Introduction to DNA and Protein-DNA interactions

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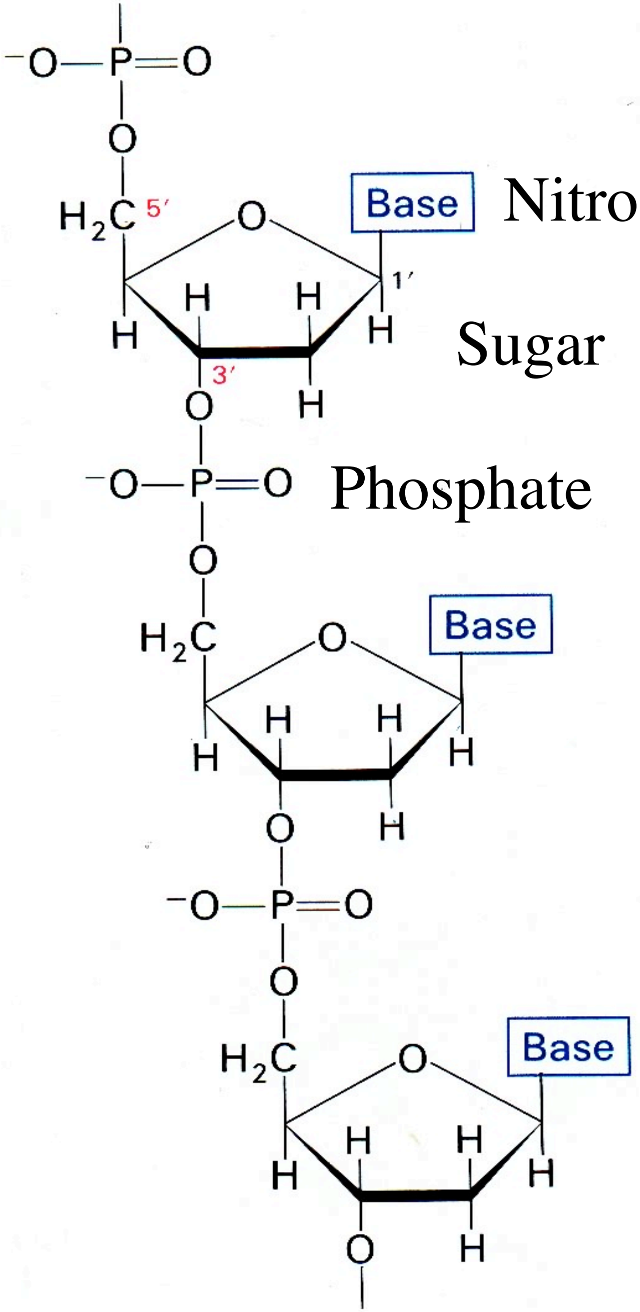
DNA Structure



Nitrogenous base

Sugar

Phosphate

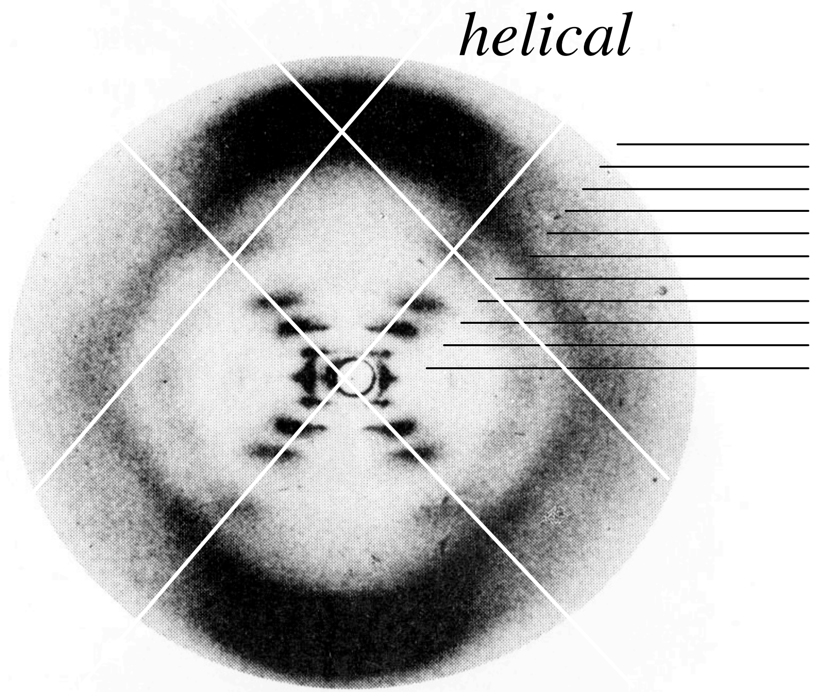
Evidence for the Double Helix

1. Fiber Diffraction data:
-Helical geometry
-3.4 A ^{o} spacing (1A^{o} = 10^{-10} m)
-34 A ^{o} pitch

2. Structure of dCTP

3. Base Tautomerism

3. Chargaff rules
- A=T, G=C



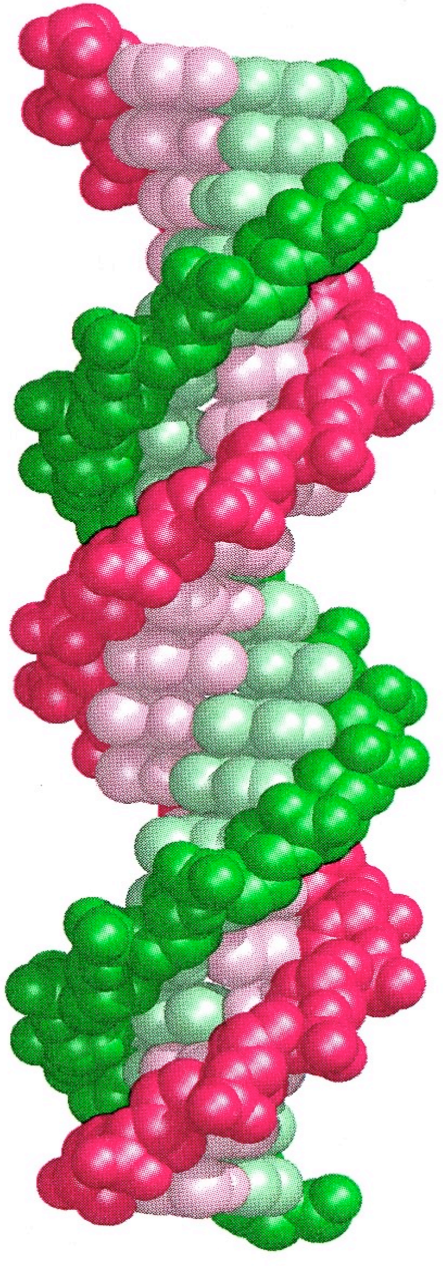
helical

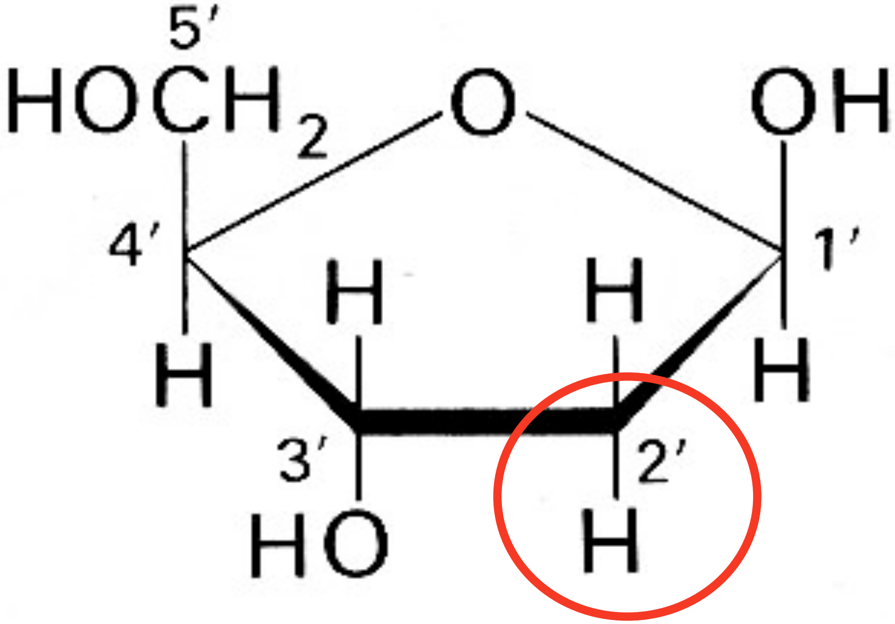
10 layer Lines Between Cross Patterns (10 Residues Per turn)

FIGURE **4.9**

Evidence for the structure of DNA. This photograph, taken by Rosalind Franklin, shows the x-ray diffraction pattern produced by wet DNA fibers. It played a key role in the elucidation of DNA structure. The cross pattern indicates a helical structure, and the strong spots at top and bottom correspond to a helical rise of 0.34 nm. The layer line spacing is one-tenth of the distance from the center to either of these spots, showing that there are 10 base pairs per repeat.

1A

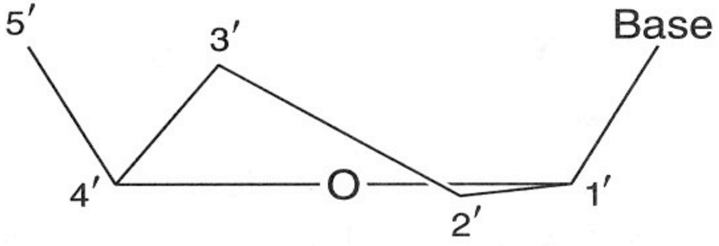




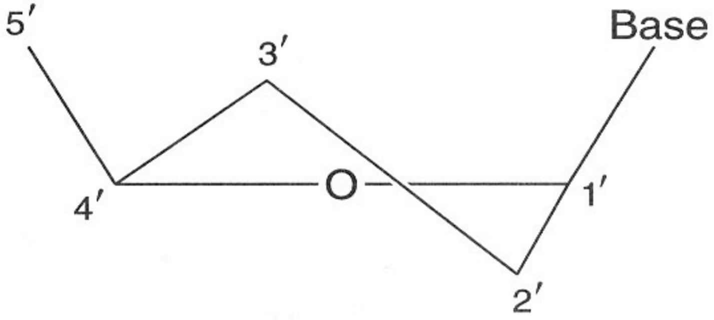
β-2’-deoxyribose

Sugar “Pucker” Conformations

A DNA

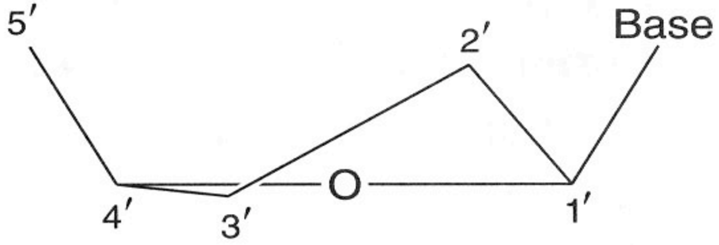


3′-*endo*

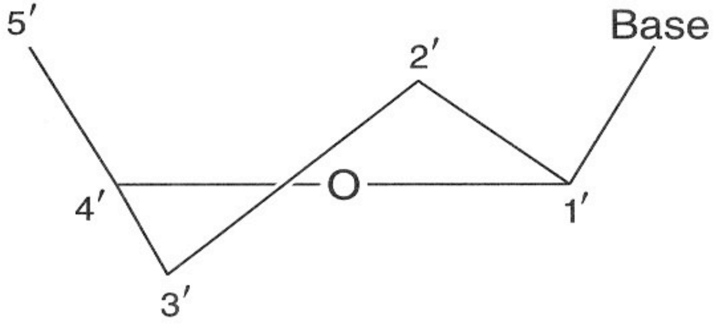


3′-*endo*, 2′-*exo*

B DNA

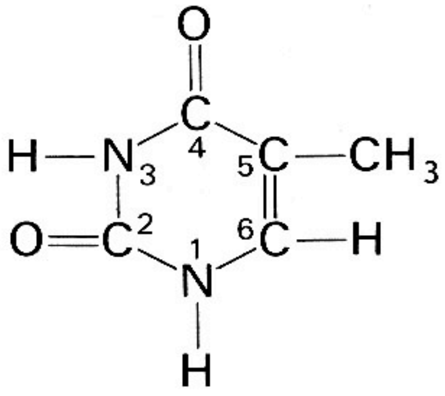


2′-*endo*

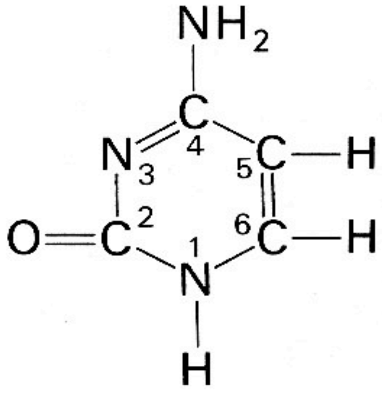


2′-*endo*, 3′-*exo*

Pyrimidines

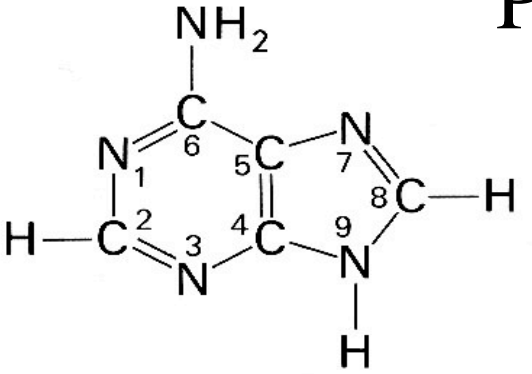


Thymine
(T)

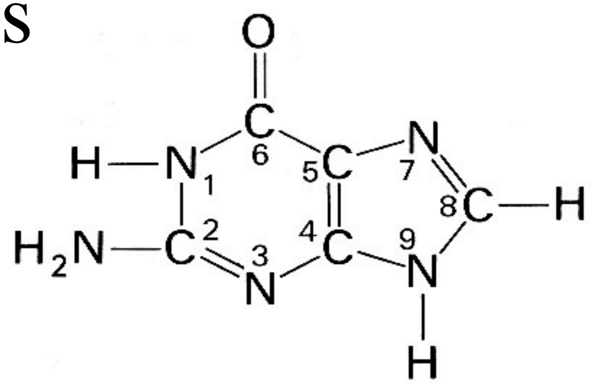


Cytosine
(C)

Purines



Adenine
(A)



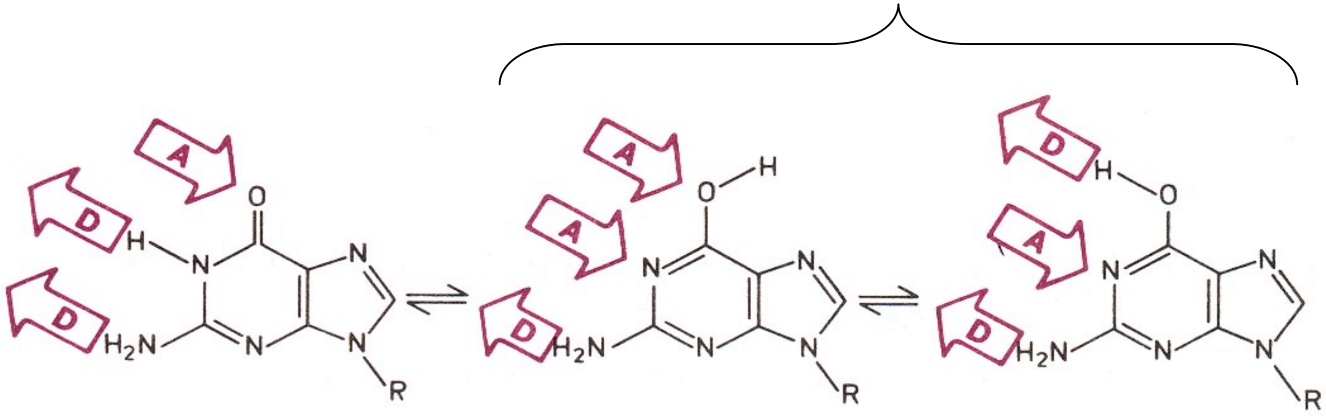
Guanine
(G)

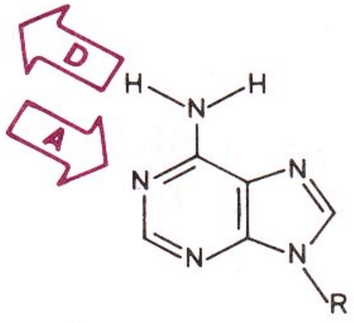
Base Tautomerization

G (Keto)

G (Enol)

