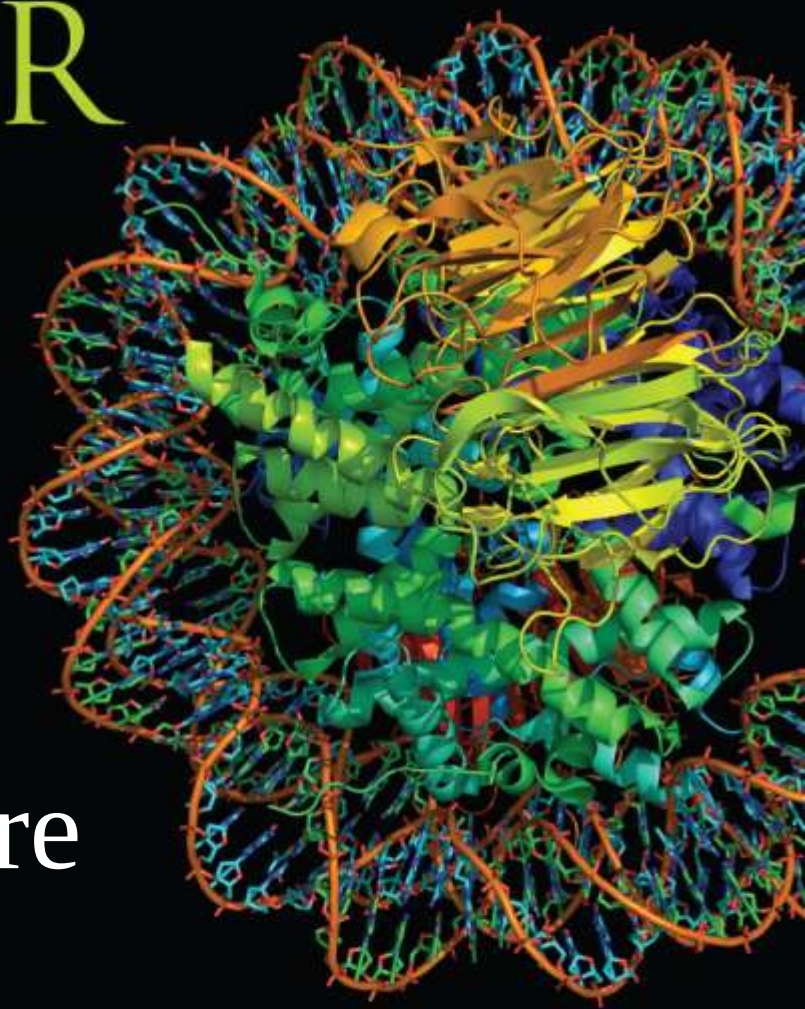
Principles of

MOLECULAR BIOLOGY

BURTON E. TROPP



Chapter 3
Nucleic Acid Structure

Lecture 3 & 4:
Nucleic Acid Structure-Chapter 3
Dr. Nabil Bashir

OUTLINES
Nucleic Acid Structure

Topic	Page	Notes
3.1: DNA Size And Fragility	81-82	
3.2: Major And Minor Grooves	82-83	
3.4:DNA Denaturation And Renaturation	84-89	
3.9:Non-B DNA Conformation	98-102	
3.10: RNA Structure	104-105	

Nucleic Acid Structure

- DNA Structure
  - Size
  - Fragility
  - Structure
  - Denaturation/Renaturation
  - Superhelicity
- RNA Structure
  - RNA secondary and tertiary structure
  - Catalytic RNA

