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\textsubscript{(n)}

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

![Pyrimidine](image)

Pyrimidine

![Purine](image)

Purine
See Fig 33.5 Major pyrimidines and purines

PYRIMIDINES

Uracil (2,4-Dioxopyrimidine)
Thymine (2,4-Dioxo-5-methylpyrimidine)
Cytosine (2-Oxo-4-aminopyrimidine)

PURINES

Adenine (6-Aminopurine)
Guanine (2-Amino-6-oxopurine)

Tautomers of adenine and cytosine

Amino versus Imino
**Tautomers of guanine, thymine and uracil**

**Lactam versus Lactim**

**Predominant forms**

- **Guanine**
- **Thymine**
- **Uracil**

**Ribose and Deoxyribose**

**See 33.3 Figure**

- **Ribose** (β-β-Ribofuranose)
- **Deoxyribose** (2-Deoxy-β-β-ribofuranose)

↓ **RNA**

↓ **DNA**
Nucleosides

See Fig. 33.6 Nucleoside structures

(a)

Adenosine

Guanosine

Cytidine

Uridine

(b)

Dexyadenosine

Dexyguanosine

Dexycytidine

Dexythymidine (Thymidine)
Two conformations of nucleosides & nucleotides are possible due to rotation around the glycosidic bond: 
**syn** and **anti**

The _________ conformation predominates

See Fig 33.6

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” : '
Structures of the deoxyribonucleoside-5’-monophosphates

See Fig 33.7
In vivo the negatively charged phosphates on nucleotides are complexed with cations or positively charged proteins
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
**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 for transforming principle!

Evidence that DNA is the genetic material in cells!!

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

Oswald Avery, Colin MacLeod, Maclyn McCarty (1944) Up to this time it was believed that protein in chromosomes was the transforming principle. They isolated transforming principle from heat-killed extract of *S pneumococci* & found that it was a “mucoid acid of the desoxyribose type”. It had chemical, optical, ultracentrifugal, diffusive & electrophoretic properties of DNA. It was not destroyed or lost upon extraction for lipid or protein or treatment with proteases or nuclease (RNase). It was destroyed by treatment with DNase! DNA was the genetic material!!!!.

Experiments by Roger Hershoff (1951) and Alfred Hershey & Martha Chase (1952) demonstrated that it was DNA in the T2 bacteriophage that was transferred to *E.coli* and led to infection by virus.

Erwin Chargaff (1940s) found that in prokaryotes and eukaryotes the molar ratio of dA = dT & dC = dG.

Chargaff Rule

dA = dT

dC = dG
Both set of phage were infective

Experiments on T2 phage by Herriott, and Hershey and Chase

Story of DNA as Genetic Material

Discovery of the structure of double stranded DNA, 1953

James Watson, Francis Crick, Rosalind Franklin, Maurice Wilkins

Confirmed DNA is the genetic material in cells.
Nature (1953) 171:737  
April

Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid.

Nature (1953) 171:964  
May

Genetical implications of the structure of deoxyribonucleic acid.
Adjacent nucleotides can hydrogen bond to each other

see Fig 33.12

(Deoxy)Adenosine

(Deoxy)Cytidine

(Deoxy)Guanosine

(Deoxy)Thymidine

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
Chargaff Rule

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

<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>Purine/pyrimidine</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>26.0</td>
<td>24.9</td>
<td>25.2</td>
<td>23.9</td>
<td>1.09</td>
<td>0.99</td>
<td>50.1</td>
<td>1.04</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>15.1</td>
<td>34.9</td>
<td>35.4</td>
<td>14.6</td>
<td>1.03</td>
<td>0.99</td>
<td>70.3</td>
<td>1.00</td>
</tr>
<tr>
<td>Yeast</td>
<td>31.7</td>
<td>18.3</td>
<td>17.4</td>
<td>32.6</td>
<td>0.97</td>
<td>1.05</td>
<td>35.7</td>
<td>1.00</td>
</tr>
<tr>
<td>Cow</td>
<td>29.0</td>
<td>21.2</td>
<td>21.2</td>
<td>28.7</td>
<td>1.01</td>
<td>1.00</td>
<td>42.4</td>
<td>1.01</td>
</tr>
<tr>
<td>Pig</td>
<td>29.8</td>
<td>20.7</td>
<td>20.7</td>
<td>29.1</td>
<td>1.02</td>
<td>1.00</td>
<td>41.4</td>
<td>1.01</td>
</tr>
<tr>
<td>Human</td>
<td>30.4</td>
<td>19.9</td>
<td>19.9</td>
<td>30.1</td>
<td>1.01</td>
<td>1.00</td>
<td>39.8</td>
<td>1.01</td>
</tr>
</tbody>
</table>
Complementary base pairing and stacking in DNA

• **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]
Double helix emphasizing the charge on the phosphate groups

Fig 33.11
Watson and Crick discovered the structure of **B-DNA**.