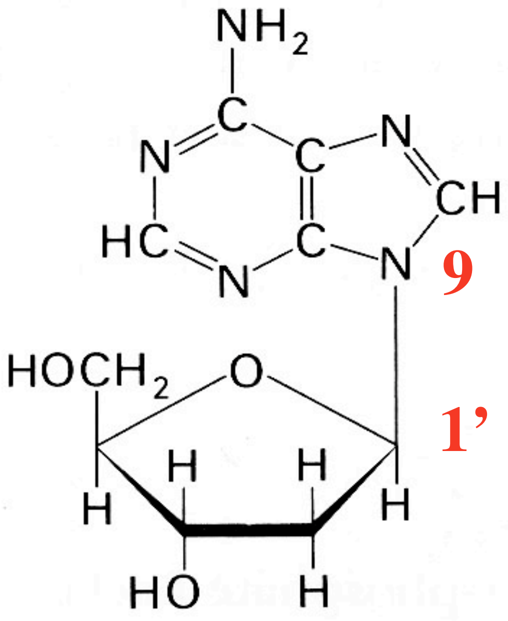
A



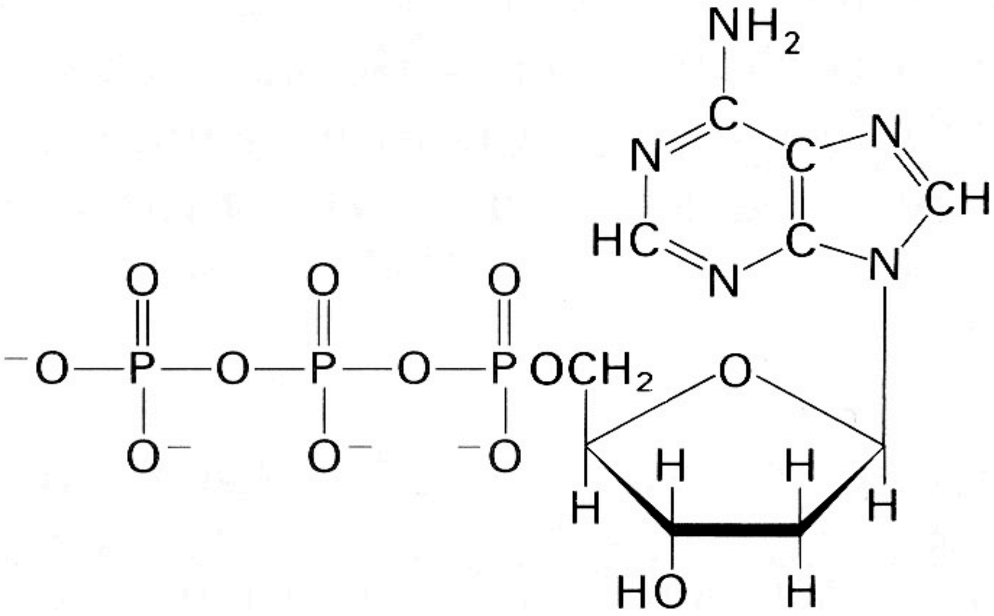


99.99%

0.01%



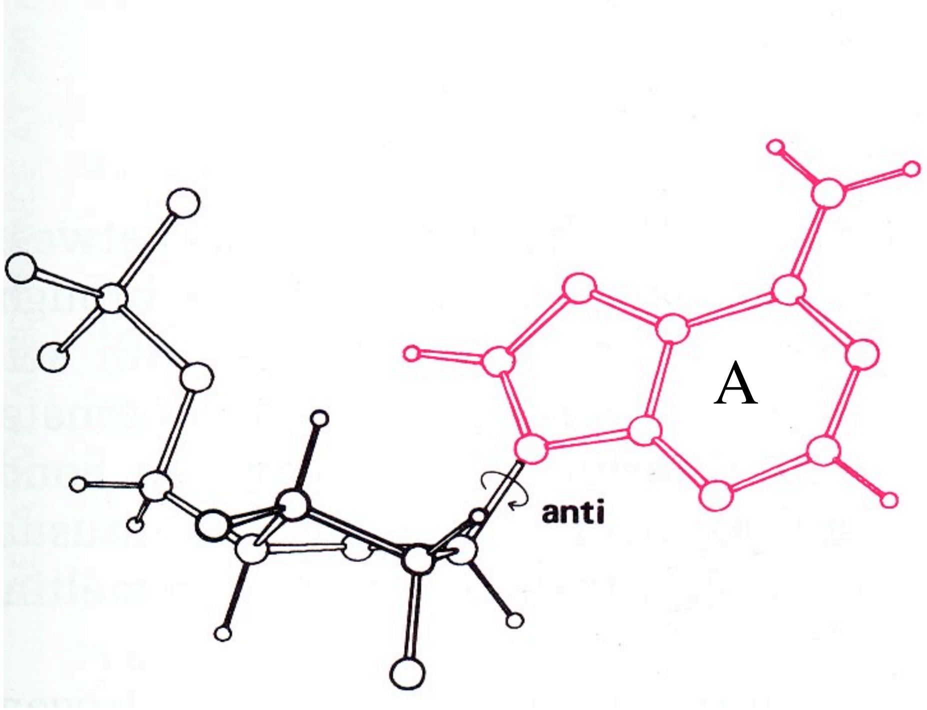
Deoxyadenosine
(A nucleoside)



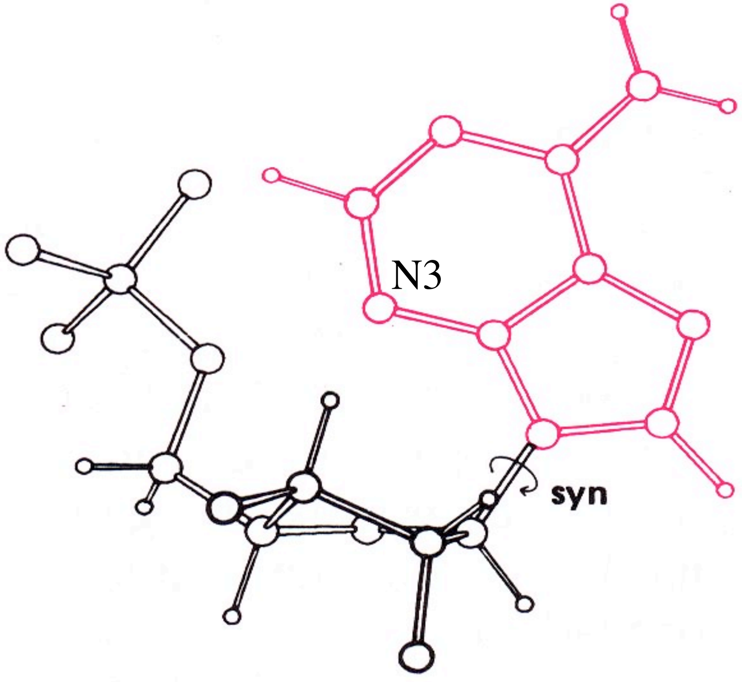
Deoxyadenosine 5′-triphosphate
(dATP)
(A nucleotide)

Base	**Nucleoside**	**Nucleotide**
Adenine	(Deoxy)adenosine	(d)A (mono, di-, tri) phosphate
Guanine	(Deoxy)guanosine	(d)G (mono, di-, tri) phosphate
Thymine	(Deoxy)thymidine	(d)T (mono, di-, tri) phosphate
Cytosine	(Deoxy)cytidine	(d)C (mono, di-, tri) phosphate

Rotation About the N-Glycosidic Bond

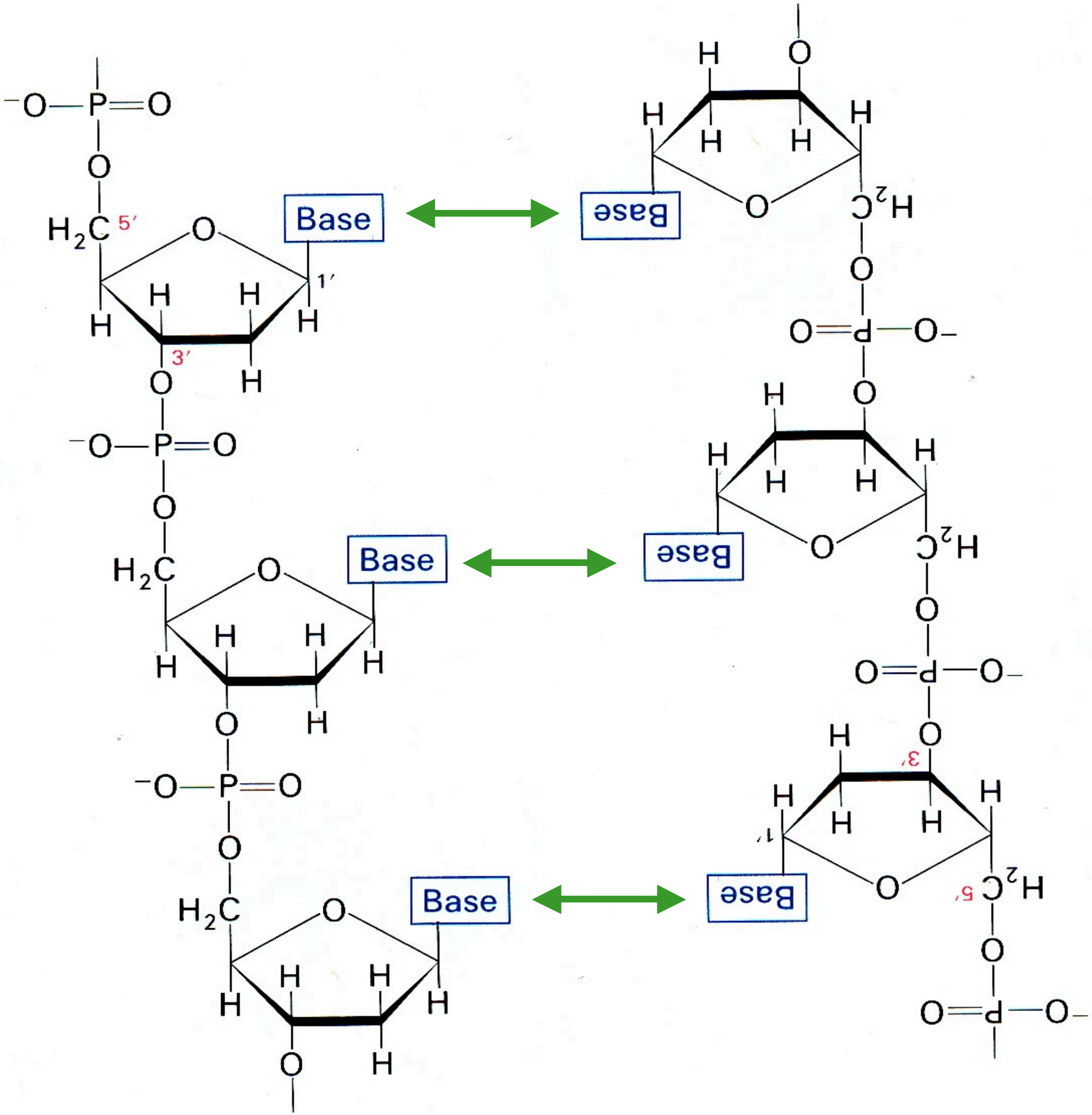


A,B DNA



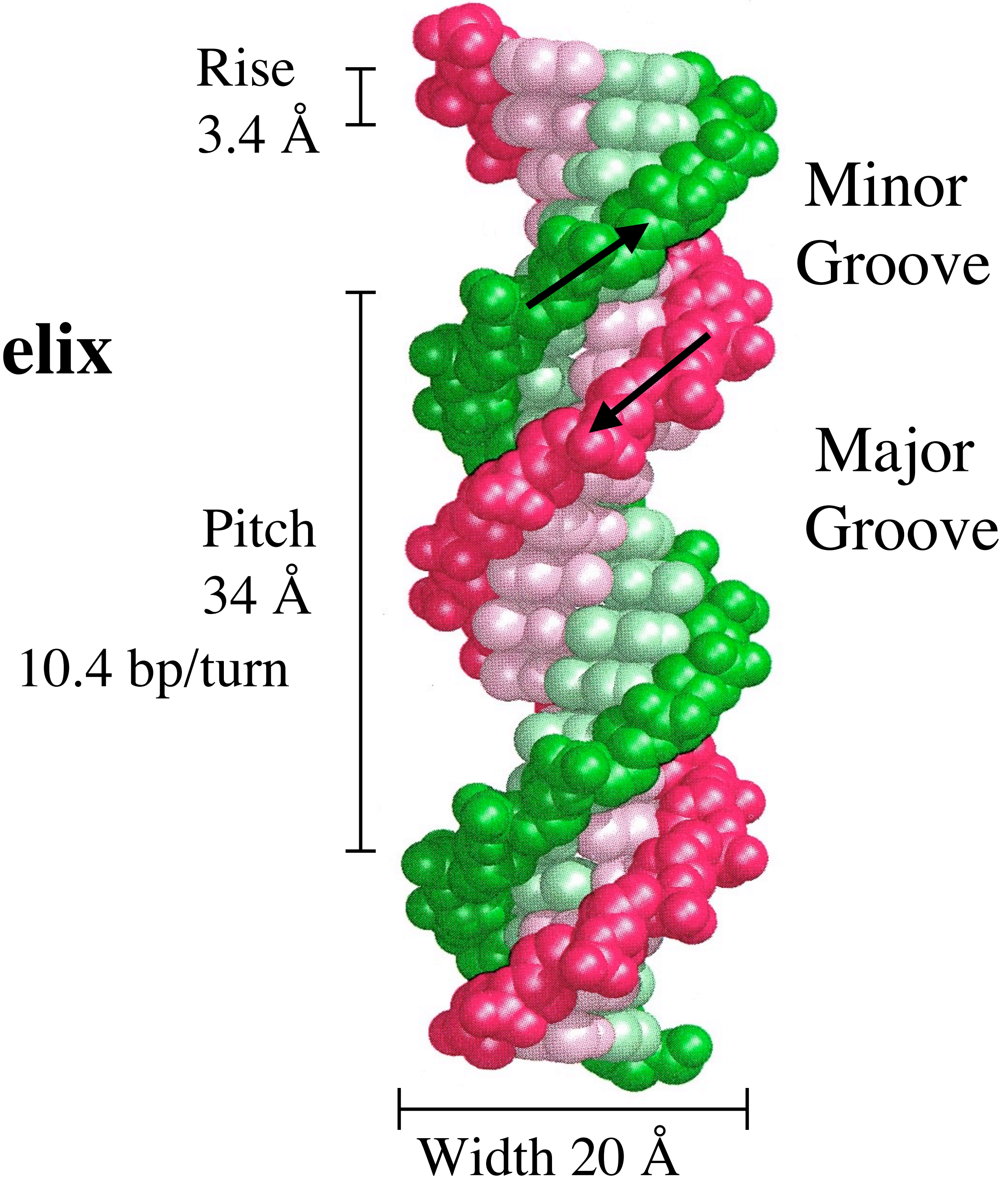
Z DNA (G only)

