Original report by Watson and Crick describing their prediction of DNA structure

Nature, April 25, 1953

Nucleotide building blocks

base

mono, di, or tri phosphate

RNA

DNA

mono, di, or tri phosphate

phosphate

RNA

DNA

RNA
Canonical bases in DNA and RNA

**PURINES**
- Adenine (A)
- Guanine (G)

**PYRIMIDINES**
- Uracil (U)
- Thymine (T)
- Cytosine (C)

RNA only

DNA only

Important features of a polynucleotide chain

- The backbone has negative charge.
- The bases have hydrogen bond acceptors and donors.
- The chain has polarity: 5′-3′.
- Chemically reactive phosphodiester linkage & glycosidic bond.
Watson-Crick model for DNA structure

1. Nucleotide unit
   - Building blocks of DNA:
     - Sugar + Base + Phosphate
   - DNA strand:
     - 5' G C A 3'

2. Strand has 5'-3' polarity

3. Duplex DNA strands are antiparallel and complementary.
   - Backbone outside;
   - H-bonded bases stacked inside.

4. The strands twist in a right-handed helix

Common geometry for all 4 base pairs

- Similar width
- Similar angle of base from sugar (glycosidic bond)
- G:C has 3 H-bonds while A:T has 2 H-bonds
**B-form dsDNA features:**

- A-T and G-C base pairs
- 2 strands with anti-parallel polarity
- bases in, backbone out
- right-handed helical twist

**ALSO:**
- bases perpendicular to helix axis
- major and minor grooves
- 10 bp/36 angstrom helical repeat
- 20 angstrom wide: bp cross center

**Energetic motivations for structure formation:**

- Base pairing: ‘horizontal’ in this view
- Base stacking: ‘vertical’ in this view
- Electrostatic repulsion of phosphate backbone: gives a helical twist
Denature/melt/unpair and Renature/anneal/hybridize

- Double-stranded DNA is thermodynamically more stable than the separated strands (under physiological conditions)
- Complete unpairing of the 2 strands is denaturation; the reverse reaction is renaturation
- Heat or chaotropic agents (urea) promote denaturation; removing them promotes renaturation
- Melting point ($T_m$) - temperature at which 1/2 of the DNA has become single stranded
- Melting curves can be followed at $\text{Abs}_{260\text{nm}}$

Melting curve

Denatured DNA absorbs more than dsDNA
RNA forms base-pairs: secondary structure

Some of the modified bases found in RNA, produced by post-transcriptional reactions.
A tRNA molecule

(A) The cloverleaf secondary structure
(B, C) The L-shaped tertiary structure, based on x-ray diffraction analysis.
(D) Primary sequence

Three-dimensional structure of phenylalanine tRNA
Averaged structure of A form helix
RNA adopts this form due to ribose 2′-OH

Ball-and-stick  Space filling

Twist/bp ~32.7°
~11 bp/turn
Bases tilted
~20°
0.26 nM rise/bp

3' Minor groove (shallow and wide)
5'

Major groove (deep and narrow)

Averaged structure of B form helix
DNA prefers B form but can adopt A form

Ball-and-stick  Space filling

Twist/bp ~34°
~10 bp/turn
Bases tilted
~0°
0.34 nM rise/bp

5’

Minor groove (narrow)

Major groove (wide)
Comparison of helical parameters

<table>
<thead>
<tr>
<th>Structure type</th>
<th>Pitch (Å)</th>
<th>Helical symmetry$^a$</th>
<th>Axial rise ($h$) and turn angle</th>
<th>Groove width(^g) (Å)</th>
<th>Groove depth(^g) (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural and synthetic DNAs</td>
<td></td>
<td></td>
<td>$h$ (Å)</td>
<td>$t$ (°)</td>
<td>Minor</td>
</tr>
<tr>
<td>A</td>
<td>28.2</td>
<td>11(_1)</td>
<td>2.56</td>
<td>32.7</td>
<td>11.0</td>
</tr>
<tr>
<td>B</td>
<td>33.8</td>
<td>10(_1)</td>
<td>3.38</td>
<td>36.0</td>
<td>5.7</td>
</tr>
</tbody>
</table>

- B-form major groove depth is accessible to protein side chains, while A-form major groove is too deep: proteins can bind dsRNA sequence-specifically without distorting the helix.
- A-form is more thermodynamically stable per base-pair: shorter duplex needed for stable RNA secondary structure.
Restriction enzymes cut dsDNA at specific sequences: restriction sites

EcoRI

Cleavage

Sticky ends

5' G A A T T C
3' C T T A A G
5' A A T T C
3' G T T A A

major groove

minor groove

KEY:
- = H-bond acceptor
- = H-bond donor
○ = hydrogen atom
▲ = methyl group
EcoRI reads dsDNA in the major groove

- Homodimer of 2 identical protein subunits (purple and yellow)
- Bound to a palindromic DNA sequence (same sequence on blue and green strands)

Looking into the minor groove
Looking into the major groove

Many restriction enzymes have been identified

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source Microorganism</th>
<th>Recognition Site</th>
<th>Ends Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>PstI</td>
<td>Providencia stuartii</td>
<td>-C·T·G·C·A·G·G·A·C·G·T·C·</td>
<td>Sticky</td>
</tr>
<tr>
<td>SacI</td>
<td>Streptomyces achromogenes</td>
<td>-G·A·G·C·T·C·G·A·G·A·G·A·C·</td>
<td>Sticky</td>
</tr>
<tr>
<td>SafI</td>
<td>Streptomyces albus</td>
<td>-G·T·C·G·A·C·A·G·C·T·G·T·C·</td>
<td>Sticky</td>
</tr>
<tr>
<td>Smal</td>
<td>Serratia marcescens</td>
<td>-C·C·C·G·G·G·G·G·G·C·C·C·C·C·</td>
<td>Blunt</td>
</tr>
</tbody>
</table>

- Three types of ends: 5’ overhang, blunt and 3’ overhang
- Cognate methyl transferases protect host genome from digestion;
  Restriction-modification systems degrade “foreign” DNA.
Gel electrophoresis separates DNA by size

DNA restriction fragments

Molecules move through pores in gel at a rate inversely proportional to their chain length

Place mixture in the well of an agarose or polyacrylamide gel. Apply electric field

Agarose: big fragments (>300 bp)
Acrylamide: smaller fragments

Mobility proportional to log MW.

Subject to autoradiography or incubate with fluorescent dye

Signal corresponding to DNA band

Common DNA fluorescent dyes

B-DNA before intercalation

B-DNA after intercalation

Intercalating agents

Ethidium bromide or Acridine orange

Intercalating agents

Ethidium bromide or Acridine orange
Southern blot to detect sequence similarity

DNA restriction fragments or other forms of DNA are resolved by agarose gel electrophoresis.

The fragments are denatured to single strands. The gel is placed in buffer and covered by a nitrocellulose filter and a stack of paper towels. DNA is carried to the filter by the buffer, which is wicked up by the towels.

The DNA-bound filter is incubated with a denatured, labeled DNA probe. Strands are allowed to reanneal. If probe is complementary to the sample DNA, the filter will remain labeled when unbound probe is rinsed away.