

2015 Summer School on Polymers in Biology

DNA mechanics and structural diversity of DNA

@ KIAS, 22 Jun – 3 July

Seok-Cheol Hong

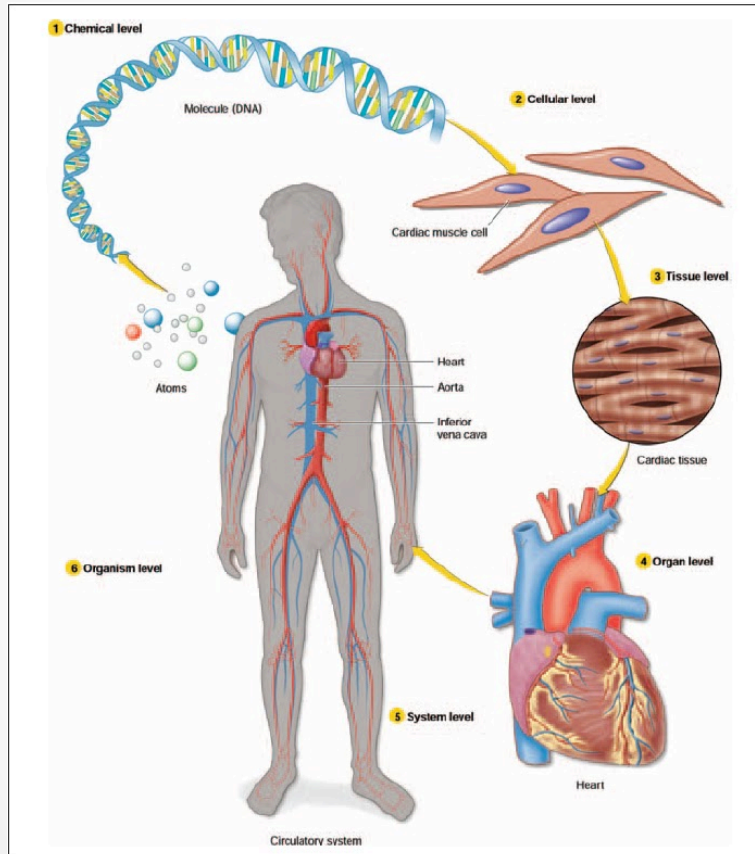
Korea University



Lecture 1

- Hierarchy of biological organization
- Biomolecules: 1D polymers
- Examples of Polymers in Biology: DNA, RNA, Proteins, and Polysaccharides
- DNA: genetic material; double helix
- Central Dogma
- DNA thermodynamics
-

Hierarchy of biological organization



Cells in the human body organize themselves into increasingly complex structures and systems. (From Premkumar K. *The Massage Connection Anatomy and Physiology*. Baltimore: Lippincott, Williams & Wilkins, 2004.)

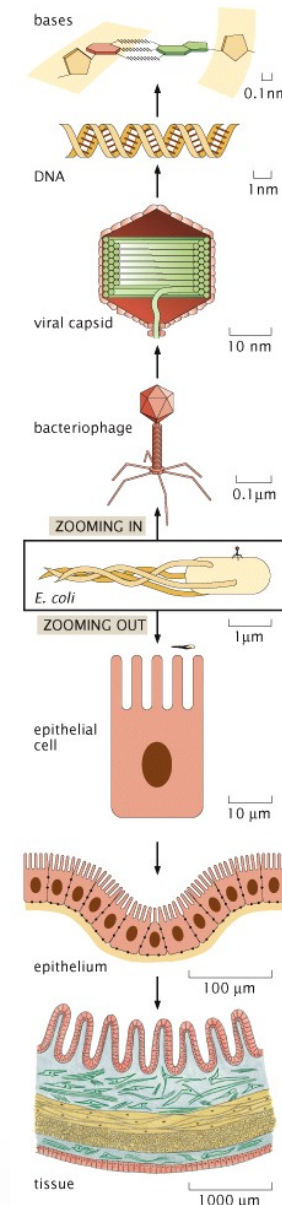


Figure 2.15 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Biomolecules are polymeric.