Most common form of DNA under physiological conditions.

**Forms of DNA**

- **A**: Dehydrated DNA
- **B**: DNA in vivo
- **Z**: DNA in some GC rich regions as left-handed DNA
Fig 33.17

**B-DNA**

**A-DNA**

**DNA in vivo**

**Dehydrated DNA**

RNA:DNA hybrid
ds RNA

Fig 33.17

**Z-DNA**

in some GC rich regions
left-handed DNA

Fig 33.17
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>~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>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>

*Syn and anti refer to the orientation of the N-glycosidic bond between the base and deoxyribose. In the syn orientation, the base is above the deoxyribose. Pyrimidines can be in anti orientations only, whereas purines can be anti or syn.
**Major groove:** wider - 12Å; deeper – 8.5Å

**Minor groove:** 6Å wide; 7.5Å deep

**Fig 33.19**

---

**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
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
  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

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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.

Relaxed; (10.4 base pairs per turn of the double helix)

Supercoiled (underwound) [supercoils are caused by underwinding or overwinding]

All base paired

Locally unwound region

Local unwinding

Supercoiling

DNA with two negative supercoils and n turns of the helix.

Closed, circular DNA with no supercoils

Closed, circular DNA with no supercoils, n-2 turns of the helix, and a locally unwound region.

See Fig. 33.22

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

Histone octamer

![Histone octamer diagram](image)
Nucleosome core particle Fig. 33.26

Nucleosome gives 10-fold packing

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

Solenoid give further 4-fold packing
Histone-depleted chromosome scaffold. Attachment of DNA to RNA-protein scaffold gives further 200-fold packing.

Protein scaffold

Loops attached to scaffold

Representation of the compaction of DNA into a eukaryotic chromosome

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 10⁸ bp.

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

\[ 5'\ldots pGpCpAp\ldots 3' + H_2O \rightarrow 5'\ldots pG + pCpAp\ldots 3' \]

Cleavage of 5’ ester of Guanylate

\[ 5'\ldots pGpCpAp\ldots 3' + H_2O \rightarrow 5'\ldots p + GpCpAp\ldots 3' \]

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

Alkaline Hydrolysis of RNA
2',3'-Cyclic nucleoside monophosphate

+ 

(From previous page)
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

\[5'\text{AAGAATTCCG}3'\]
\[3'\text{ATCTTAAGCC}5'\]

<table>
<thead>
<tr>
<th>Specificities of some common restriction endonucleases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source</strong></td>
</tr>
<tr>
<td>Acetobacter pasteurianus</td>
</tr>
<tr>
<td>Bacillus amyloliquefaciens H</td>
</tr>
<tr>
<td>Escherichia coli RY13</td>
</tr>
<tr>
<td>Escherichia coli R245</td>
</tr>
<tr>
<td>Haemophilus aegyptius</td>
</tr>
<tr>
<td>Haemophilus influenzae R4</td>
</tr>
<tr>
<td>Haemophilus parainfluenzae</td>
</tr>
<tr>
<td>Klebsiella pneumonieae</td>
</tr>
<tr>
<td>Nocardia asteroides-caviarum</td>
</tr>
<tr>
<td>Providencia stuartii</td>
</tr>
<tr>
<td>Serratia marcescens Sb</td>
</tr>
<tr>
<td>Xanthomonas haidrii</td>
</tr>
<tr>
<td>Xanthomonas walsbyi</td>
</tr>
</tbody>
</table>

39
• Methylation and restriction at the EcoR1 site

(a) Replication
Following DNA replication, the GAATTTC site is hemimethylated.

H₂C
5’~~~NNGAATTCCNN~~~3’
3’~~~NNCTTAAGNN~~~5’

EcoRI
GAATTC
CTTAAG

Methylation
A methylase catalyzes methylation of the second adenine residue in the recognition site.

H₂C
5’~~~NNGAATTCCNN~~~3’
3’~~~NNCTTAAGNN~~~5’

(b) Restriction
The endonuclease recognizes the GAATTTC sequence and cleaves both strands of the foreign DNA to produce fragments with staggered ends.

H₂C
5’~~~NNG3’
3’~~~NNCTTAAGNN~~~5’

EcoRI
GAATTC
CTTAAG

5’~~~NNG3’
3’~~~NNCTTAAGNN~~~5’

5’~~~NNCTTAAGNN~~~5’
3’~~~NNCTTAAGNN~~~5’
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 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