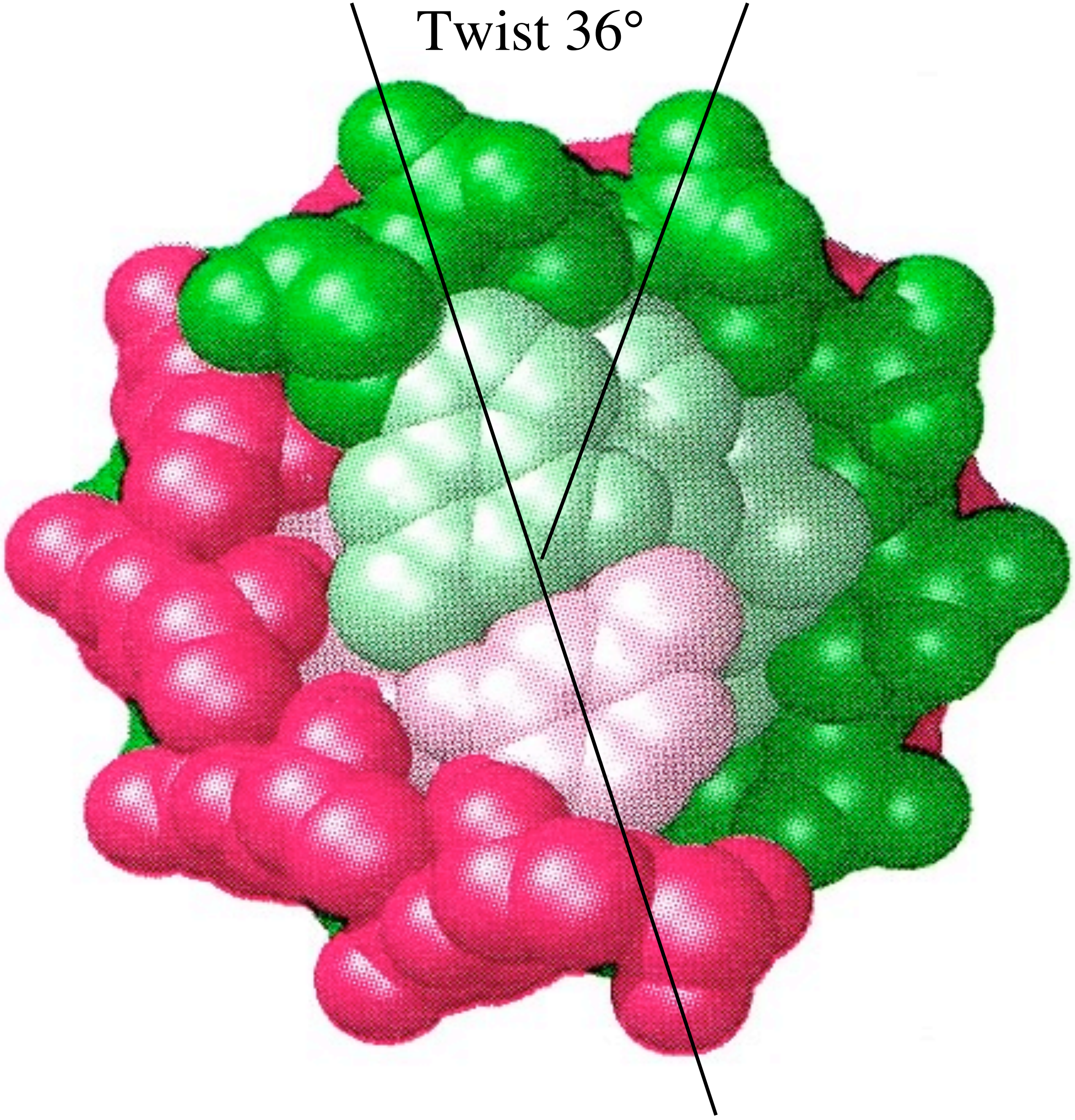
Phosphodiester Backbone

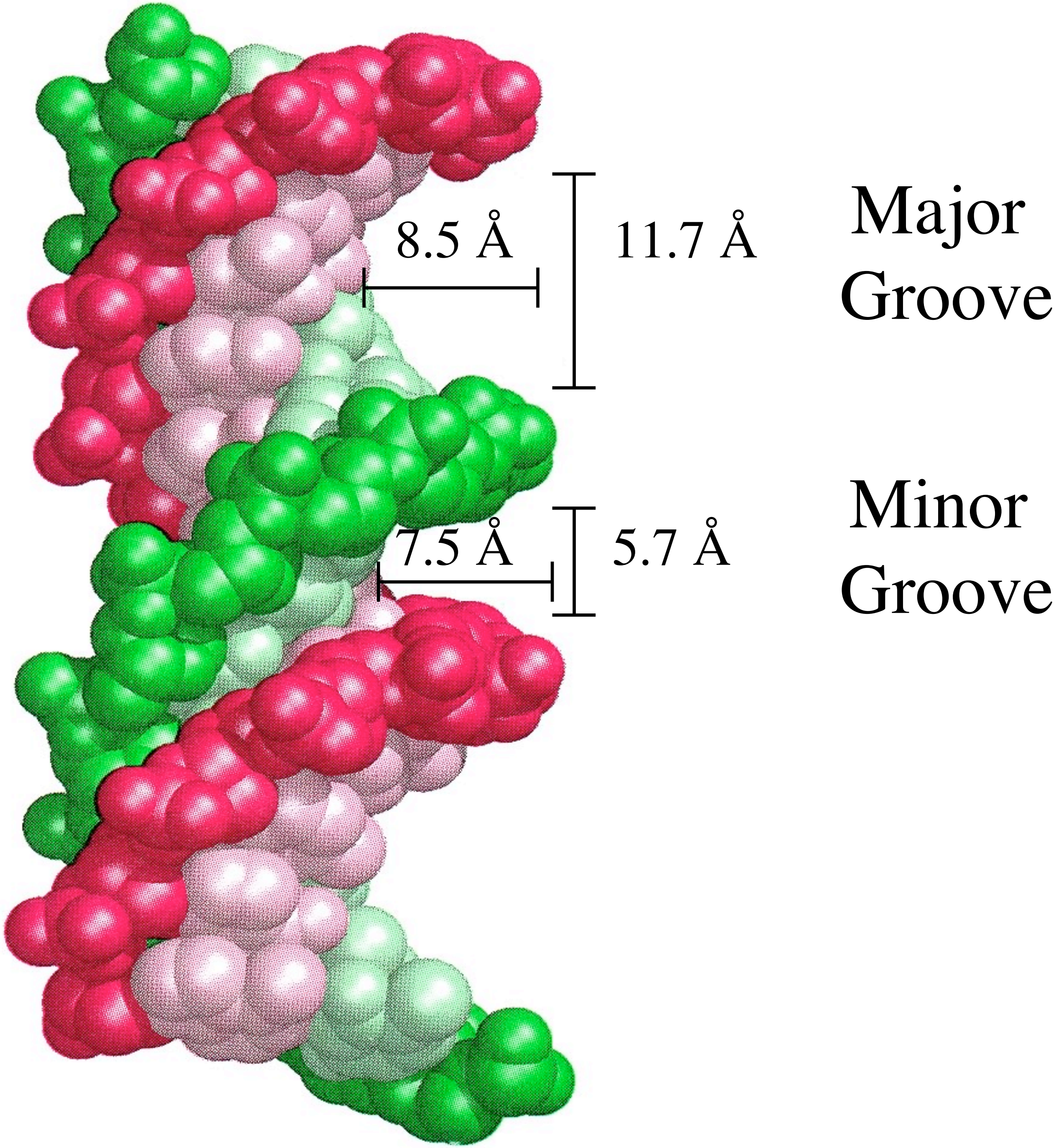


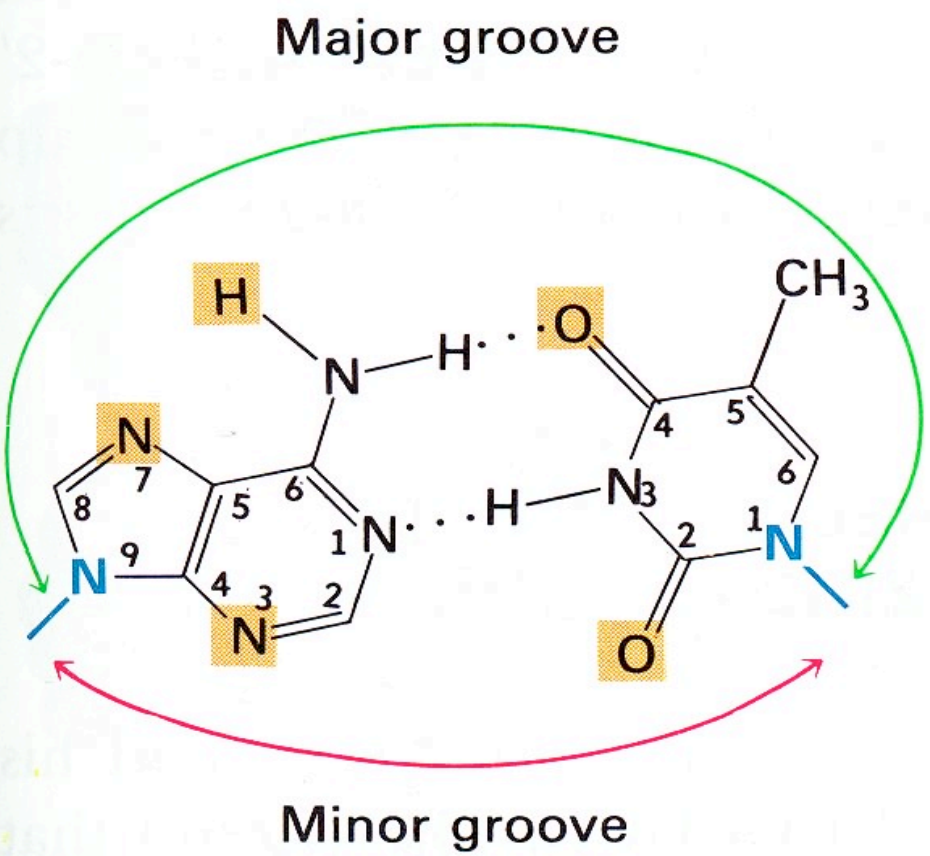
B-DNA: A right Handed double helix

Why?

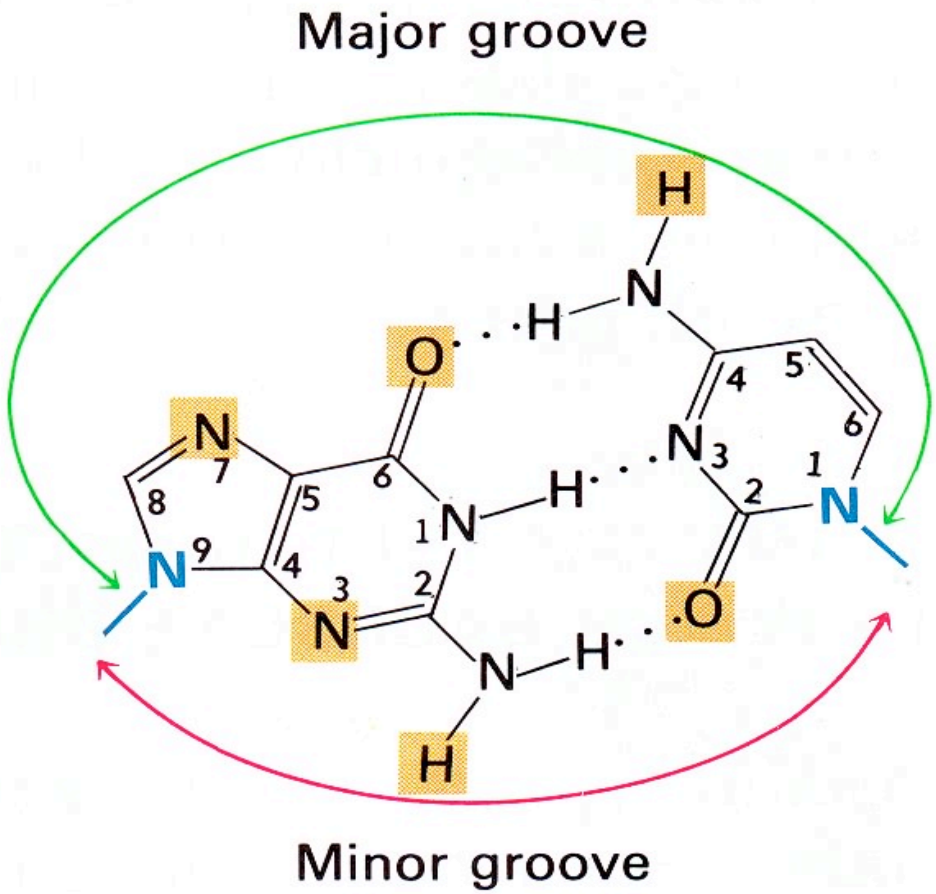



Twist 36°


Major
Groove
8.5 Å
11.7 Å
Minor
Groove
7.5 Å
5.7 Å

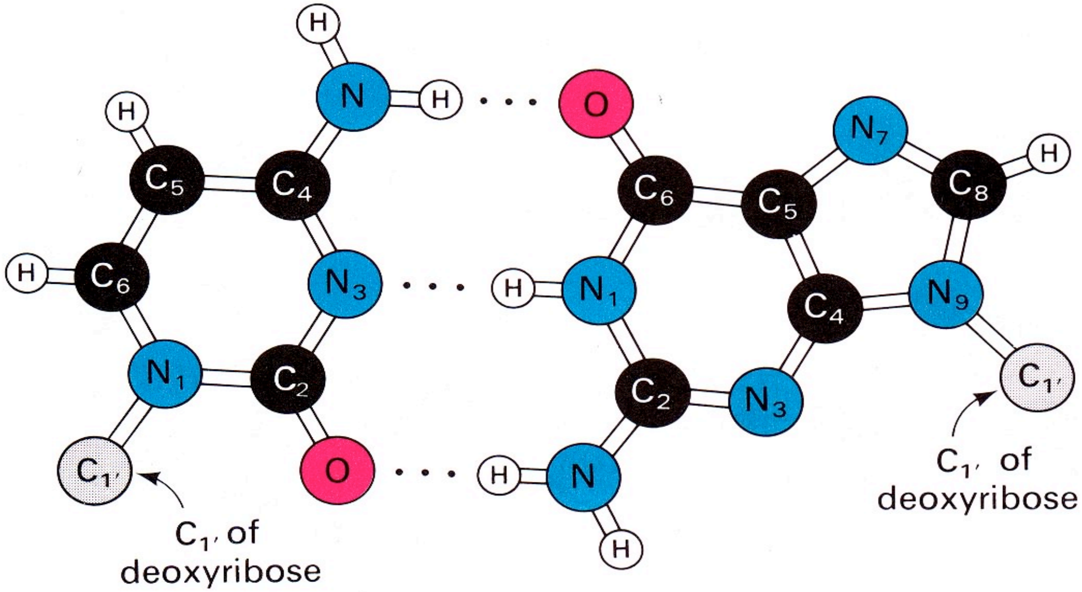


Adenine : Thymine

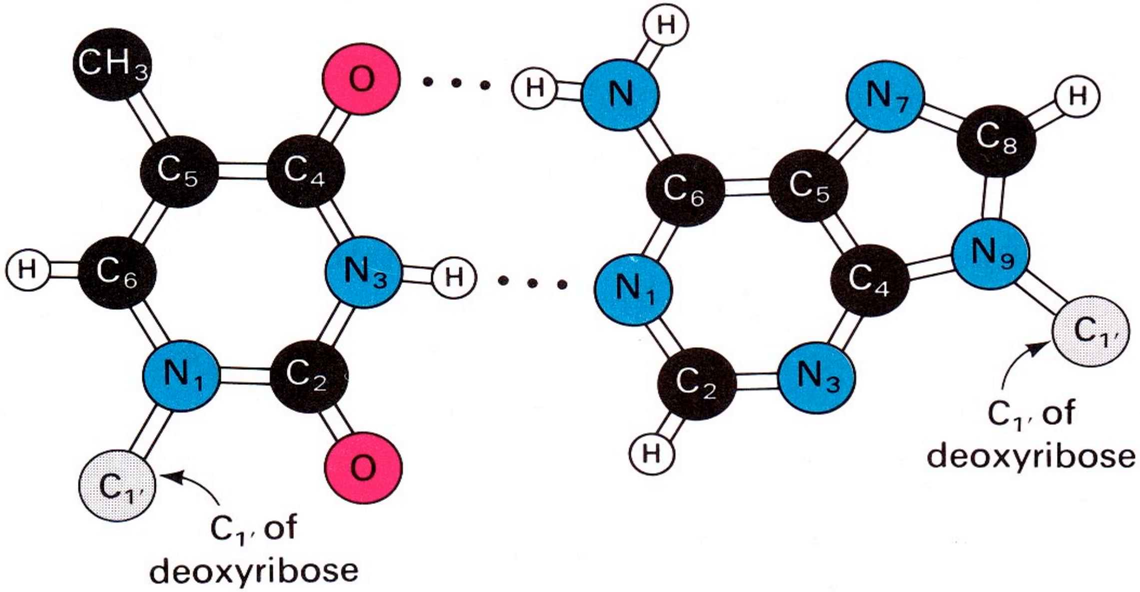


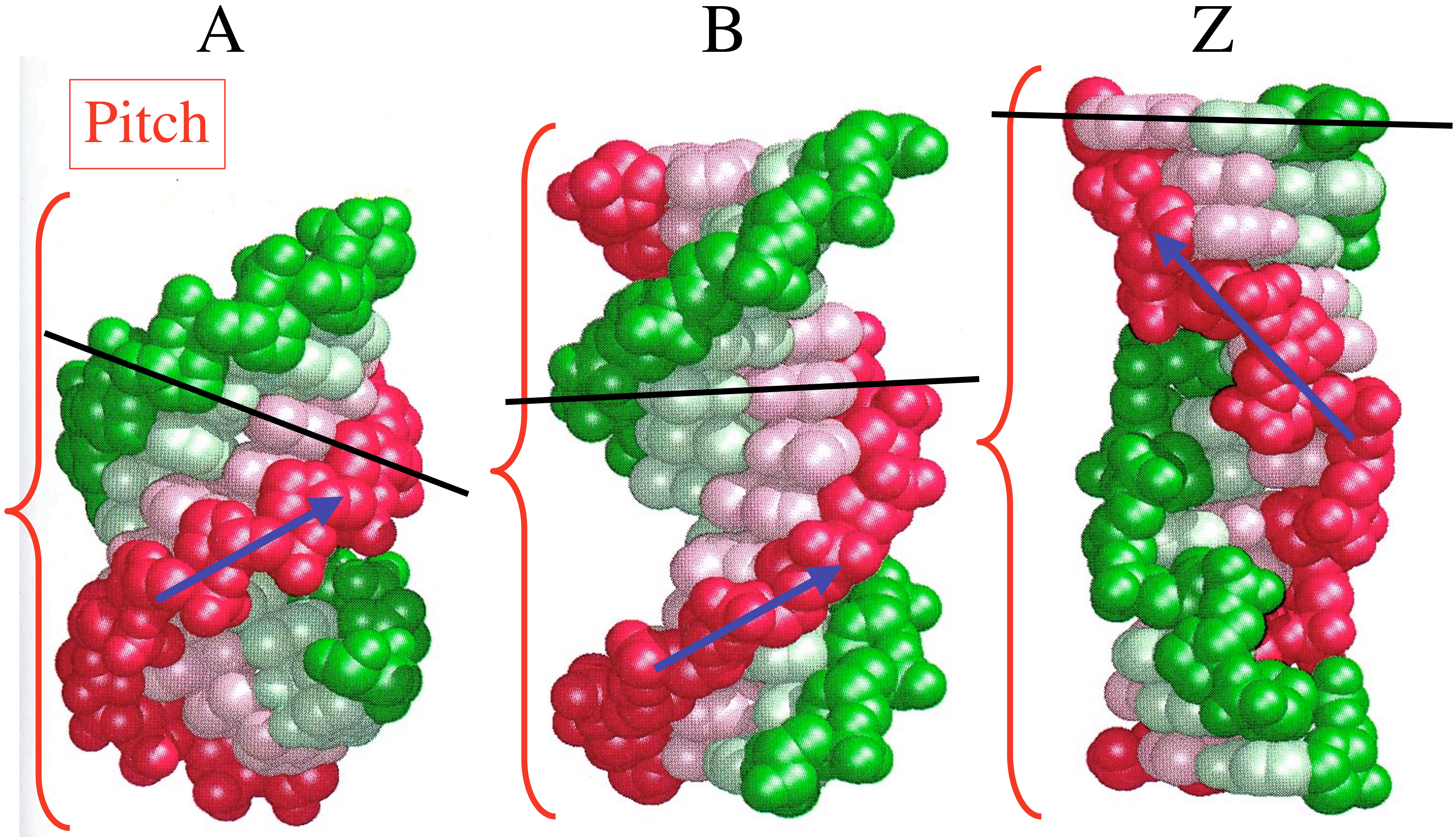
Guanine : Cytosine

C-G


H
N
H
O
H
C5
C4
N7
C8
H
C6
C5
H
C6
N3
H
N1
C4
N9
N1
C2
C2
N3
C1′
O
H
N
C1′
H
C1′ of deoxyribose
C1′ of deoxyribose