DNA

Protein

Polysaccharide

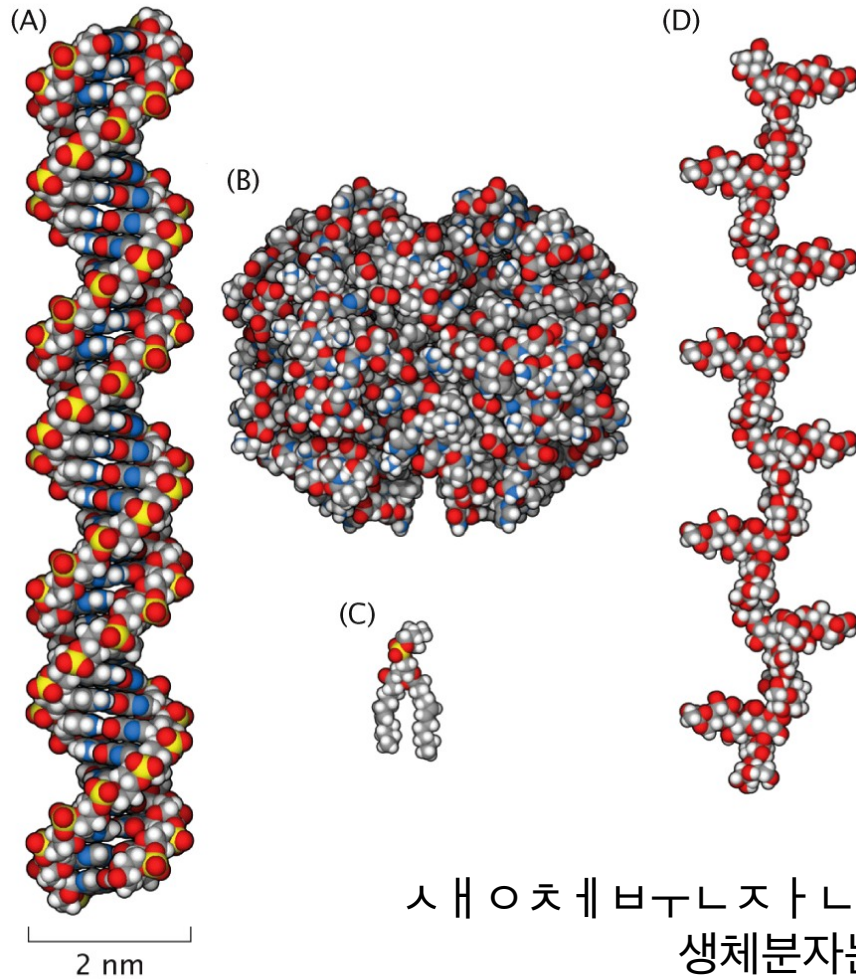
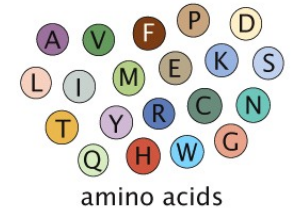
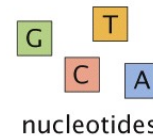


Figure 1.1 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

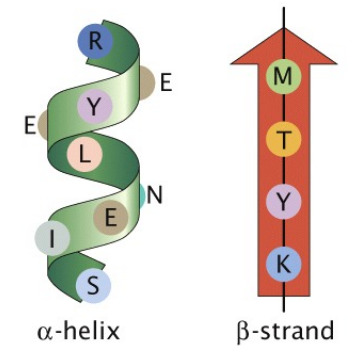
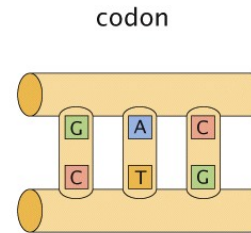
NUCLEIC ACIDS

PROTEINS

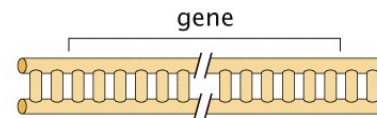
ALPHABET



WORDS

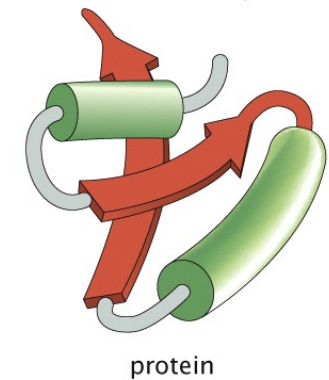


SENTENCES



스 || 오 처 || 버 T L 자 | L _ L 7 ⊥ 버 T L 자 | 이 | 다 |
 생체분자는 고분자이다.

Figure 1.2 Physical Biology of the Cell, 2ed. (© Garland Science 2013)



Polymers in Biology

- Proteins: hetero-polymer of 20 amino acids
- DNA: double-stranded (anti-parallel) polymer of 4 deoxyribo-nucleotides (A, T, C, and G).
- RNA: single-stranded (or double-stranded) polymer of 4 ribonucleotides (A, U, C, and G).
- Polysaccharides: polymer of sugars (-ose) (glucose, galactose, ...)

	Letter	Word	Sentence
Nucleic acids	A, T, C, and G	Triplet (codons)	Genes
Proteins	20 amino acids	Specific motif	Folded, biologically active enzymes

DNA: bases, nucleotides, pairing, and double helix

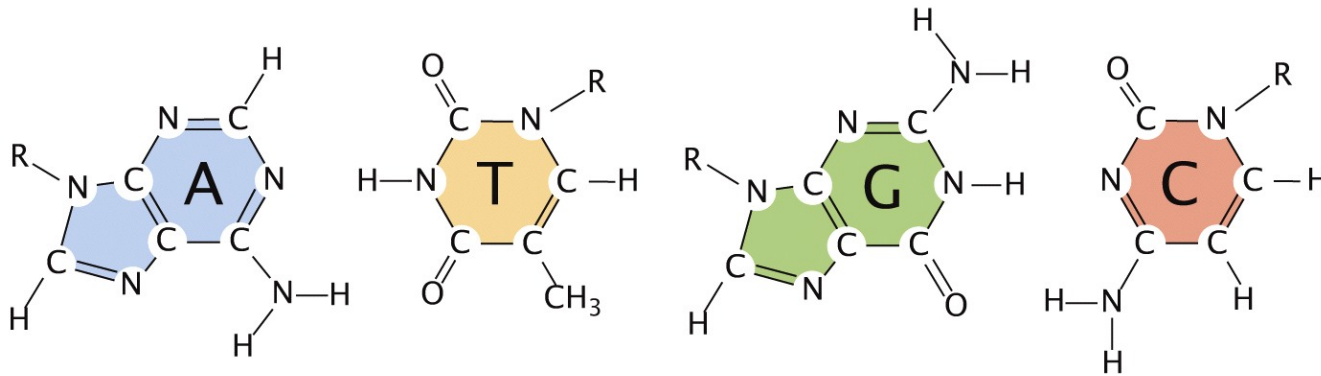


Figure 1.3a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

- A: Adenine
- T: Thymine
- G: Guanine
- C: Cytosine

- A pairs with T
- G pairs with C
- DNA is highly (negatively) charged.