DNA Size and Fragility

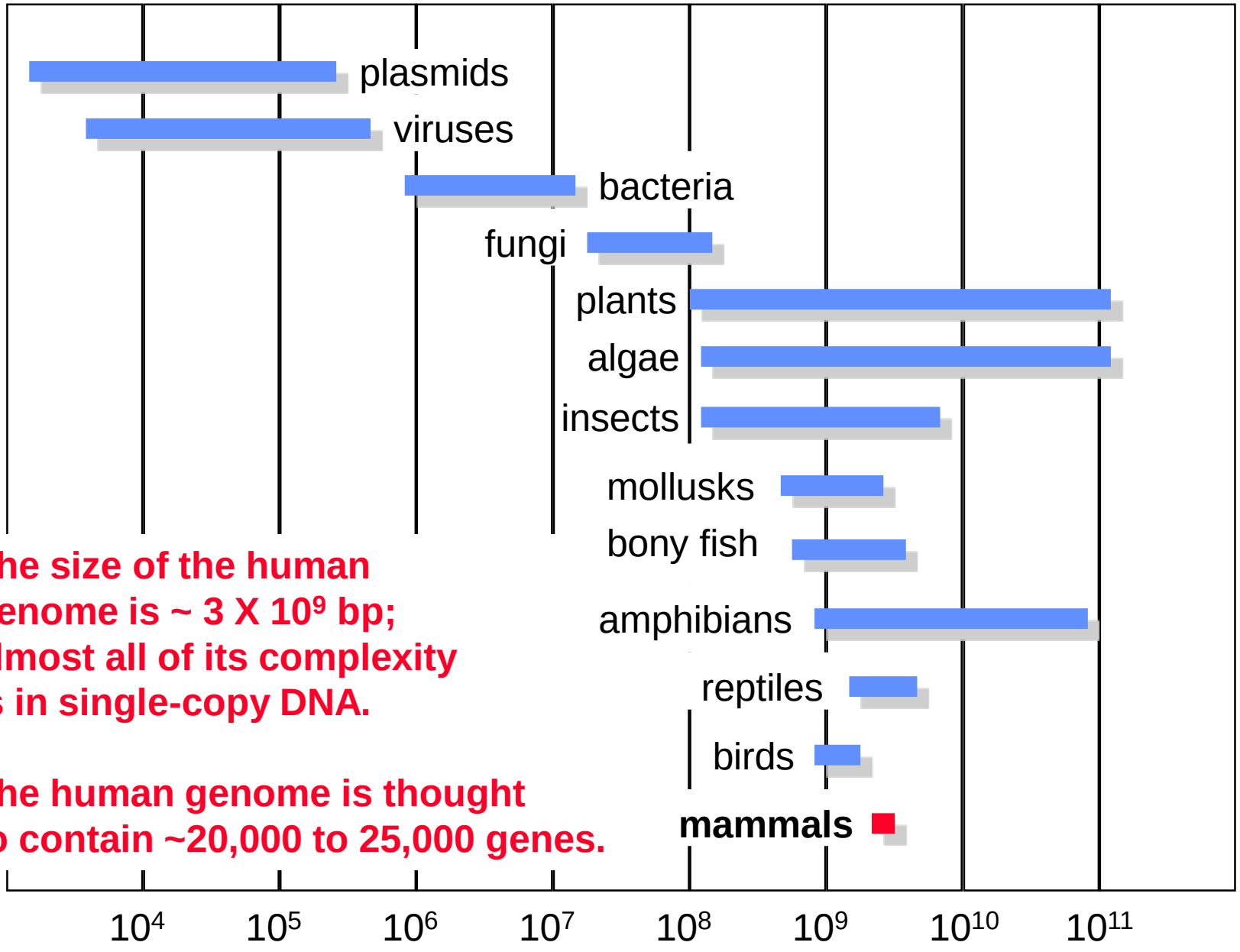
DNA in prokaryotic(circular) & eukaryotics(linear).

DNA molecules vary in size

Length of a DNA molecule can be calculated

- Distance between base pairs = 0.34nm
  - pBR322 plasmid (4,361 bp) = 1.48μm
  - Human Chromosome 1 (245,522,847 bp) = 8.3cm!

Genome sizes in nucleotide pairs (base-pairs)



The size of the human genome is ~ 3 X 10^9 bp; almost all of its complexity is in single-copy DNA.

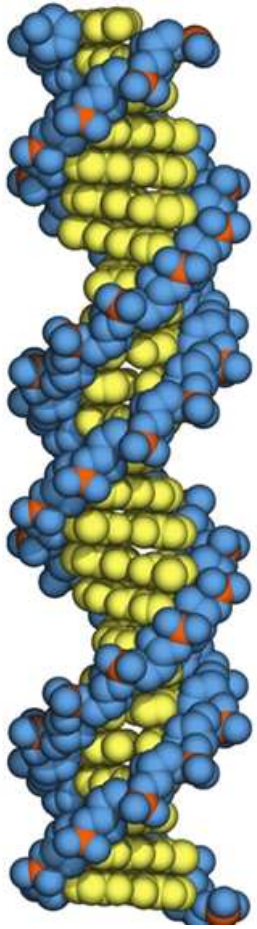
The human genome is thought to contain ~20,000 to 25,000 genes.