T-A


H
CH3
O
H
N
N7
H
C8
C5
C4
C6
C5
H
C6
N3
H
N1
C4
N9
N1
C2
C2
N3
C1′
H
O
C1′
C1′ of deoxyribose
C1′ of deoxyribose

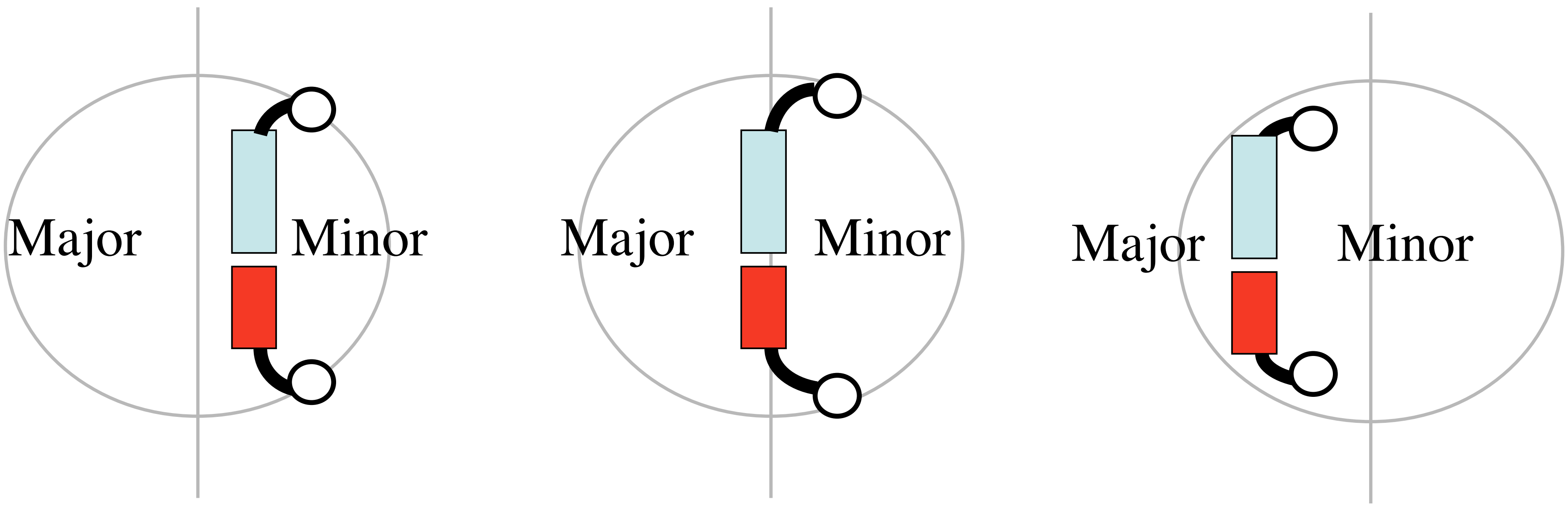


Pitch

Base Inclination

Handedness

Base Displacement Determines Groove *Depth*



A DNA

Major

Minor

dx = -4 Å

B DNA

Major

Minor

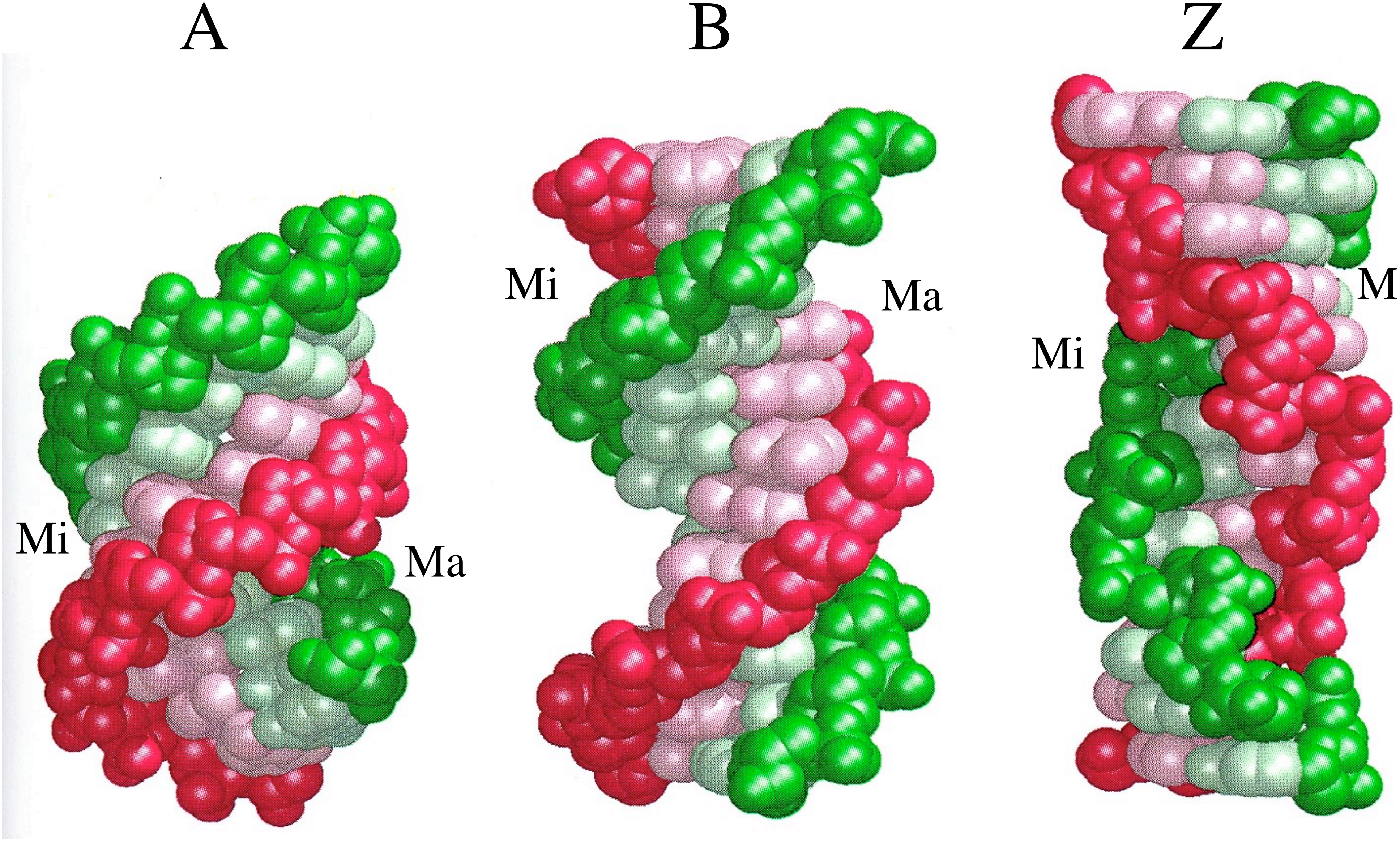
dx = 0.8 Å

Z DNA

Major

Minor

dx = +3-4 Å


A
B
Z
Mi
Ma
Mi
Ma
Mi
Ma

Z-DNA Phosphate Backbone is Kinked

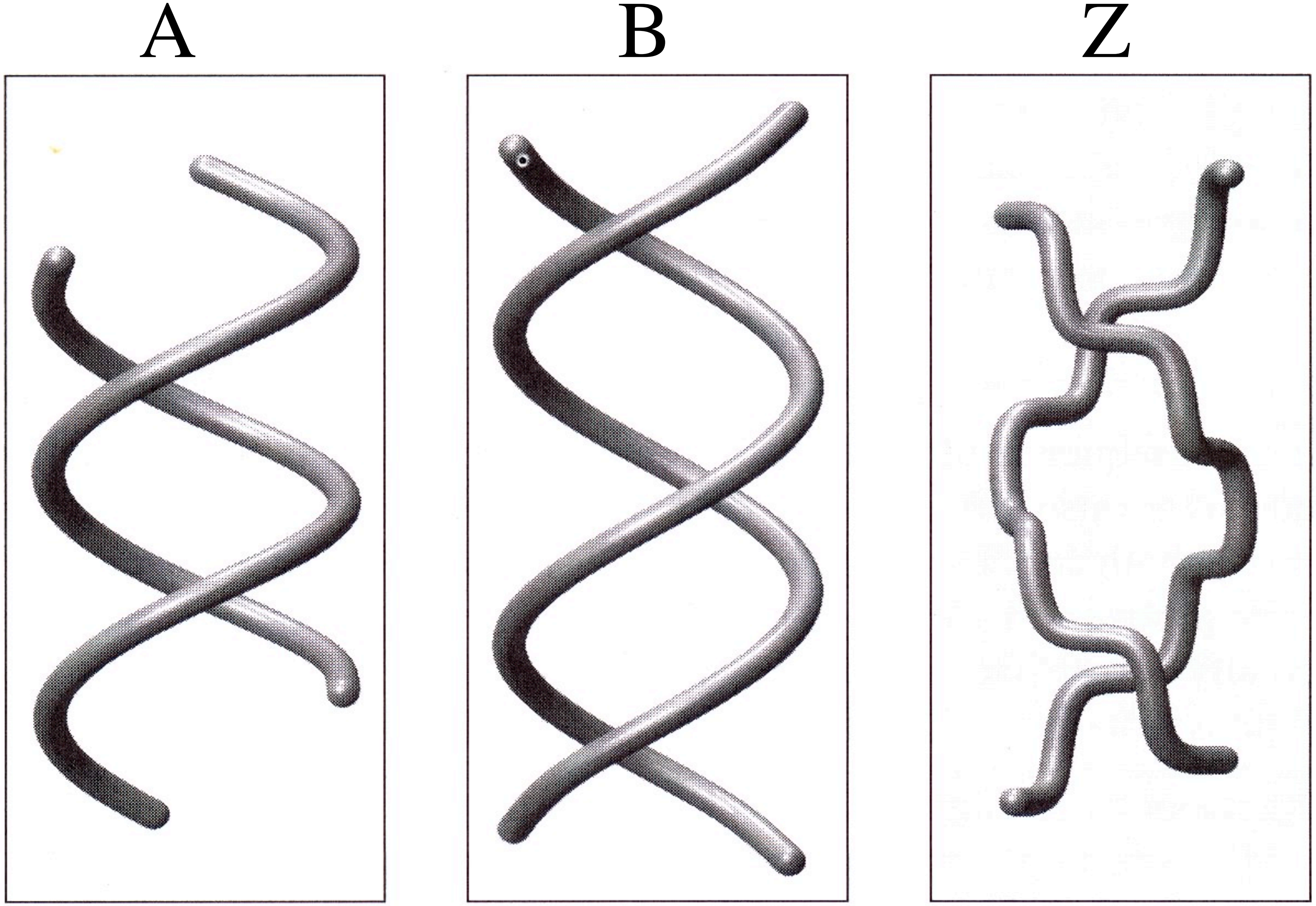


Table 4.1
Average Structural Parameters for Various Helical Forms

	A-DNA	B-DNA	Z-DNA
Helix handedness	Right	Right	Left
bp/repeating unit	1	1	2
bp/turn	11	10	12
Helix twist, (°)	32.7	36.0	−10[a], −50[b]
Rise/bp, (Å)	2.9	3.4	−3.9[a], −3.5[b]
Helix pitch, (Å)	32	34	45
Base pair inclination, (°)	12	2.4	−6.2
P distance from helix axis, (Å)	9.5	9.4	6.2[a], 7.7[b]
X displacement from bp to helix axis, Å	−4.1	0.8	3.0
Glycosidic bond orientation	anti	anti	anti[c], syn[d]
Sugar conformation	C3′-*endo*	C2′-*endo*[e]	C2′-*endo*[c] C3′-*endo*[d]
Major groove depth	13.5	8.5	Convex
width, (Å)	2.7	11.7	
Minor groove depth	2.8	7.5	9
width, (Å)	11.0	5.7	4

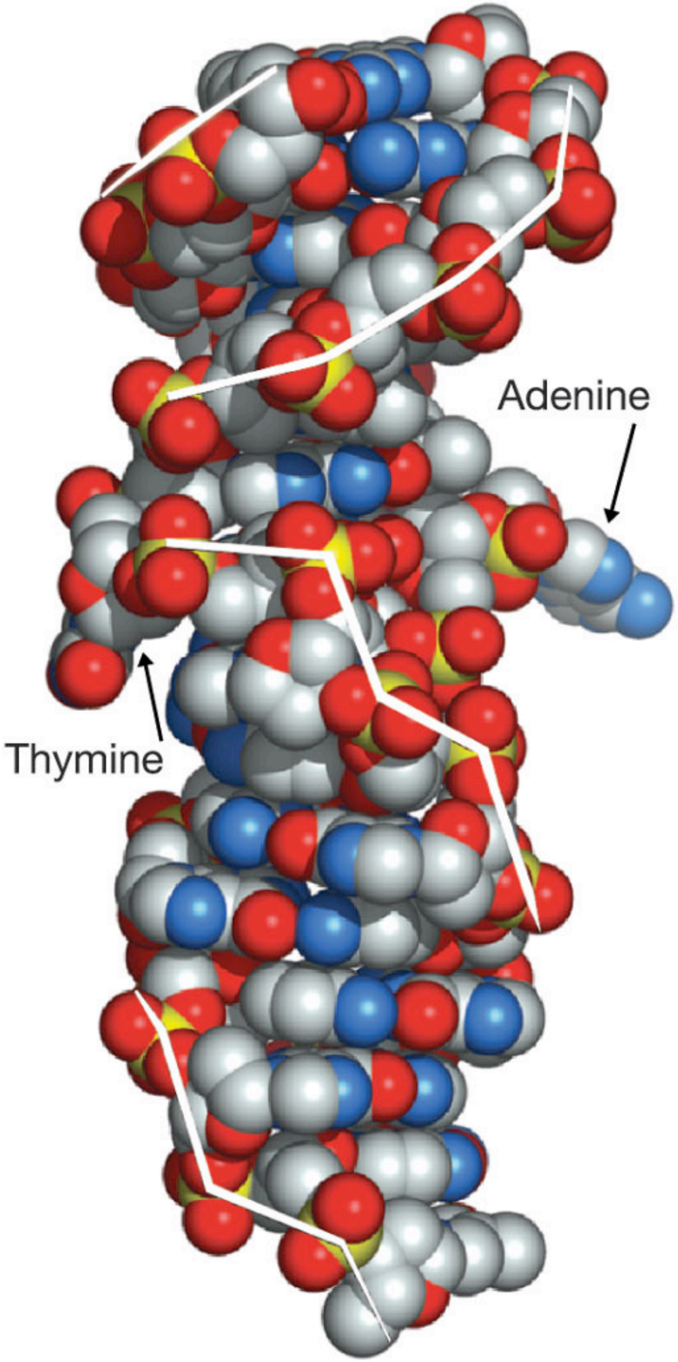
[a] CpG step.

[b] GpC step.

[c] cytosine.

[d] guanine.

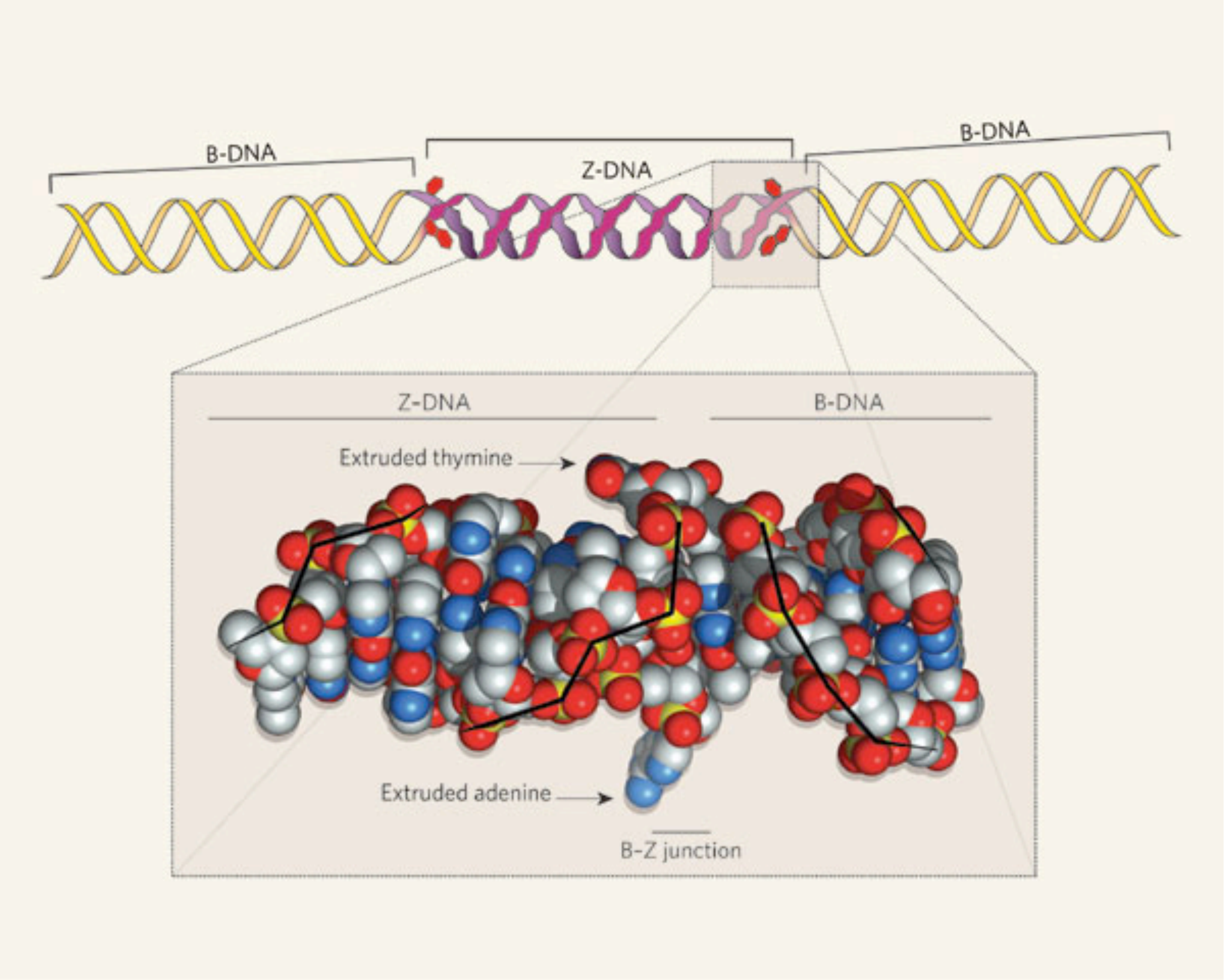
[e] There is a range of conformations.



