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)

C, H, O, N, P

DNA and RNA are made up of nucleotides

__________: base + sugar + phosphate

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

__________: base + sugar

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

Purines and Pyrimidines

See Fig. 33.5
See Fig 33.5 Major pyrimidines and purines

**PYRIMIDINES**
- Uracil (2-Deoxy-uracil)
- Thymine (2-Deoxy-2-thymine)
- Cytosine (2-Deoxy-cytosine)

**PURINES**
- Adenine (6-Aminopurine)
- Guanine (2-Aminopurine)

Tautomers of adenine and cytosine

Amino versus Imino

Predominant forms

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

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 ________

To distinguish the labeling of the sugar carbons from the base ring positions, the carbons in sugars in nucleotides and nucleosides are called “prime”; ' 
Nomenclature of bases, nucleosides, and nucleotides

<table>
<thead>
<tr>
<th>Base</th>
<th>Ribonucleoside</th>
<th>Ribonucleoside (5'-monophosphate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine (A)</td>
<td>Adenine</td>
<td>Adenine 5'-monophosphate (AMP; adenylic acid)</td>
</tr>
<tr>
<td>Guanine (G)</td>
<td>Guanosine</td>
<td>Guanosine 5'-monophosphate (GMP; guanylic acid)</td>
</tr>
<tr>
<td>Cytosine (C)</td>
<td>Cytidine</td>
<td>Cytidine 5'-monophosphate (CMP; cytidylate)</td>
</tr>
<tr>
<td>Uracil (U)</td>
<td>Uridine</td>
<td>Uridine 5'-monophosphate (UMP; uridylic acid)</td>
</tr>
</tbody>
</table>

Note anti-conformation

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

*Anticlockwise orientations.*
BCMB 3100 - Nucleic Acids - Chapter 19

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

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

Representation of first two (5’-end) residues of the tetranucleotide pdApdGpdTpC

Backbone of Nucleic Acids

Fig 33.4

Story of DNA as Genetic Material


**Evidence for transforming principle!**

- **S** have capsular polysaccharide = death (pathogenic)
- **R** do NOT have capsule = live (NOT pathogenic)

**Dead S ≠ death**

**Live R ≠ death**

**Dead S + Live R = death !!!!**

---

**Evidence that DNA is the genetic material in cells!!**

- Oswald Avery, Colin MacLeod, Maclyn McCarty (1944):
  - It was believed that protein in chromosomes was the transforming principle.
  - They isolated transforming principle from heat-killed extract of S pneumoniae and found that it was a "nucleic acid or deoxyribonucleic acid".
  - It was extracted and characterized and found to be DNA.

**Evidence that DNA is the genetic material**

- Chargaff Rule:
  - \( dA = dT \)
  - \( dC = dG \)

**Story of DNA as Genetic Material**

**Discovery of the structure of double stranded DNA, 1953**

James Watson, Francis Crick, Rosalind Franklin, Maurice Wilkins
Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid.

Genetical implications of the structure of deoxyribonucleic acid.

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

### Chargaff Rule

<table>
<thead>
<tr>
<th>Source</th>
<th>A</th>
<th>G</th>
<th>C</th>
<th>T</th>
<th>A/T</th>
<th>G/C</th>
<th>G+C</th>
<th>Percentage of (G+C)</th>
<th>Pitch (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>30.4</td>
<td>19.9</td>
<td>19.9</td>
<td>30.4</td>
<td>1.01</td>
<td>1.00</td>
<td>1.00</td>
<td>59K</td>
<td>34 Å</td>
</tr>
<tr>
<td>Mouse</td>
<td>31.5</td>
<td>18.3</td>
<td>18.3</td>
<td>31.5</td>
<td>1.05</td>
<td>1.65</td>
<td>1.85</td>
<td>70.5</td>
<td>34 Å</td>
</tr>
<tr>
<td>Yeast</td>
<td>31.5</td>
<td>18.3</td>
<td>18.3</td>
<td>31.5</td>
<td>1.05</td>
<td>1.65</td>
<td>1.85</td>
<td>70.5</td>
<td>34 Å</td>
</tr>
<tr>
<td>E.coli</td>
<td>31.5</td>
<td>18.3</td>
<td>18.3</td>
<td>31.5</td>
<td>1.05</td>
<td>1.65</td>
<td>1.85</td>
<td>70.5</td>
<td>34 Å</td>
</tr>
<tr>
<td>R. mutans</td>
<td>31.5</td>
<td>18.3</td>
<td>18.3</td>
<td>31.5</td>
<td>1.05</td>
<td>1.65</td>
<td>1.85</td>
<td>70.5</td>
<td>34 Å</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++)

[Note: role of histones in eukaryotes]
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:
- Dehydrated DNA
- RNA:DNA hybrid
- ds RNA
- DNA in vivo
- in some GC rich regions left-handed DNA
Table 33.1 Comparison of A-, B-, and Z-DNA

<table>
<thead>
<tr>
<th>Helix type</th>
<th>A</th>
<th>B</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Broadest</td>
<td>Intermediate</td>
<td>Narrowest</td>
</tr>
<tr>
<td>Rise per base pair</td>
<td>2.3 Å</td>
<td>3.4 Å</td>
<td>3.8 Å</td>
</tr>
<tr>
<td>Helix diameter</td>
<td>~36 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>35.4 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 orientations only, whereas purines can be anti or syn.