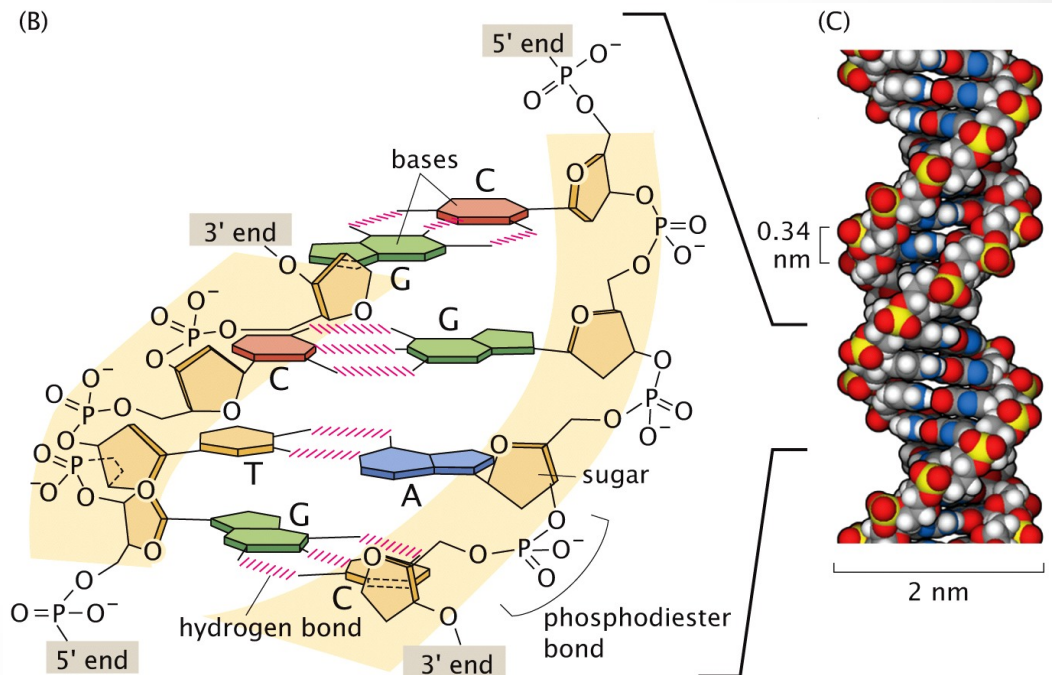
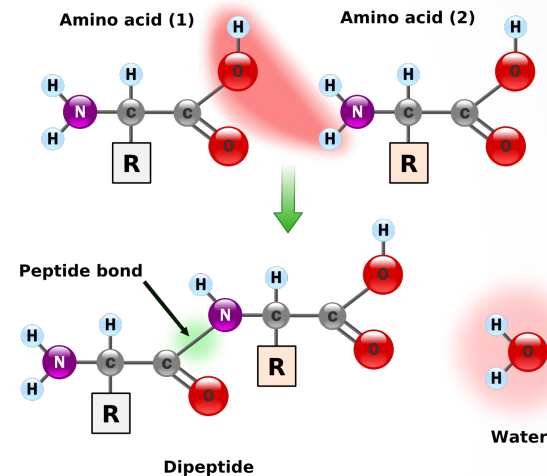
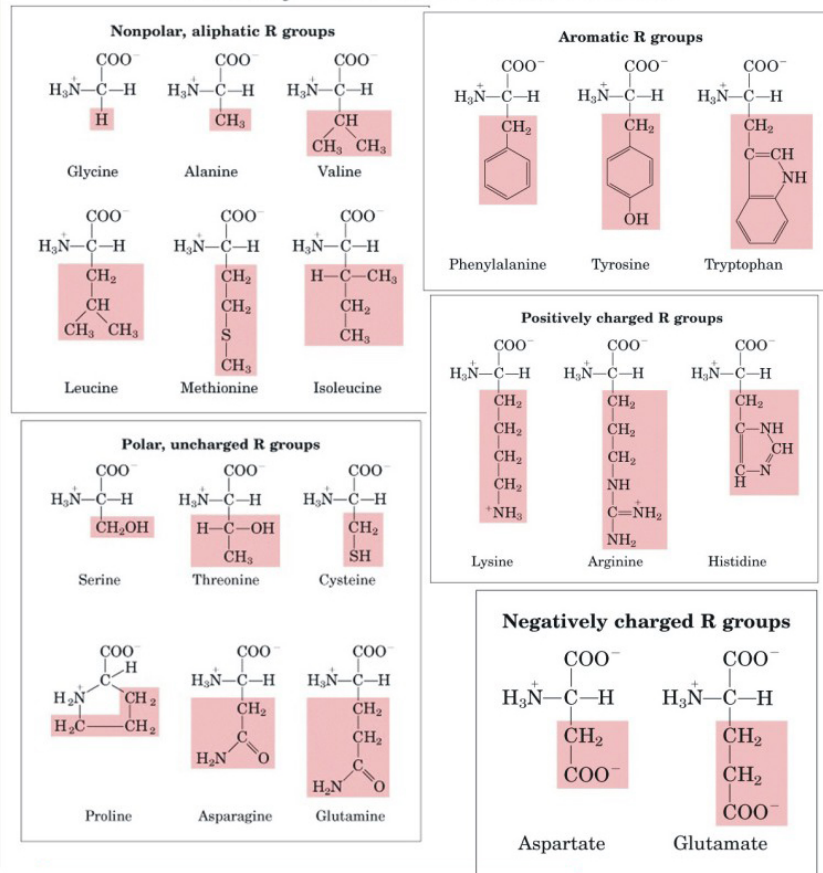