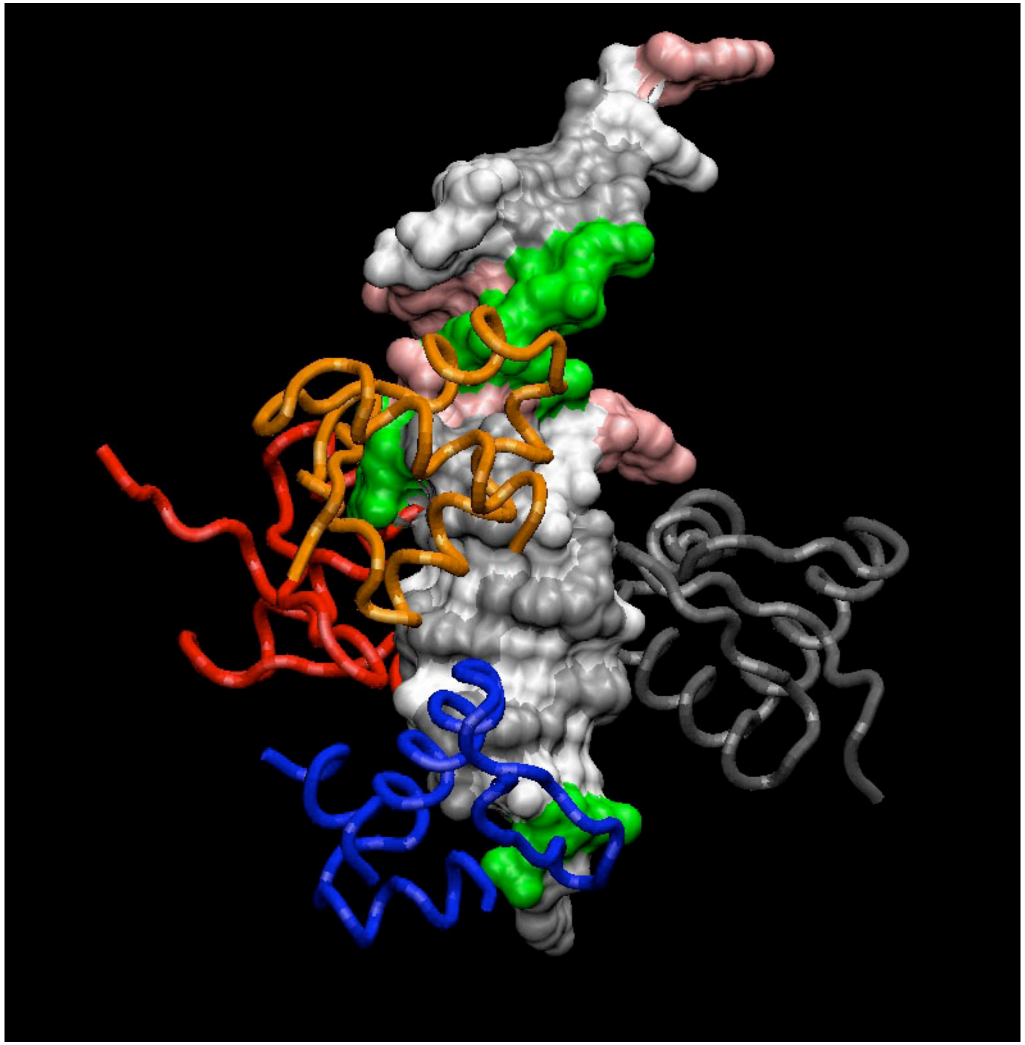
The structure of the B-Z junction

Crystal structure of a junction between B-DNA
and Z-DNA reveals two extruded bases
Sung Chul Ha, Ky Lowenhaupt, Alexander
Rich, Yang-Gyun Kim and Kyeong Kyu Kim,
Nature 437, 1183-1186 (20 October 2005)

A region of left-handed Z-DNA is connected to right-handed B-DNA



B-Z junction with bound protein from adenosine deaminase ADR1

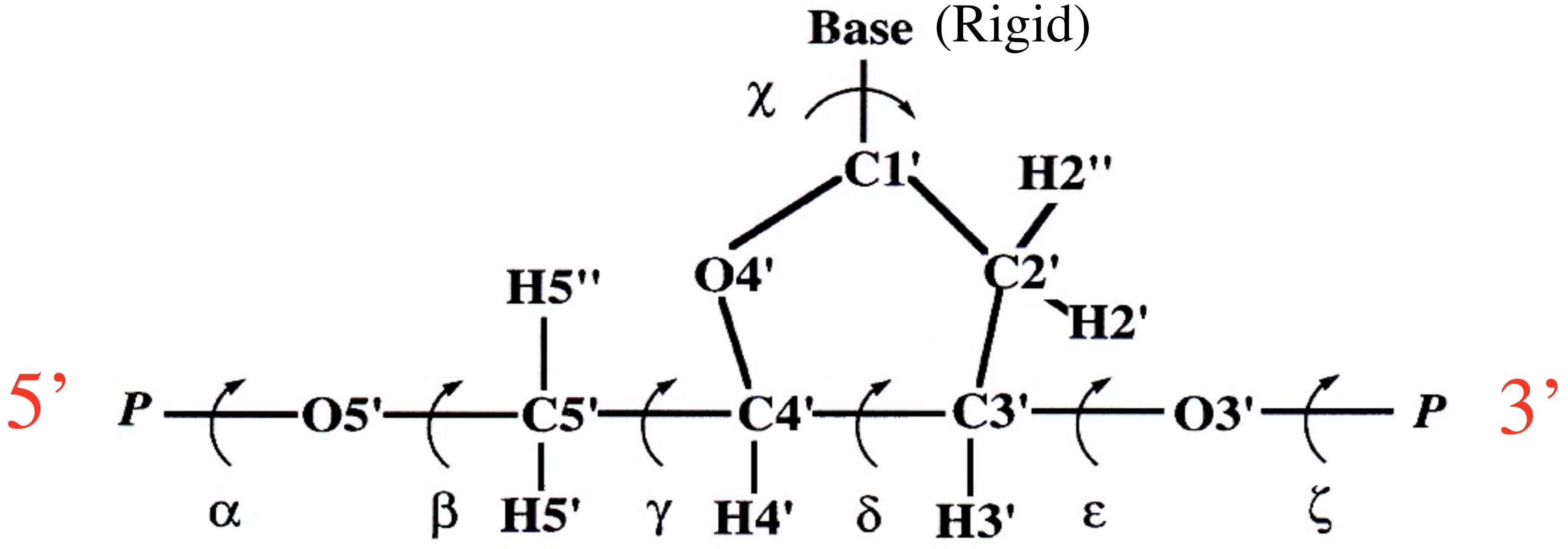


Question: is all B-DNA structurally identical?

Implications of structural variation

Implications of flexibility

Degrees of freedom:
7 Torsion angles and sugar conformation

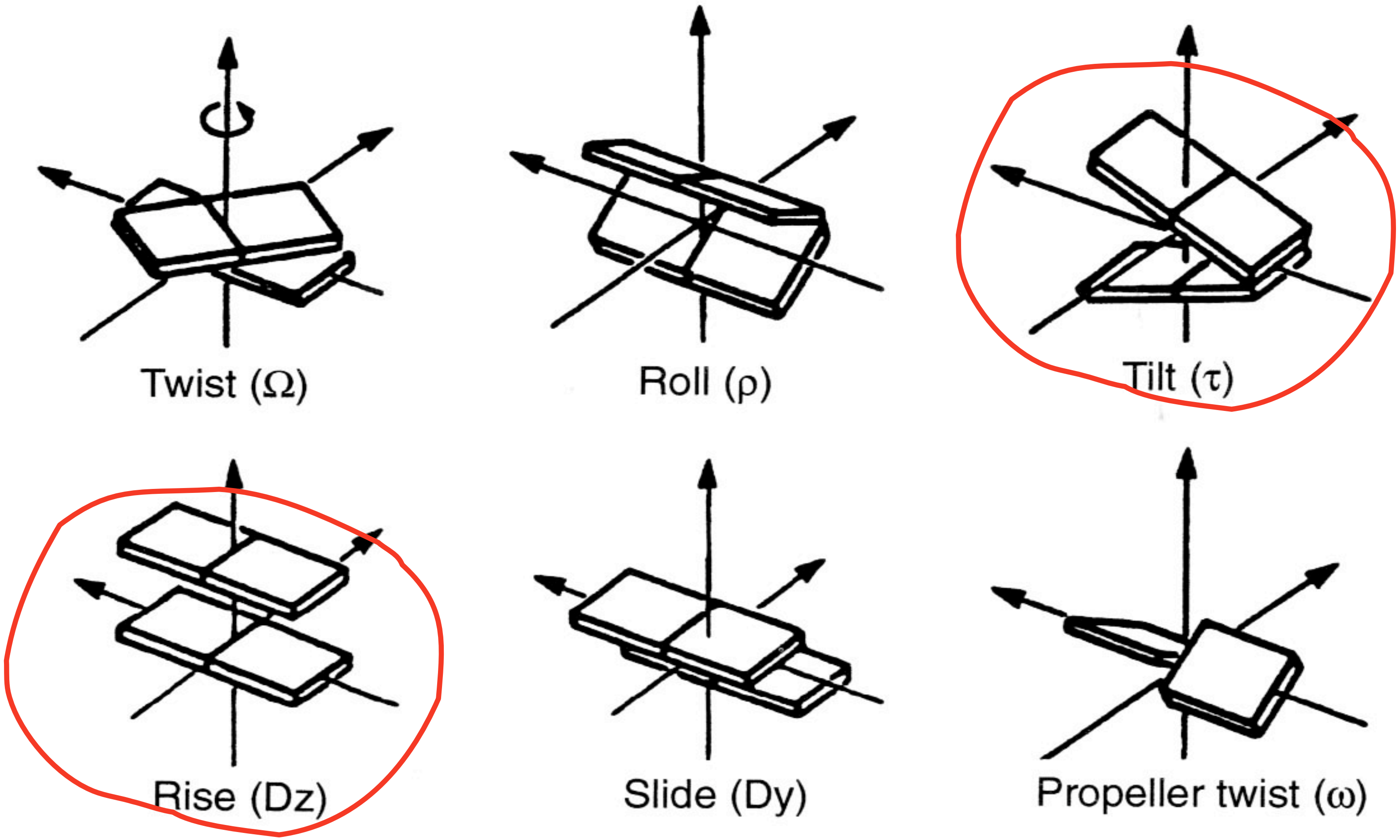


Structural Variation Defined by Bases

normal

frequent

never

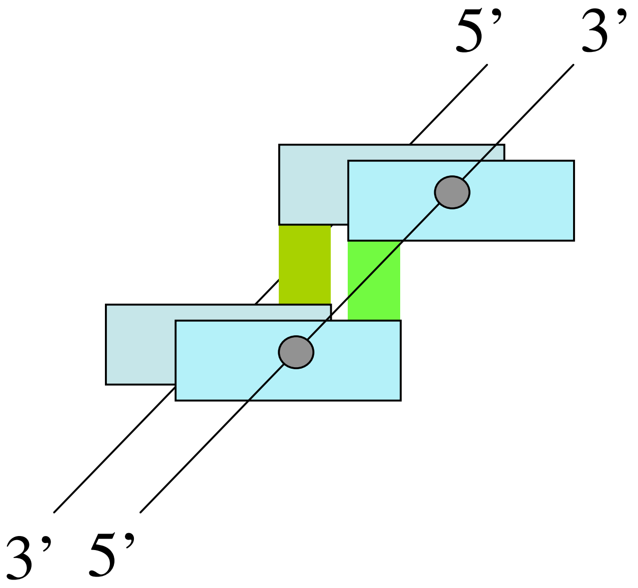


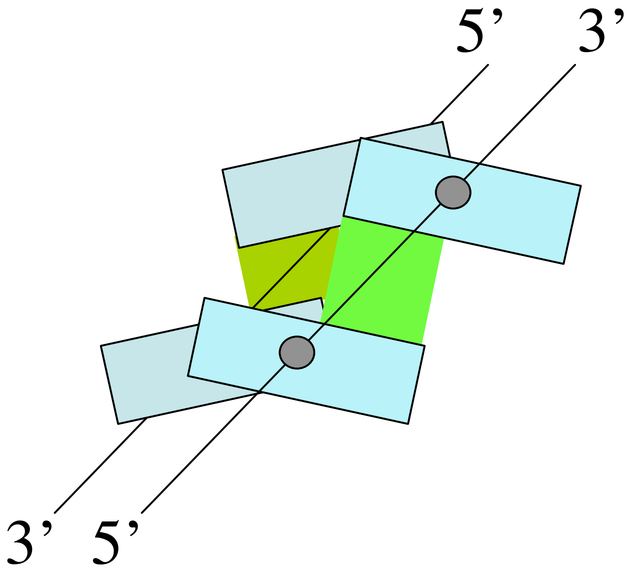
Never
(except in intercalation)