Fig 33.17

**DNA in vivo**
- Dehydrated DNA
- RNA-DNA hybrid
- ds RNA

**B-DNA**
- Top view
- Side view

**A-DNA**
- Top view
- Side view

**Z-DNA**
- Top view
- Side view
- Zigzag backbones

**Fig 33.17**

**Fig 33.20**

**Z-DNA**
- in some GC rich regions
- left-handed DNA
Major groove: wider - 12Å; deeper – 8.5Å

Minor groove: 6Å wide; 7.5Å deep

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>$4.2 \times 10^6$ bp</td>
<td>same</td>
</tr>
<tr>
<td>fruit fly</td>
<td>$62 \times 10^6$ bp</td>
<td>$130 \times 10^6$ bp</td>
</tr>
<tr>
<td>mitochondria</td>
<td>$0.015 \times 10^6$ bp</td>
<td>same</td>
</tr>
<tr>
<td>(from mammals; can be up to $2.5 \times 10^8$ in plants)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(circular in mammals; can be linear or circular in plants)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>$240 \times 10^6$ bp</td>
<td>$3200 \times 10^6$ bp</td>
</tr>
<tr>
<td>(46 chromosomes)</td>
<td></td>
<td></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 $OD_{260}$

Melting curve for DNA

Temperature at which amount of dsDNA = ssDNA is $T_m$$($_______)$

$T_m$ for poly GC is greater than $T_m$ for poly AT
BCMB 3100 - Nucleic Acids - Chapter 33

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

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

ss = single stranded

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
  - snRNA, snoRNA, SmY, RNase P, others
- Regulatory RNAs
  - aRNA (antisense RNA), miRNA (microRNA), siRNA (small interfering RNA), others
- Parasitic RNAs
- Other RNAs

BCMB 3100 - Nucleic Acids - Chapter 33

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

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.

If DNA in bacteria were not packed, it would extend 1000x longer than the bacteria!

Supercoiling is part of this compaction mechanism.
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

<table>
<thead>
<tr>
<th>Type</th>
<th>Molecular weight</th>
<th>Number of residues</th>
<th>Number of basic residues</th>
<th>Number of acidic residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit thymus H1</td>
<td>21 600</td>
<td>213</td>
<td>65</td>
<td>10</td>
</tr>
<tr>
<td>Calf thymus H2A</td>
<td>14 600</td>
<td>129</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>Calf thymus H2B</td>
<td>13 800</td>
<td>125</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td>Calf thymus H3</td>
<td>15 300</td>
<td>135</td>
<td>33</td>
<td>11</td>
</tr>
<tr>
<td>Calf thymus H4</td>
<td>11 300</td>
<td>102</td>
<td>27</td>
<td>7</td>
</tr>
</tbody>
</table>

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.

Electron micrograph of chromatin
Electron micrograph of chromatin
*(note error in legend – this is actually salt-treated chromatin)*

**Fig. 33.24**

**Histone octamer**

**Nucleosome core particle** **Fig. 33.26**

**Nucleosome**

Nucleosome gives 10-fold packing

146 bp

54 bp
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.

Representation of the compaction of DNA into a eukaryotic chromosome.
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 $2.4 \times 10^8$ 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

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

\[ 5'\ldots p\text{GpCpAp…}3' + H_2O \rightarrow 5'\ldots p\text{G} + p\text{CpAp…}3' \]

Cleavage of 5’ ester of Guanylate

\[ 5'\ldots p\text{GpCpAp…}3' + H_2O \rightarrow 5'\ldots p + p\text{GpCpAp…}3' \]

Alkaline Hydrolysis of RNA

DNA is stable in basic solution

RNA is unstable in base

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

(From previous page)

2,3’-Cyclic nucleotide monophosphate

(1)

2’-Nucleotide monophosphate

(2)
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

$$5'\text{AAGAATTCCG}3'$$
$$3'\text{ATCTTAAGCC}5'$$

- Methylation and restriction at the EcoRI site

<table>
<thead>
<tr>
<th>Source</th>
<th>Enzyme</th>
<th>Recognition sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetobacter pasteurianus</td>
<td>Ase I</td>
<td>GGCGCCGG</td>
</tr>
<tr>
<td>Bacillus amyloiduricites</td>
<td>Bam HI</td>
<td>GGATCC</td>
</tr>
<tr>
<td>Escherichia coli RY3</td>
<td>Eco RI</td>
<td>GGATCC</td>
</tr>
<tr>
<td>Escherichia coli R245</td>
<td>Eco RI</td>
<td>GCCCTT</td>
</tr>
<tr>
<td>Haemophilus parasuis</td>
<td>Hpa II</td>
<td>GGCACT</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>Hinf I</td>
<td>GGCACT</td>
</tr>
<tr>
<td>Haemophilus parainfluenza</td>
<td>Hp II</td>
<td>GGCACT</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>Kpn I</td>
<td>GGCTAC</td>
</tr>
<tr>
<td>Nocardioides cyriacius</td>
<td>Nco I</td>
<td>GGCGCGCC</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Pst I</td>
<td>GCGCGC</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>Sma I</td>
<td>GGCGCGCC</td>
</tr>
<tr>
<td>Xanthomonas arabidopsis</td>
<td>Xba I</td>
<td>GCAGTA</td>
</tr>
<tr>
<td>Xanthomonas phascolarum</td>
<td>Xho I</td>
<td>GCGCGC</td>
</tr>
</tbody>
</table>

- The endonuclease recognizes the GAATTCC sequence and cleaves both strands of the foreign DNA to produce fragments with staggered ends,
D. *Eco*R1 Binds Tightly to DNA

- *Eco*R1 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 λ, showing cleavage sites of some restriction enzymes

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

DNA Fingerprinting