Figure 1.3b Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Proteins: amino acids and polypeptides

Twenty standard Amino Acids



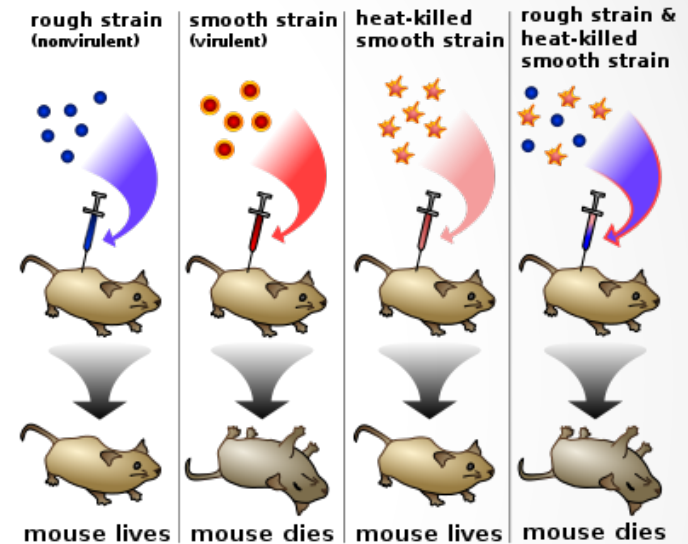
Advantages of Polymers

- Restricted set of building blocks
 - Nucleic acids – 4 nucleotides
 - Proteins – 20 amino acids
- Limited set of (bio)chemical reactions: removal of water; phosphodiester bond for DNA; peptide bond for protein
- Huge diversity of (a small distinct classes of) macromolecules
 - DNA: 10 nucleotides $\rightarrow 4^{10} \sim 10^6$
 - Protein: 10 amino acids $\rightarrow 20^{10} \sim 10^{13}$
- Small building blocks (\sim nm or less)
- Large, complicated macromolecules
-

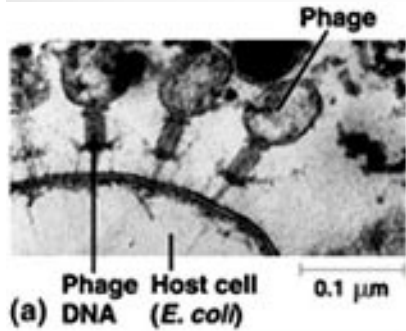


DNA (deoxy-ribonucleic acid)

- Discovery: Friedrich Miescher (1869)
 - Collected white blood cell from pus
 - Lysed cells and isolated nuclei
 - Found a substance he called nuclein
 - Found nuclein in every cell type he tested
 - Rich in Phosphor
- Genetic material
 - Griffith (1928)
 - Avery-MacLeod-McCarty (1944)
 - Hershey & Chase (blender experiment) (1952)



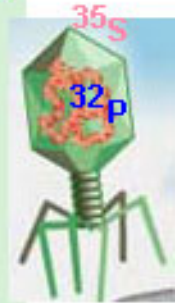
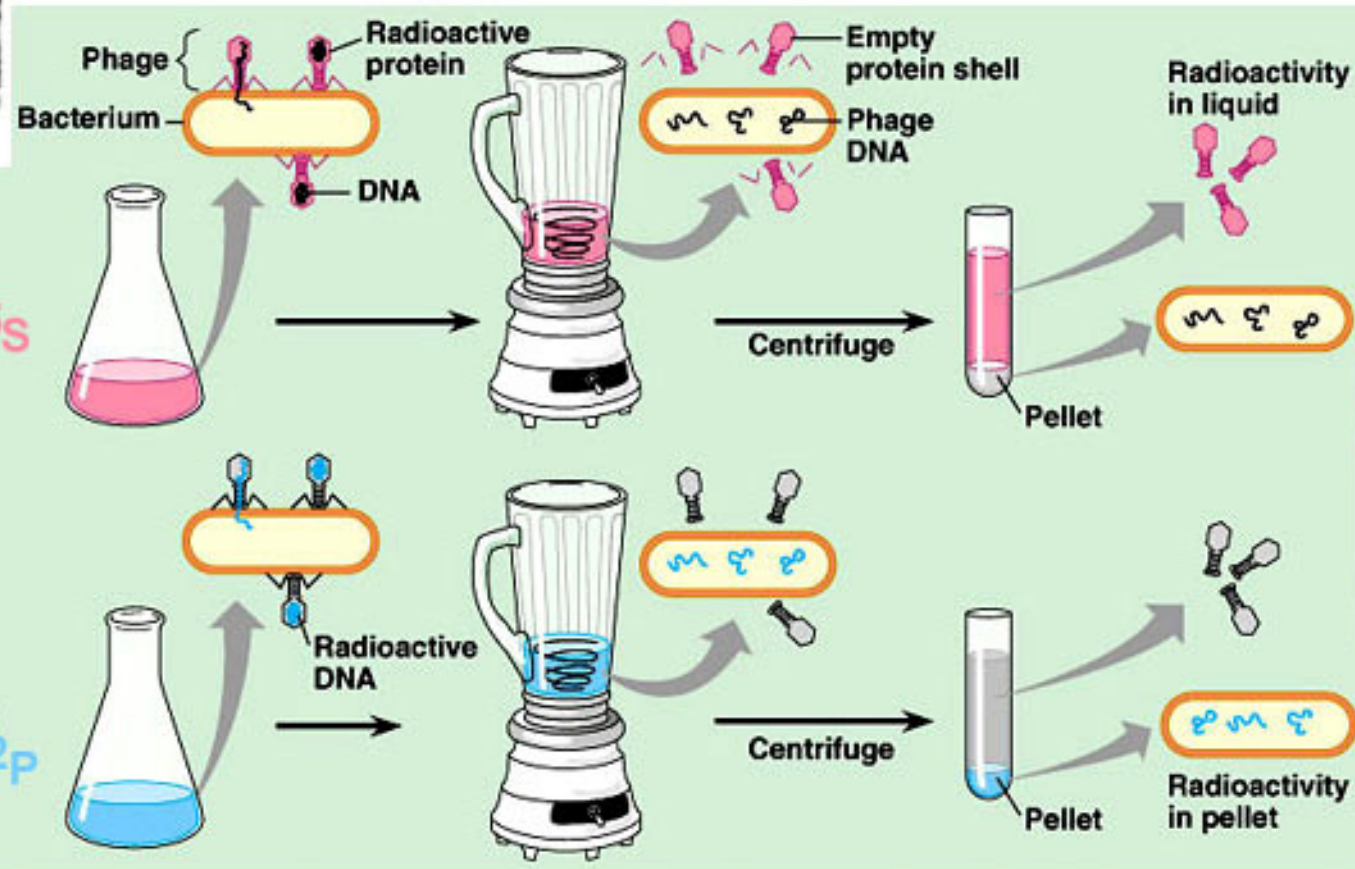
Hershey-Chase experiment