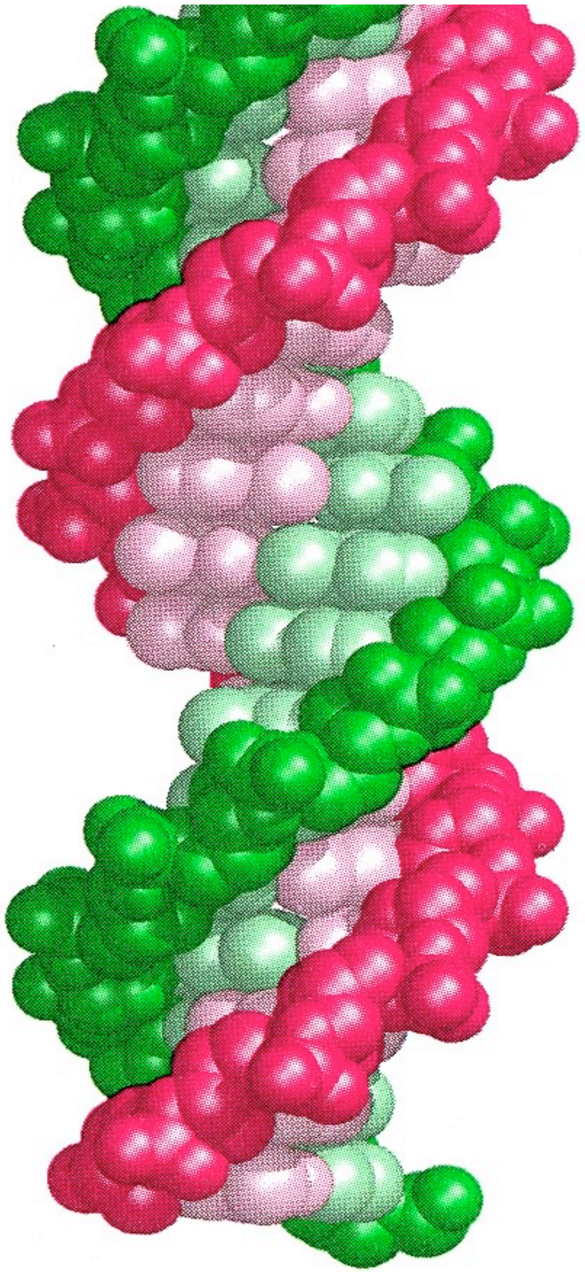
Common

Common

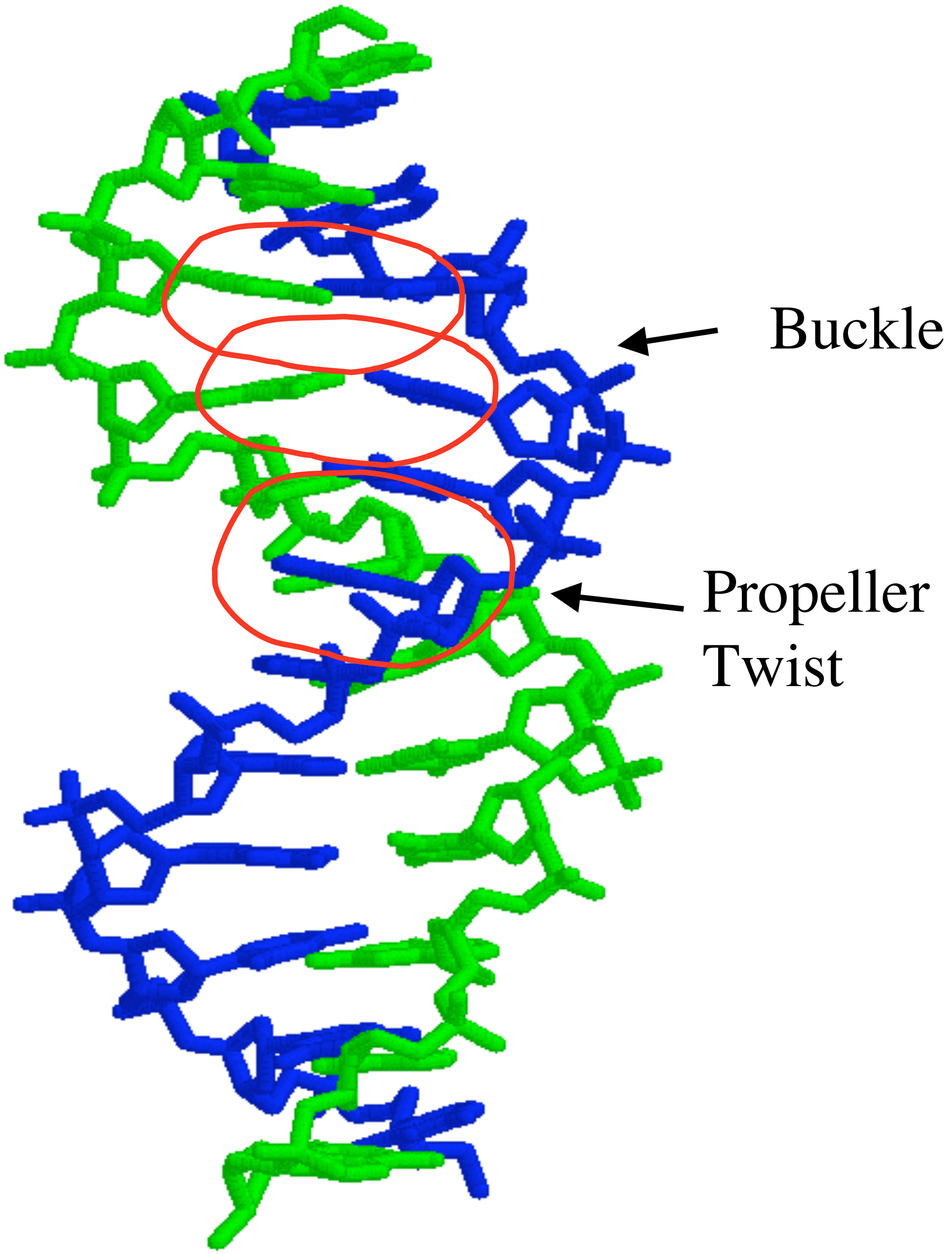
Propeller Twist Maximizes Base Stacking





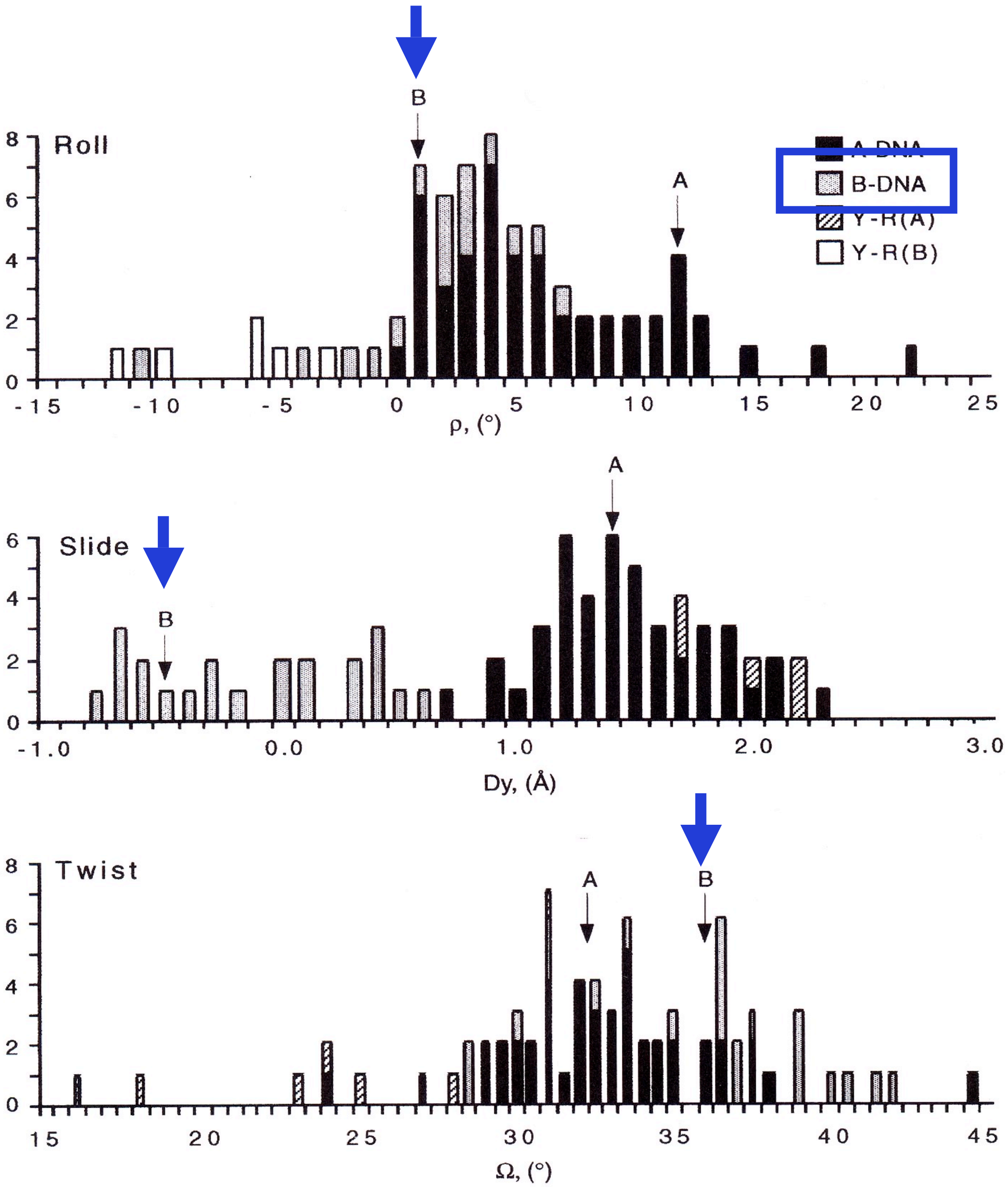


Textbook



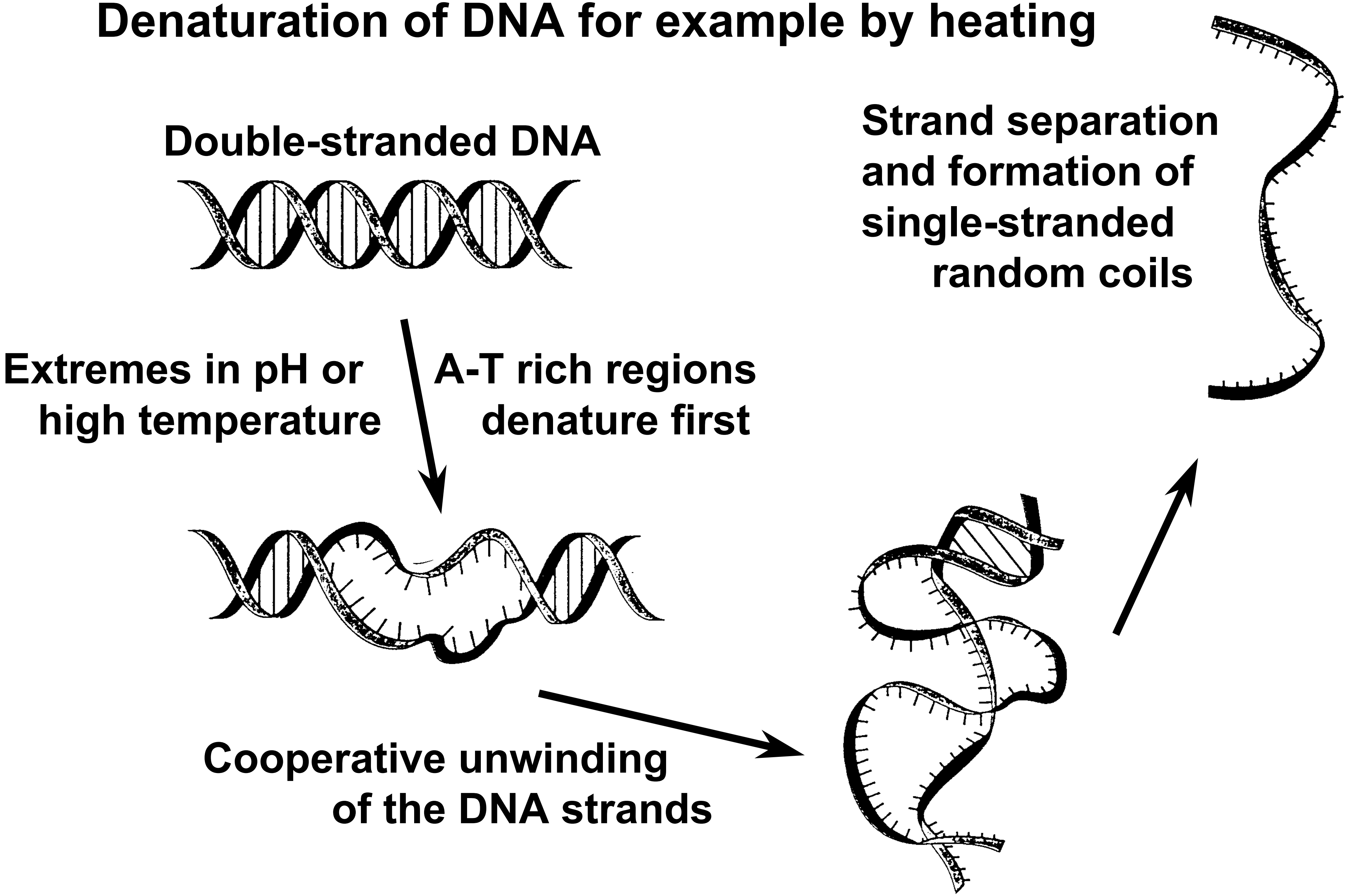
Real Life

Naturally Occurring Variations in Roll, Slide, Twist



DNA Stability

Denaturation of DNA for example by heating



Double-stranded DNA

Extremes in pH or
high temperature

A-T rich regions
denature first

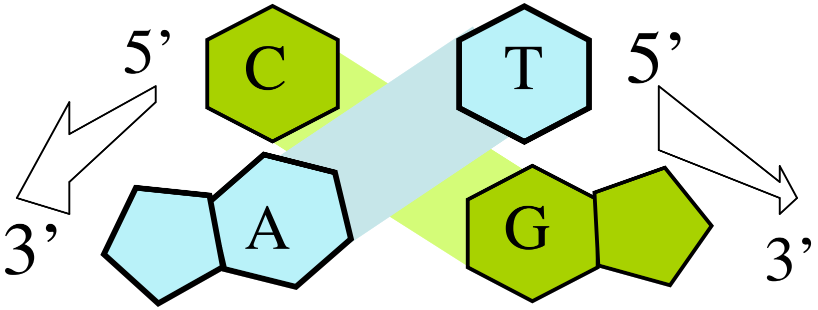
Cooperative unwinding
of the DNA strands

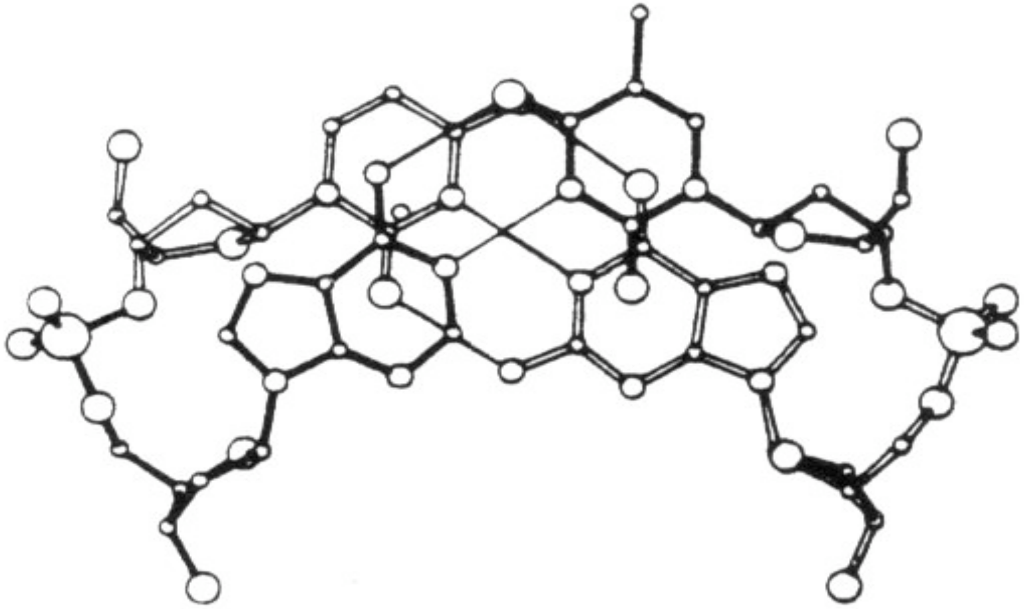
Strand separation
and formation of
single-stranded
random coils

Pyrimidine-Purine Steps Have Little Base Stacking

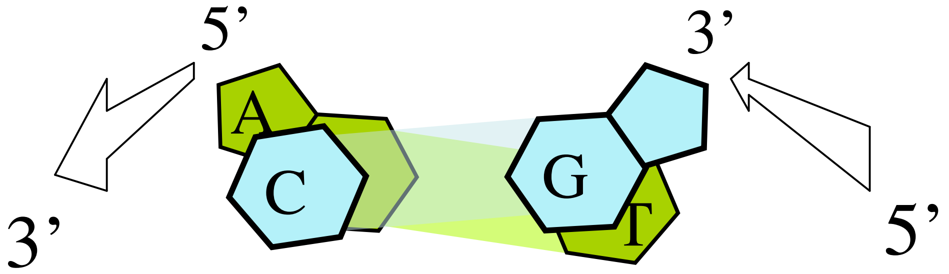
Step Definition: Going along one strand of DNA in 5’to 3’ direction

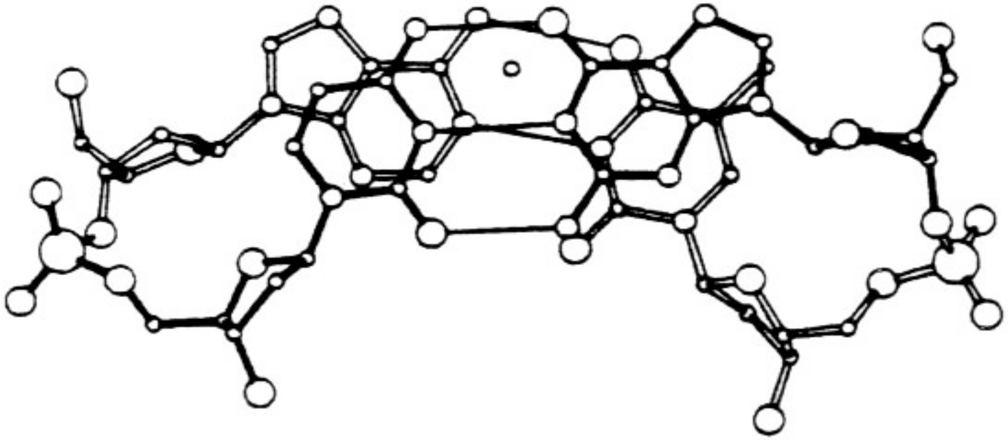
Four Possibles: P-Y, P-P, Y-P, Y-Y





Purine-Pyrimidine Steps Have Extensive Base Stacking





Absorbance measurements with a spectrophotometer

$I = I_0 10^{-\varepsilon c d}$ $A = \varepsilon \cdot c \cdot d$

I intensity of light, I_0 incoming light, ε extinction coefficient, *c* concentration, *d* path length

