome would be 8.2 cm long if it were not packaged as chromatin (as opposed to 2-10 µm)!!

http://www.answers.com/topic/chromosome

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Nucleases and Hydrolysis of Nucleic Acids

• **Nucleases** - hydrolyze phosphodiester bonds
  - **RNases** (RNA substrates)
  - **DNases** (DNA substrates)

• May cleave either the 3'- or the 5'- ester bond of a 3'–5' phosphodiester linkage

• **Exonucleases** start at the end of a chain

• **Endonucleases** hydrolyze sites within a chain

• **Nuclease cleavage sites**

  • Cleavage at bond A generates a 5'-phosphate and a 3' OH terminus
  • Cleavage at bond B generates a 3'-phosphate and a 5'-hydroxyl terminus

  A = cleavage of 3'- ester bond
  B = cleavage of 5'- ester bond

Cleavage of 3' ester of Guanylate


Cleavage of 5' ester of Guanylate

DNA is stable in basic solution

RNA is unstable in basic solution

Alkaline Hydrolysis of RNA

RNA is less stable than DNA because RNA is sensitive to hydrolysis in basic solutions

Ribonuclease-Catalyzed Hydrolysis of RNA

RNase A cleaves 5' ester to right of pyrimidines


Enzyme-catalyzed cleavage by RNase A results, specifically, in a 3'-phosphate product
site-specific endodeoxyribonucleases causing cleavage of both strands of DNA at points within or near the specific site recognized by the enzymes; important tools in genetic engineering

______________________: catalyze both methylation of host DNA and cleavage of non-methylated DNA at recognition site

______________________: cleave non-methylated DNA at recognition site

Most restriction enzymes recognize palindromes: inverted sequences with two-fold symmetry over two strands

\[
\begin{align*}
\text{5'} \text{AAGAATTCCG} \text{3'} \\
\text{3'} \text{ATCTTAAGCC} \text{5'}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Source</th>
<th>Enzyme*</th>
<th>Recognition sequence$^\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetobacter pasteurianus</td>
<td>Apal</td>
<td>GAGGCGGC</td>
</tr>
<tr>
<td>Bacteriophage T4</td>
<td>BstBI</td>
<td>GAGGCGGC</td>
</tr>
<tr>
<td>Escherichia coli 5953</td>
<td>EcoRI</td>
<td>GAATTC</td>
</tr>
<tr>
<td>Escherichia coli 2073</td>
<td>EcoRI</td>
<td>GAATTC</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>HinfI</td>
<td>AGCCCT</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>HinfII</td>
<td>AGCCCT</td>
</tr>
<tr>
<td>Haemophilus parathyreus</td>
<td>HpaII</td>
<td>CGCCGG</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>KpnI</td>
<td>GGCGGC</td>
</tr>
<tr>
<td>Neisseria rattus equi</td>
<td>NdeI</td>
<td>GGGGCC</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>PstI</td>
<td>GGGGCC</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>SacI</td>
<td>GGGGCG</td>
</tr>
<tr>
<td>Tannohomonas acidilactica</td>
<td>XacI</td>
<td>GGGGCG</td>
</tr>
<tr>
<td>Xanthomonas velutina</td>
<td>XbaI</td>
<td>GGGGCG</td>
</tr>
</tbody>
</table>

- Methylation and restriction at the EcoR1 site

**EcoR1**

\[
\begin{align*}
\text{5'} \text{NGAAATTCCN} \text{3'} \\
\text{3'} \text{NCTTAAGN} \text{5'}
\end{align*}
\]

(a) R.C.

\[
\begin{align*}
\text{5'} \text{NGAAATTCCN} \text{3'} \\
\text{3'} \text{NCTTAAGN} \text{5'}
\end{align*}
\]

**EcoR1**

\[
\begin{align*}
\text{5'} \text{NGAAATTCCN} \text{3'} \\
\text{3'} \text{NCTTAAGN} \text{5'}
\end{align*}
\]

- Methylation and replication

\[
\begin{align*}
\text{5'} \text{NGAAATTCCN} \text{3'} \\
\text{3'} \text{NCTTAAGN} \text{5'}
\end{align*}
\]

D. **EcoR1 Binds Tightly to DNA**

- EcoR1 has 2 identical subunits (purple and yellow)
- Bound to a fragment of DNA (strands blue and green)
Uses of Restriction Endonucleases

- Developing restriction maps (indicates specific cleavage sites in a DNA fragment)
- Map of bacteriophage $\lambda$, showing cleavage sites of some restriction enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Size (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApaI</td>
<td>10.0</td>
</tr>
<tr>
<td>BspI</td>
<td>6.9</td>
</tr>
<tr>
<td>KpnI</td>
<td>5.5</td>
</tr>
<tr>
<td>XhoI</td>
<td>9.5</td>
</tr>
<tr>
<td>XbaI</td>
<td>15.0</td>
</tr>
</tbody>
</table>

- DNA Fingerprinting

- Restriction digest of bacteriophage $\lambda$
- Four restriction enzymes used
- Sizing gel separates fragments (smallest move fastest)