- 1 Mix radioactively labeled phages with bacteria. The phages infect the bacterial cells.
- 2 Agitate in a blender to separate phages outside the bacteria from the cells and their contents.
- 3 Centrifuge the mixture so bacteria form a pellet at the bottom of the test tube.
- 4 Measure the radioactivity in the pellet and the liquid.

Batch 1:
Phages grown with radioactive sulfur (^{35}S)

^{35}S



(b) The experiment showed that T2 proteins remain outside the host cell during infection, while T2 DNA enters the cell.

Critical information for DNA

- Erwin Chargaff (1948): discovered the ratio of A and T = 1:1 & the ratio of G and C = 1:1 by utilizing then newly developed paper chromatography and UV spectrophotometry.
- X-ray scattering from DNA fiber (Franklin (~1952)):



Rosalind Franklin

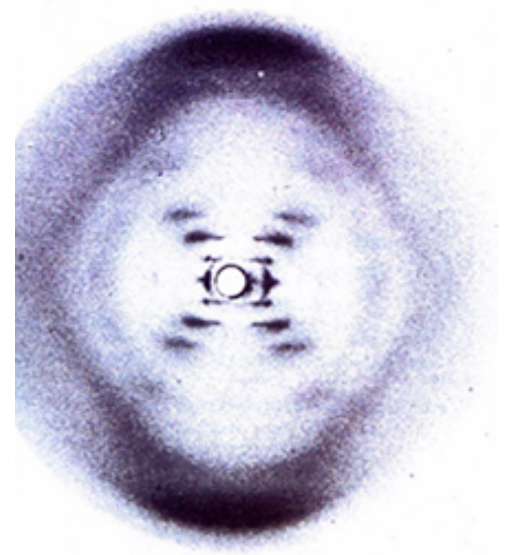
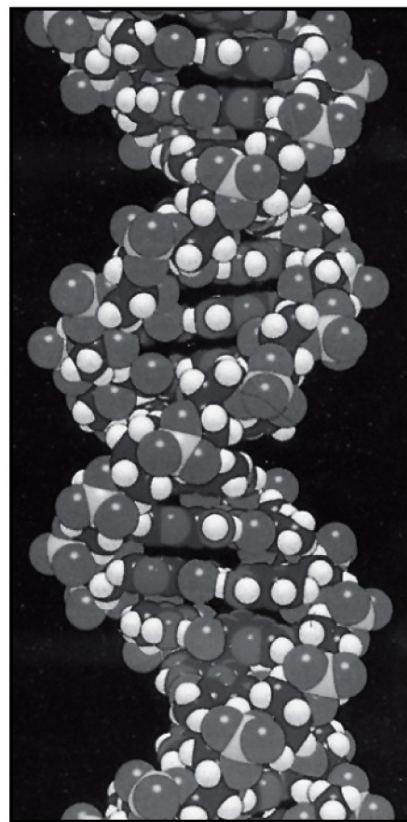