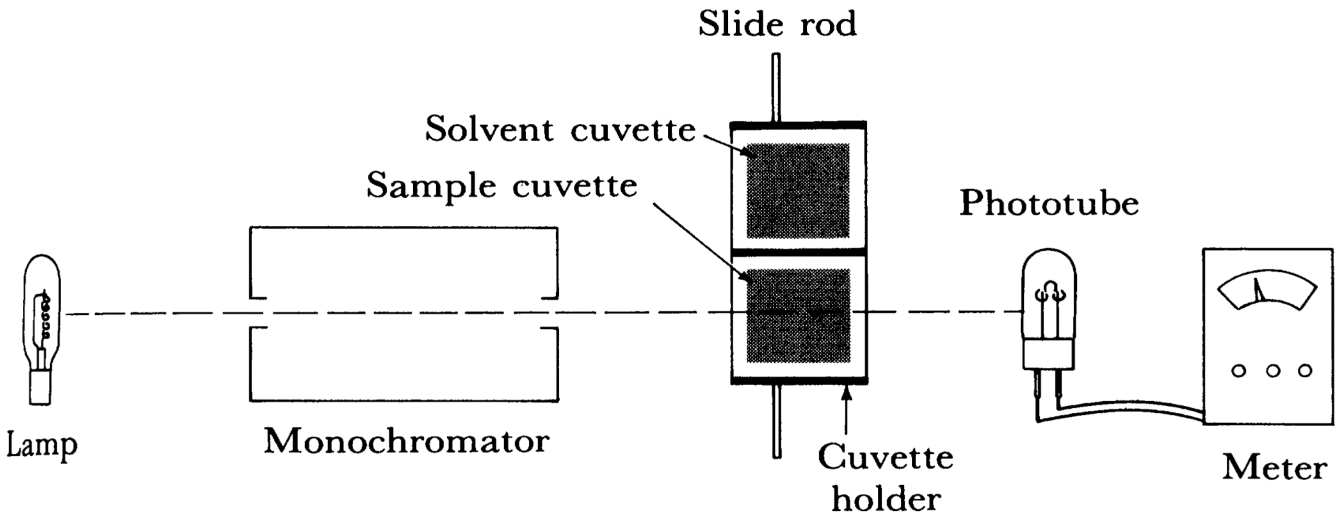
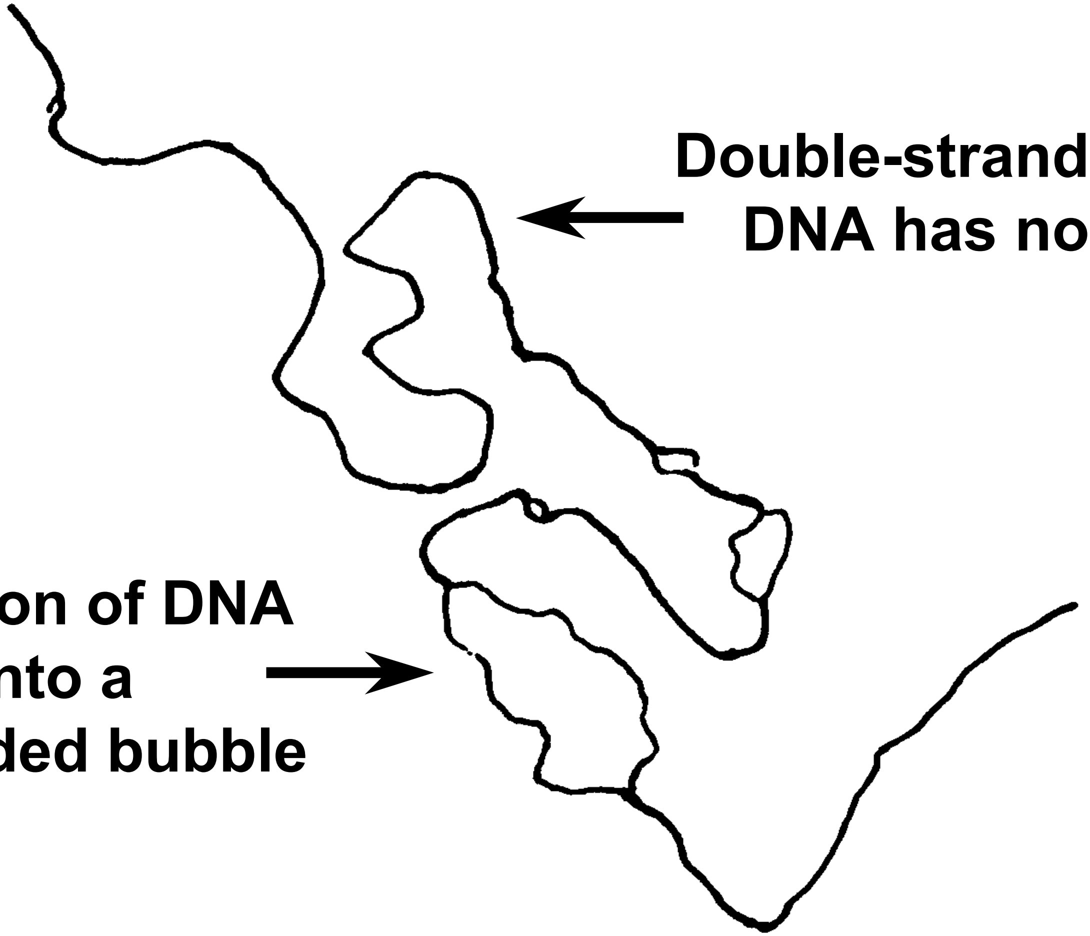


Figure 14-5

A spectrophotometer. Light from a lamp passes through a monochromator for wavelength selection. Sample and solvent are contained in two cuvettes in a cuvette holder. Light passes through a cuvette and falls on a phototube whose output is recorded on a meter. The cuvette holder is on a slide so that each cuvette can be separately placed in the beam.

Electron micrograph of partially melted DNA

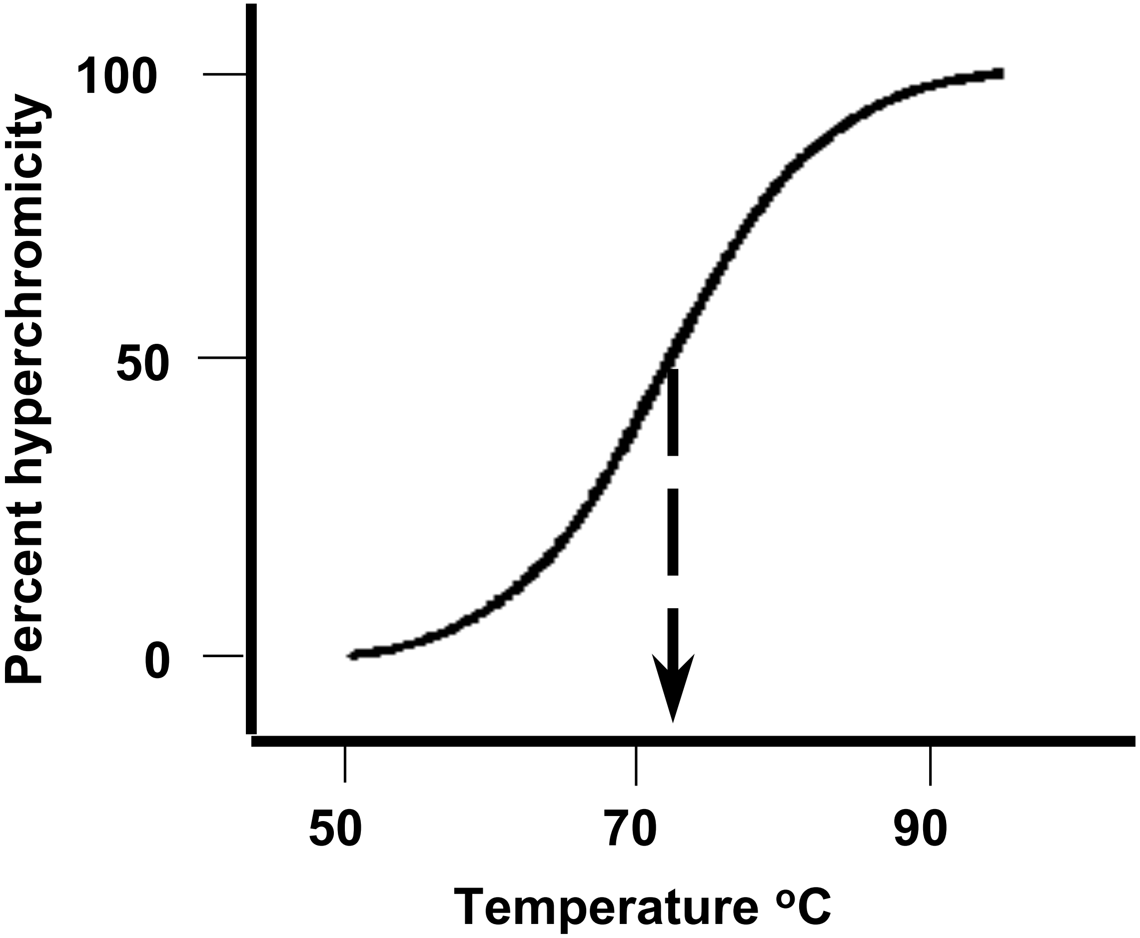


Double-stranded, G-C rich DNA has not yet melted

A-T rich region of DNA has melted into a single-stranded bubble

- A-T rich regions melt first, followed by G-C rich regions

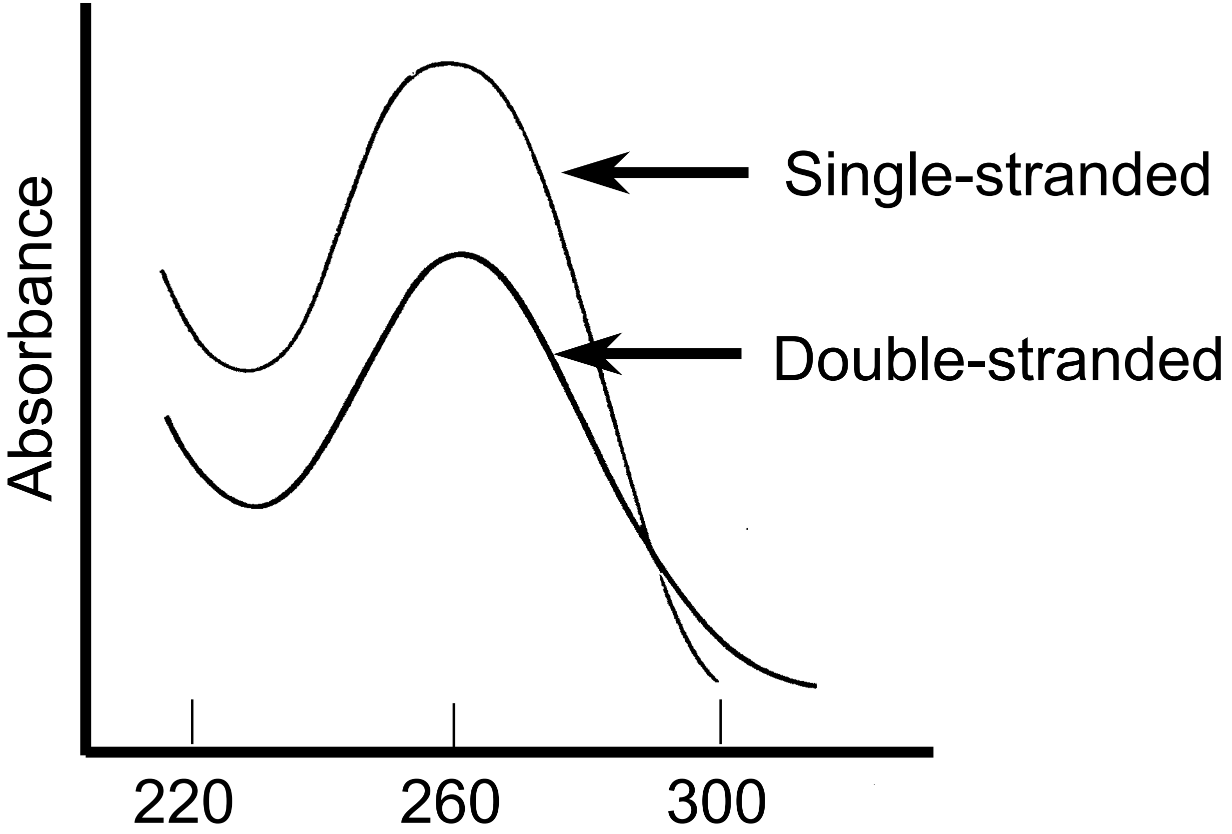
- **DNA melting curve**



- **T_m is the temperature at the midpoint of the transition**

- hyperchromicity

$$\%h = 100\frac{A(\text{melted species}) - A(\text{duplex})}{A(\text{duplex})}$$



The absorbance at 260 nm of a DNA solution increases when the double helix is melted into single strands.

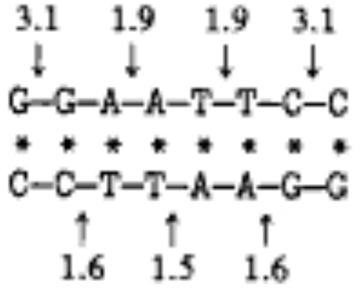
Values of the Nearest-Neighbor Parameters for DNA in 1 M NaCl

Sequence	ΔH (kcal/ mol)	ΔS (cal/ K·mol)	ΔG (kcal/ mol)
AA/TT	-7,9	-22,2	-1,00
AC/TG	-8,4	-22,4	-1,44
AG/TC	-7,8	-21,0	-1,28
AT/TA	-7,2	-20,4	-0,88
CA/GT	-8,5	-22,7	-1,45
CC/GG	-8,0	-19,9	-1,84
CG/GC	-10,6	-27,2	-2,17
CT/GA	-7,8	-21,0	-1,28
GA/GT	-8,2	-22,2	-1,30
GC/GG	-9,8	-24,4	-2,24
GG/CC	-8,0	-19,9	-1,84
GT/CA	-8,4	-22,4	-1,44
TA/AT	-7,2	-21,3	-0,58
TC/AG	-8,2	-22,2	-1,30
TG/AC	-8,5	-22,7	-1,45
TT/AA	-7,9	-22,2	-1,00
Init.	0	0	0
Init. AT	2,3	4,1	1,03
Init. CG	0,1	-2,8	0,98
symmetric	0	-1,4	0,43
non-symmetric	0	0	0

Predicting Transition Free Energies for DNA duplexes

Scheme II
Predicting transition free energies of DNA oligomers

$$\Delta G_{\text{total}} \overset{?}{=} -(\Delta g_i + \Delta g_{\text{sym}}) + \Sigma_x \Delta g_x$$



$$\Delta G_{\text{predicted}} = -(5.0 + 0.4) + (2 \times 3.1) + (2 \times 1.6) + (2 \times 1.9) + (1 \times 1.5)$$

$$\Delta G_{\text{predicted}} = 9.3 \text{ kcal}$$

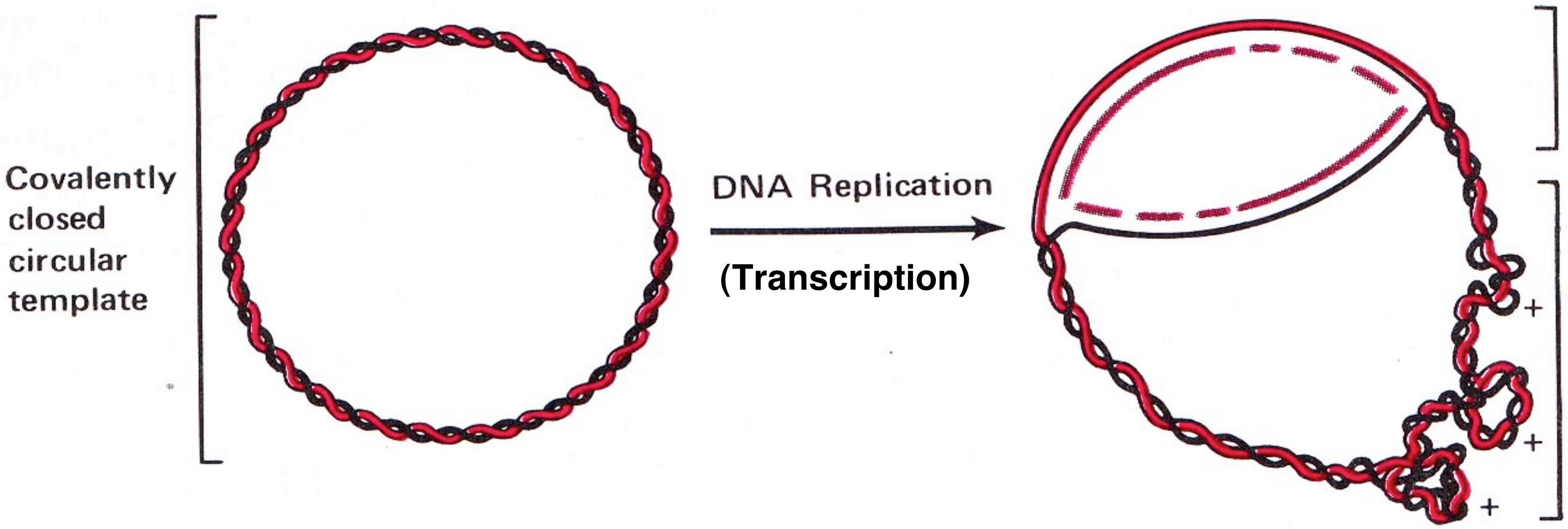
$$\Delta G_{\text{observed}} = 9.4 \text{ kcal}$$

$$\Delta G_i(\text{total}) = \sum_j n_{ij}\Delta G_j + \Delta G(\text{init}) + \Delta G_i(\text{sym})$$

$$T_{\text{M}} = \Delta H^\circ/(\Delta S^\circ + R \ln C_{\text{T}})$$

DNA Topology

DNA Unwinding Causes Topological Problems



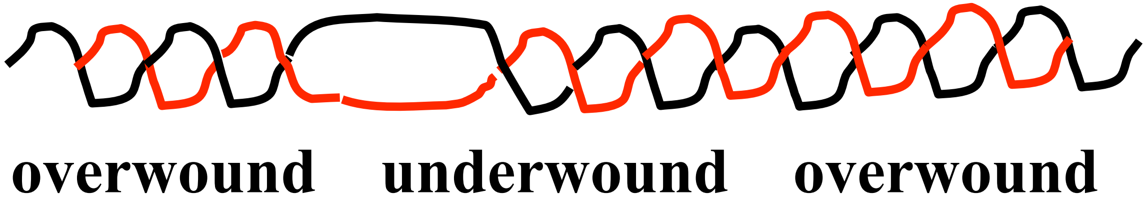
(Transcription)

Unwound Parental Duplex

Over-Wound region

The “twisting problem”

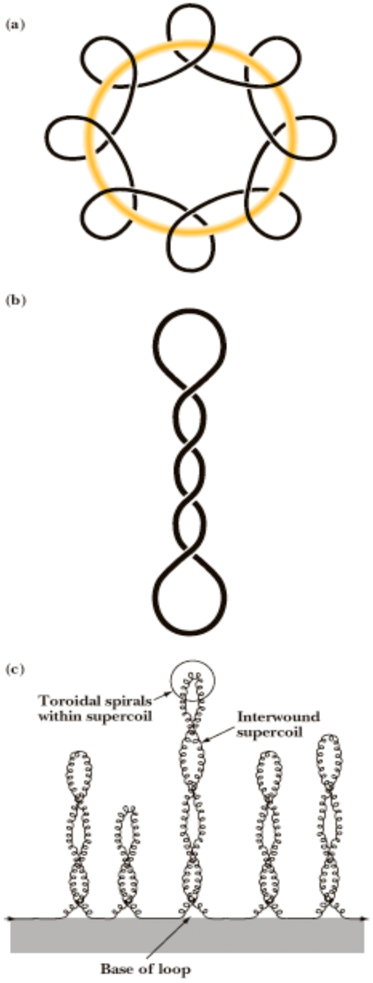
- DNA is a coiled double helix
- Replication and transcription require local DNA strand separation (**melting** of DNA).
- Local unwinding of DNA due to strand separation introduces a strain on the surrounding base pairs - they become **overwound**.