Photo 51 - The world famous x-ray that established DNA as a helix

DNA: right-handed double strand

- Watson & Crick: determine double helical structure of DNA

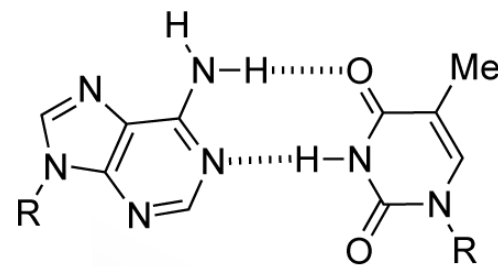


0.34 nm

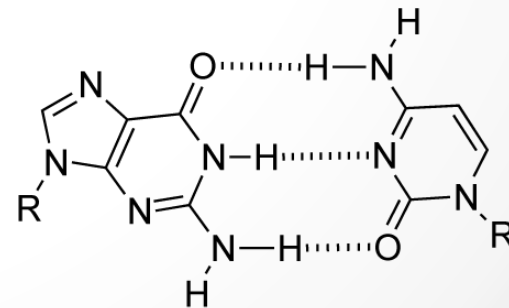
minor groove

major groove

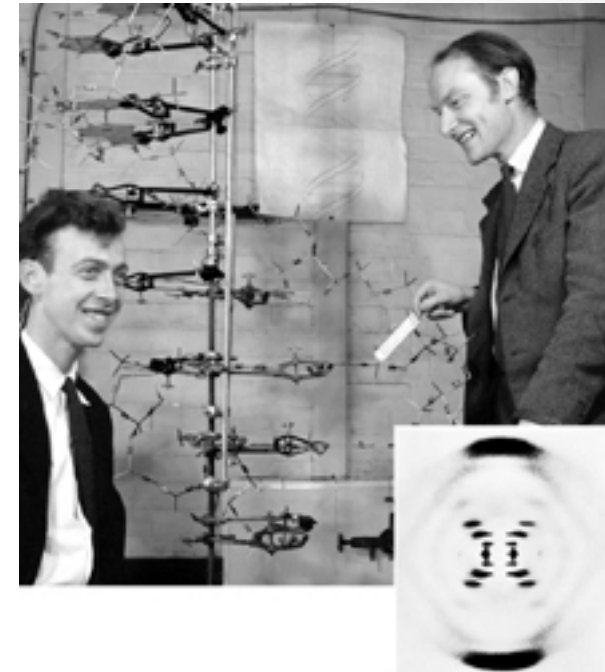
2 nm



A·T base pair



G·C base pair



Central Dogma

- Information flow from DNA to protein

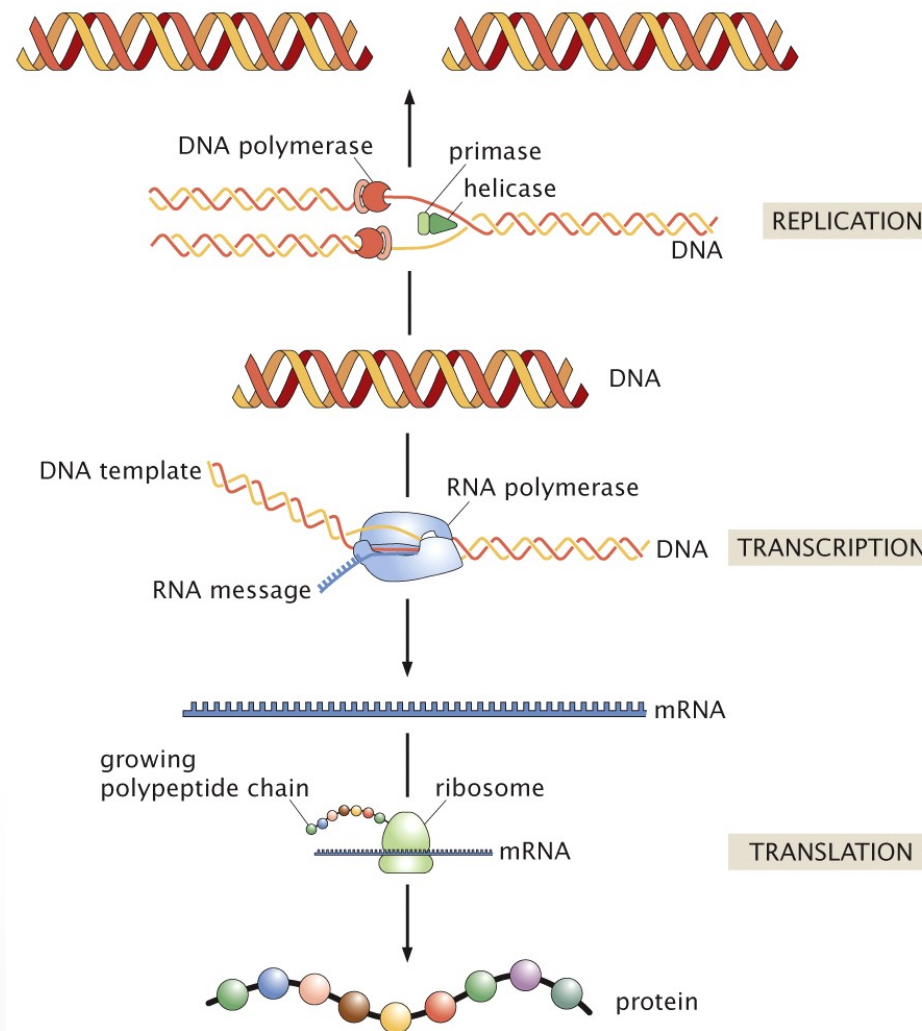


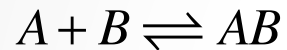
Figure 3.12 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Thermodynamics of DNA

- Interactions that stabilize DNA
- Calculation of free energy of DNA

Double helix formation by oligonucleotides without loops

Nonself-complementary $[A_0] = [B_0] = C_T / 2$



$$K = \frac{[AB]}{[A][B]} = \frac{2\alpha}{(1-\alpha)^2 C_T}$$

← Derive this.

α : fraction in duplex

C_T : total strand concentration

T_m : melting temperature (defined as T at which $\alpha = 0.5$)

$$K = \frac{2 \times 0.5}{(0.5)^2 C_T} = \frac{4}{C_T}$$

$$\Delta G = -RT \ln K = \Delta H - T \Delta S$$

$$\frac{1}{T_m} = \frac{R \ln(C_T / 4)}{\Delta H} + \frac{\Delta S}{\Delta H}$$

Nearest neighbor (NN) model

- The stability of a helix depends on the nearest neighbor interactions.



- $\Delta G_{\text{tot}} = \Delta G_{\text{init}} + \Delta G_{\text{sym}} + \sum \Delta G_{\text{NN}} + \Delta G_{\text{TERM}} = 1.96 + 0 + (\dots) + 0 = 1.96 - 6.59 = -4.63 \text{ kcal/mol}$

- $(\dots) = \begin{array}{cccc} 5' & G & C & \\ C & G & 5' & \end{array} + \begin{array}{cccc} 5' & C & A & \\ G & T & 5' & \end{array} + \begin{array}{cccc} 5' & A & T & \\ T & A & 5' & \end{array} + \begin{array}{cccc} 5' & T & A & \\ A & T & 5' & \end{array} + \begin{array}{cccc} 5' & A & C & \\ T & G & 5' & \end{array}$
 $= -2.24 - 1.45 - 0.88 - 0.58 - 1.44 = -6.59$

Look for a more reliable, up-to-date method to calculate thermodynamic quantities for DNA

Estimation of melting T

- $\Delta H = 0.2 - 9.8 - 8.5 - 7.2 - 7.2 - 8.4 = -40.9$ kcal/mol
- $\Delta S = -5.6 - 24.4 - 22.7 - 20.4 - 21.3 - 22.4 = -116.8$ eu
- $R = 8.3$ J/K/mol = 1.987 cal/K/mol
 - eu = cal/K/mol
 - 1 cal = 4.19 J
- $C_T = 10^{-6}$ M
- $T_m = 278.22 \sim 5^\circ\text{C}$ cf. IDT: $\sim 10^\circ\text{C}$

OligoAnalyzer 3.1

[Instructions](#) | [Definitions](#) | [Feedback](#)



Sequence

6 Bases

5'- GCA TAC -3'

Clear Sequence

Add To Order

Parameter sets

SpecSheet (Default)

Target type

DNA

Oligo Conc

1 μM

Na⁺ Conc

1000 mM

Mg⁺⁺ Conc

0 mM

dNTPs Conc

0 mM

Analyze

Hairpin

Self-Dimer

Hetero-Dimer

NCBI Blast

Tm Mismatch

Results

5' Mods

Internal Mods

3' Mods

Mixed Bases

RESULTS

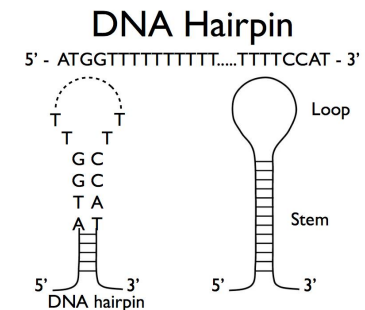
Resuspension

Dilution

SEQUENCE	5'- GCA TAC -3'
COMPLEMENT	5'- GTA TGC -3'
LENGTH	6
GC CONTENT	50 %
MELT TEMP	10.1 °C

Hairpin

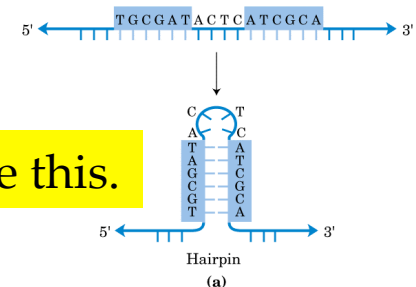
- Free energy increment for loop formation is unfavorable.
- The stability of hairpin depends on loop size, the loop sequence, and the base pair closing the loop.
- CG closing loop: very stable
- H-bond and stacking within loop probably make contribution
- ΔH (see below); $\Delta S = -(S_{\text{loop}} + S_{\text{duplex}})$
- $\Delta G_{37, \text{loop}} = \Delta G_{37, \text{length}} + \Delta G_{37, \text{mm}} - 0.8$
if first mismatch is GA or UU.
- $\Delta G_{37, \text{length}}(n) = \Delta G(n_{\text{max}}) + 1.75 RT \ln(n/n_{\text{max}})$



Unimolar $\Delta H = 4RT_m^2 \left(\frac{\partial \alpha}{\partial T} \right)_{T=T_m}$; α : fraction of single strand

$K = \frac{\alpha}{1 - \alpha}$; van't Hoff equation: $\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$

← Derive this.



Interactions determining duplex stability

- Conformational entropy: S associated with propagating a single strand stacked helix by 1 additional nucleotide was estimated as -11 eu.
 - For duplex, $S = 2 \times -11 = -22$ eu \rightarrow account for most of observed ΔS .
- Stacking
 - Stacking free energy = favorable interaction + unfavorable int. \rightarrow
fav. Int. = stacking G – unfav. Int. as large as $(-1.7$ kcal/mol – 1.9 kcal/mol (empirical) = -3.6 kcal/mol)
- Hydrogen bonding
 - The effect of hydrogen bonds is due to the difference between hydrogen bonding in a base pair and hydrogen bonding of the separated bases with water.