- In closed circular DNA molecules the coils can’t run off at the end of the chromosome

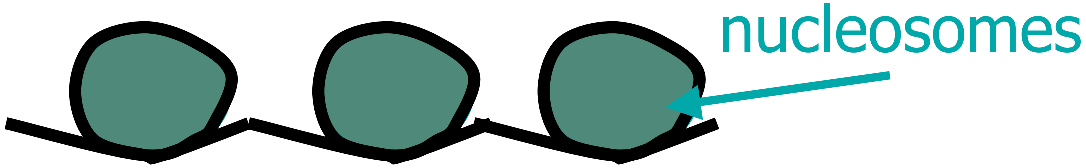
Varieties of Supercoiled DNA

Note that all black lines represents double stranded DNA



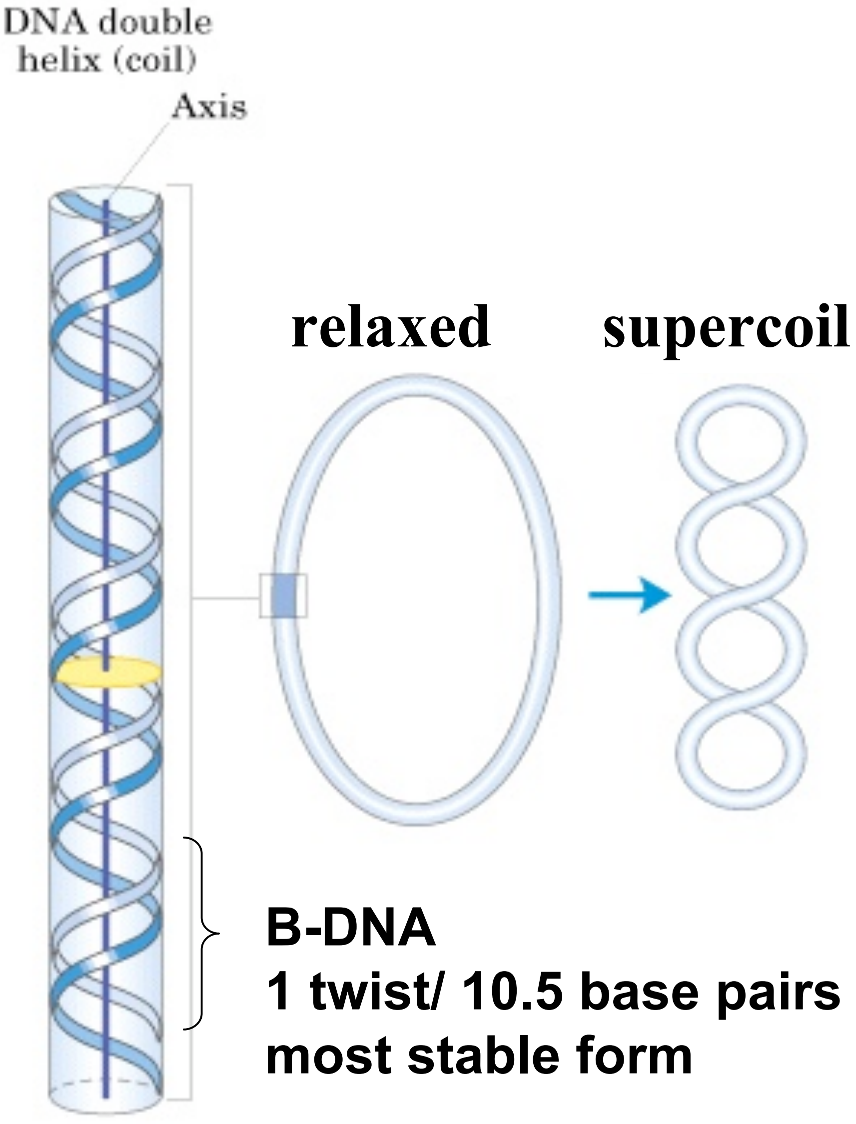
Nucleosomes

- Nucleosomes look like “beads on a string” under microscope. The beads contain a pair of four histone proteins, H2A, H2B, H3, and H4 (octamer). The string is double stranded DNA;
- The surface of the octamer contain features that guide the course of DNA such that DNA can wrap 1.65 turns around in a left-handed conformation. H1 proteins serves to seal the ends of the DNA and connects consecutive nucleosomes.



DNA supercoiling

- **DNA**:
  - coiled in form of a double helix around an axis
  - humans 2 m of DNA / cell
- **Relaxed state**
  - no bending of the DNA
- **Supercoiling**
  - coiling of the DNA helix,
  - supercoiled DNA is more compact than relaxed DNA
  - Condenses DNA which allows faster migration in agarose gels



B-DNA
1 twist/ 10.5 base pairs
most stable form

Underwinding

Underwinding:

- DNA has fewer turns than expected
- DNA is strained

Example:

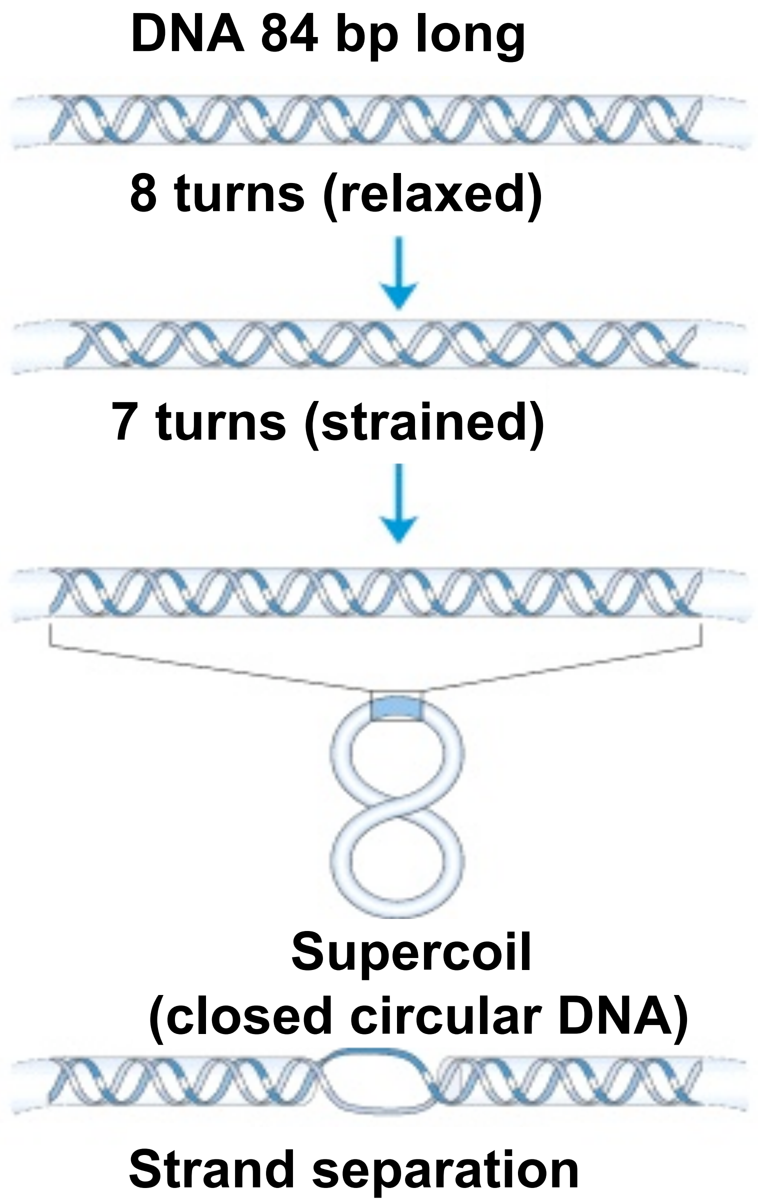
84 bp = 8 turns (@10.5 bp/ turn)

* Remove 1 turn

84 bp => 7 turns (at 12 bp/ turn)

Overcome problem

- by forming supercoils
- by separating strands



DNA 84 bp long

8 turns (relaxed)

7 turns (strained)

Supercoil
(closed circular DNA)

Strand separation

Underwinding

1. Every cell underwinds its DNA
2. Facilitates strand separation
3. Stored form of energy
4. Underwound DNA can only be maintained if:
    - the DNA is a closed circular DNA
    - the DNA is bound and stabilized by proteins

Properties of Topoisomerases

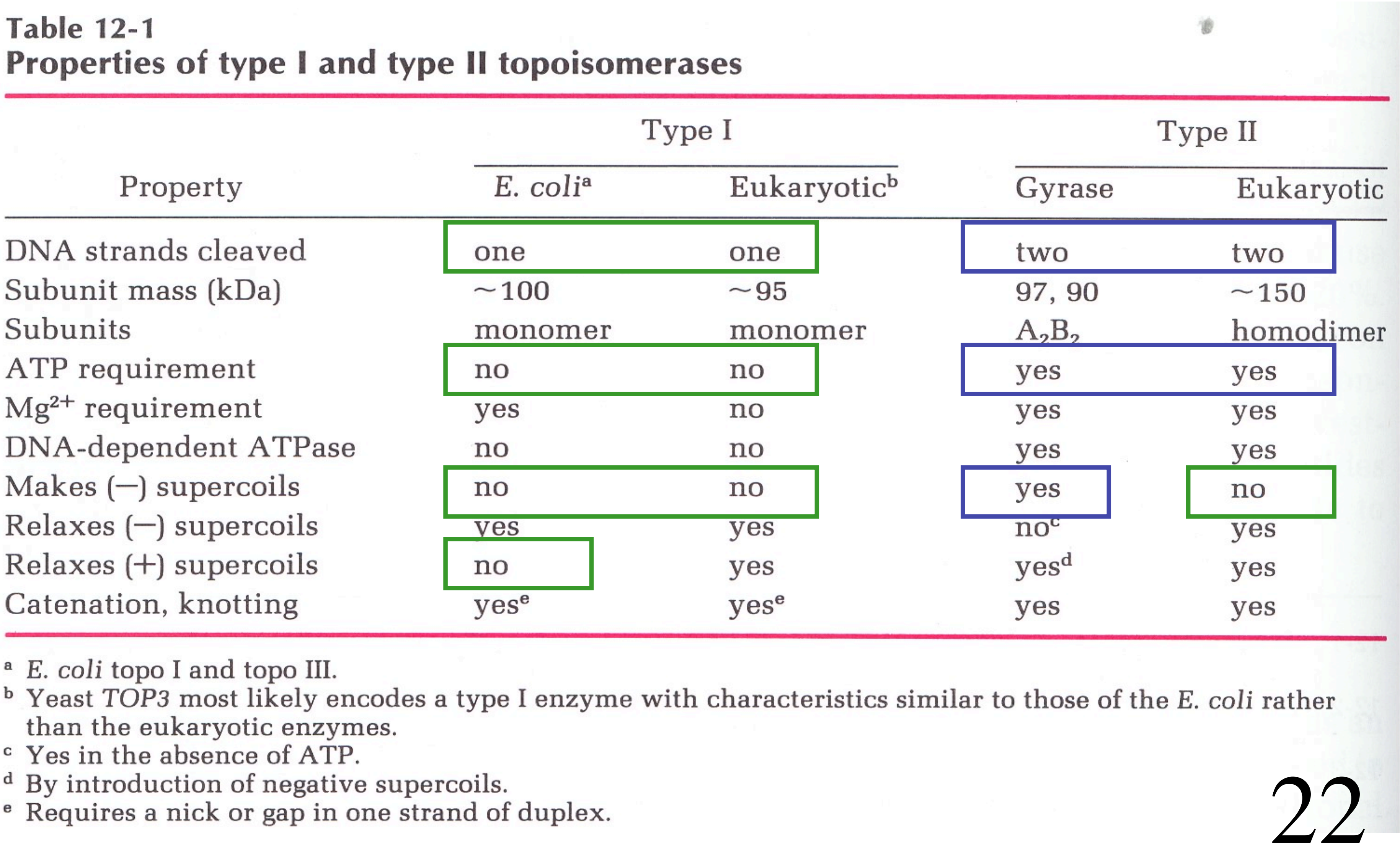


Table 12-1
Properties of type I and type II topoisomerases

	Type I		Type II	
Property	*E. coli*[a]	Eukaryotic[b]	Gyrase	Eukaryotic
DNA strands cleaved	one	one	two	two
Subunit mass (kDa)	~100	~95	97, 90	~150
Subunits	monomer	monomer	A_2B_2	homodimer
ATP requirement	no	no	yes	yes
Mg^{2+} requirement	yes	no	yes	yes
DNA-dependent ATPase	no	no	yes	yes
Makes (−) supercoils	no	no	yes	no
Relaxes (−) supercoils	yes	yes	no[c]	yes
Relaxes (+) supercoils	no	yes	yes[d]	yes
Catenation, knotting	yes[e]	yes[e]	yes	yes

[a] *E. coli* topo I and topo III.

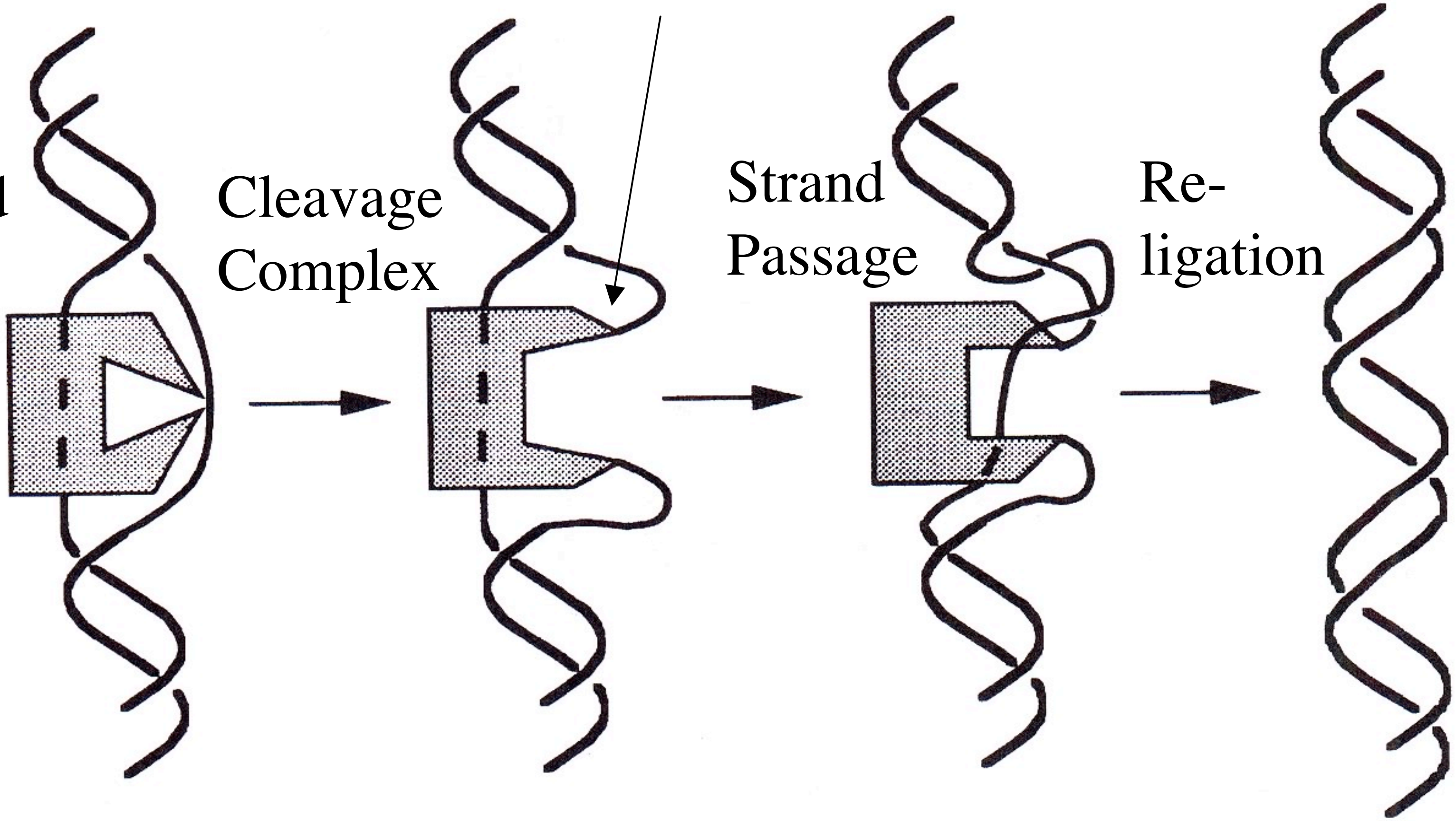
[b] Yeast *TOP3* most likely encodes a type I enzyme with characteristics similar to those of the *E. coli* rather than the eukaryotic enzymes.

[c] Yes in the absence of ATP.

[d] By introduction of negative supercoils.

[e] Requires a nick or gap in one strand of duplex.

Strand Passage Model for Topo I



Covalent Tyrosine-5’P

Unwound Complex

Cleavage Complex

Strand Passage

Re-ligation

L=2

L=3