Max value from Table

Hydrogen bonding (RNA)

- In $(GCCGGC)_2$, ΔG_{37} for each terminal base pair = $[\Delta G_{37}(GCCGGC) - \Delta G_{37}(CCGG)] / 2 = -3.3$ kcal/mol
- Stacking of each 3' dangling end in $(CCGGC)$: $[\Delta G_{37}(CCGGC) - \Delta G_{37}(CCGG)] = -0.4$ kcal/mol
- Effect of each 5' terminal G in $(GCCGG)$: $[\Delta G_{37}(GCCGG) - \Delta G_{37}(CCGG)] = -0.2$ kcal/mol
- Free energy gained from pairing the terminal G and C: $\Delta G_{37,p} = -3.3 - (-0.4 - 0.2 + 1.9) = -4.6$ kcal/mol \rightarrow -1.5 kcal/mol/HB

Table 8.5
Thermodynamic Parameters for Unpaired Terminal Nucleotides in RNA at 1 M NaCl^a

Propagation Sequence	X=A			X=C			X=G			X=U		
	ΔH°	ΔS°	ΔG_{37}°	ΔH°	ΔS°	ΔG_{37}°	ΔH°	ΔS°	ΔG_{37}°	ΔH°	ΔS°	ΔG_{37}°
<u>3' Unpaired Nucleotides</u>												
→ AX U ←	-4.9	-13.2	-0.8	-0.9	-1.2	-0.5	-5.5	-15.0	-0.8	-2.3	-5.4	-0.6
→ CX G ←	-9.0	-23.4	-1.7	-4.1	-10.7	-0.8	-8.6	-22.2	-1.7	-7.5	-20.4	-1.2
→ GX C ←	-7.4	-20.0	-1.1	-2.8	-7.9	-0.4	-6.4	-16.6	-1.3	-3.6	-9.7	-0.6
→ UX A ←	-5.7	-16.4	-0.7	-0.7	-1.8	-0.1	-5.8	-16.4	-0.7	-2.2	-6.8	-0.1
<u>5' Unpaired Nucleotides</u>												
→ XA U ←	1.6	6.1	-0.3	2.2	7.9	-0.3	0.7	3.4	-0.4	3.1	10.6	-0.2
→ XC G ←	-2.4	-6.0	-0.5	3.3	11.8	-0.3	0.8	3.4	-0.2	-1.4	-4.3	-0.1
→ XG C ←	-1.6	-4.5	-0.2	0.7	3.1	-0.3	-4.6	-14.8	0.0	-0.4	-1.2	0.0
→ XU A ←	-0.5	-0.7	-0.3	6.9	22.8	-0.1	(0.6)	(2.7)	(-0.2)	(0.6)	(2.7)	(-0.2)

^aTurner et al., (1988). The parameters ΔH° and ΔG° (in kcal mol⁻¹); ΔS° (in eu). Values in parentheses are estimated.

Environmental effects on helix stability:

salt concentration (experimental)

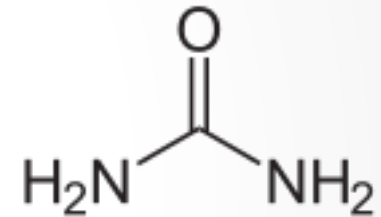
- Na^+ up to 1 M: increase stability
- Na^+ up to 0.2 M, T_m increases linearly with $\log[\text{Na}^+]$
 - Rate of increase depends on base composition
 - $dT_m/d\log[\text{Na}^+] = 18.3 - 7.04 F_{GC}$ or $19.96 - 6.65 F_{GC}$
- Above 1 M, addition of salt lowers T_m of DNA.
 - The lowering is relatively independent of cation but is strongly dependent on anion with $\text{CCl}_3\text{COO}^- > \text{SCN}^- > \text{ClO}_4^- > \text{CH}_3\text{COO}^- > \text{Br}^-, \text{Cl}^-$
 - correlate with the effect of ions on the solubility of the bases (better denaturants are more effective in base solubility)
- Mg^{2+} up to 1 – 10 mM: increase T_m .
- Beyond it, addition of Mg^{2+} lowers T_m .

Environmental effects on helix stability:

solvent effects

- Addition of cosolvent to aqueous solutions of nucleic acids destabilizes the ordered form.

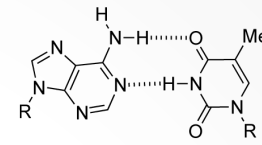
- Urea: T_m decreases at $2.5^\circ\text{C}/\text{M}$



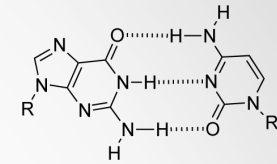
- Favorable interactions between bases and cosolvent favor denaturation.
 - The cosolvent concentrations required for 50% denaturation correlate well with enhancement of base solubility.

Environmental effects on helix stability:

pH



A-T base pair



G-C base pair

- The stability of duplex is insensitive to pH between 5 and 9.
- At low pH, bases in ssDNA bind more protons than in duplex, thus favoring ssDNA.
- At high pH, G, T, and U are deprotonated, thus precluding normal H bonding and increasing charge repulsion.
- Some sequences such as poly(dC) form duplex at low pH (C.C⁺).

Lecture 2

- Watson-Crick base pair
- Effects of chemical factors on DNA stability
- DNA sequence vs. charged polymer
- Mechanical models: Freely Jointed Chain model
- Persistence length, end-to-end extension, radius of gyration, force response



Lecture 3

- Mechanical models: Worm Like Chain model
- DNA supercoils: definition (sign, magnitude)
- Linking number, twist, writhe
- Călugăreanu-White-Fuller theorem
- Energy associated with DNA supercoiling
- Non-canonical DNA structures (induced by SC)
-

Lecture 4

- Single molecule methods (revisit)
- Hybrid single molecule technique of smFRET & MT
- Case studies: DNA mechanics via single-molecule methods
- Case studies: Non-canonical DNA and its dynamics via single-molecule methods