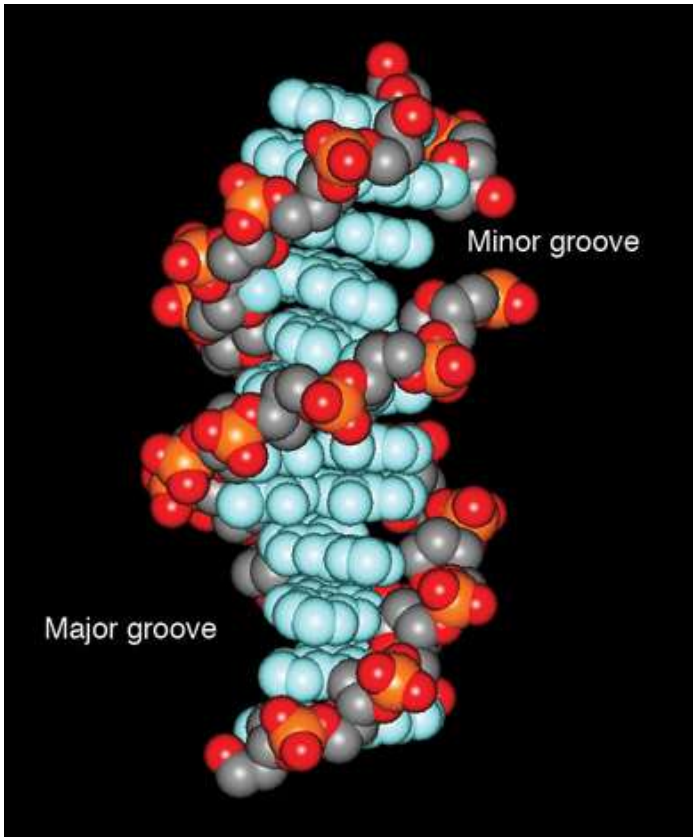
DNA Size and Fragility

DNA molecules are fragile

- Easily sheared by ordinary lab practices (pouring, pipetting, mixing)
- Average DNA length from standard isolation procedures is approx. 40,000bp

Enzymes can recognize patterns in the major and minor groove





(Structure from Protein Data Bank ID: 1BNA Drew, H. R., Wing, R. M., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R. E. 1981. Structure of a B-DNA dodecamer: conformation and dynamics. Proc Natl Acad Sci USA 78: 2179–2183. Prepared by B. E. Tropp.)

Figure 03.01: Major and minor grooves in B-DNA.

Recognition Patterns in the Major and Minor Grooves

Base sequence recognition by enzymes depends primarily on a unique pattern that G-C or A-T base pairs project it the edge of the major groove

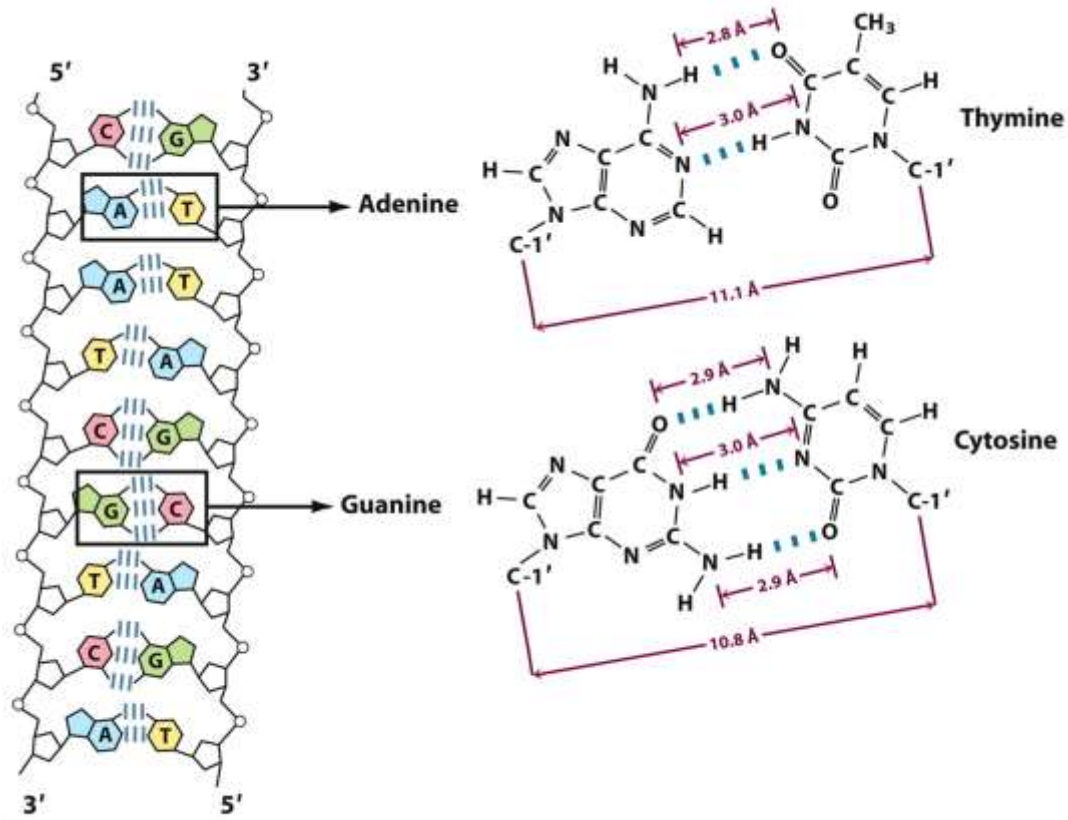


Figure 8-11
Lehninger Principles of Biochemistry, Sixth Edition
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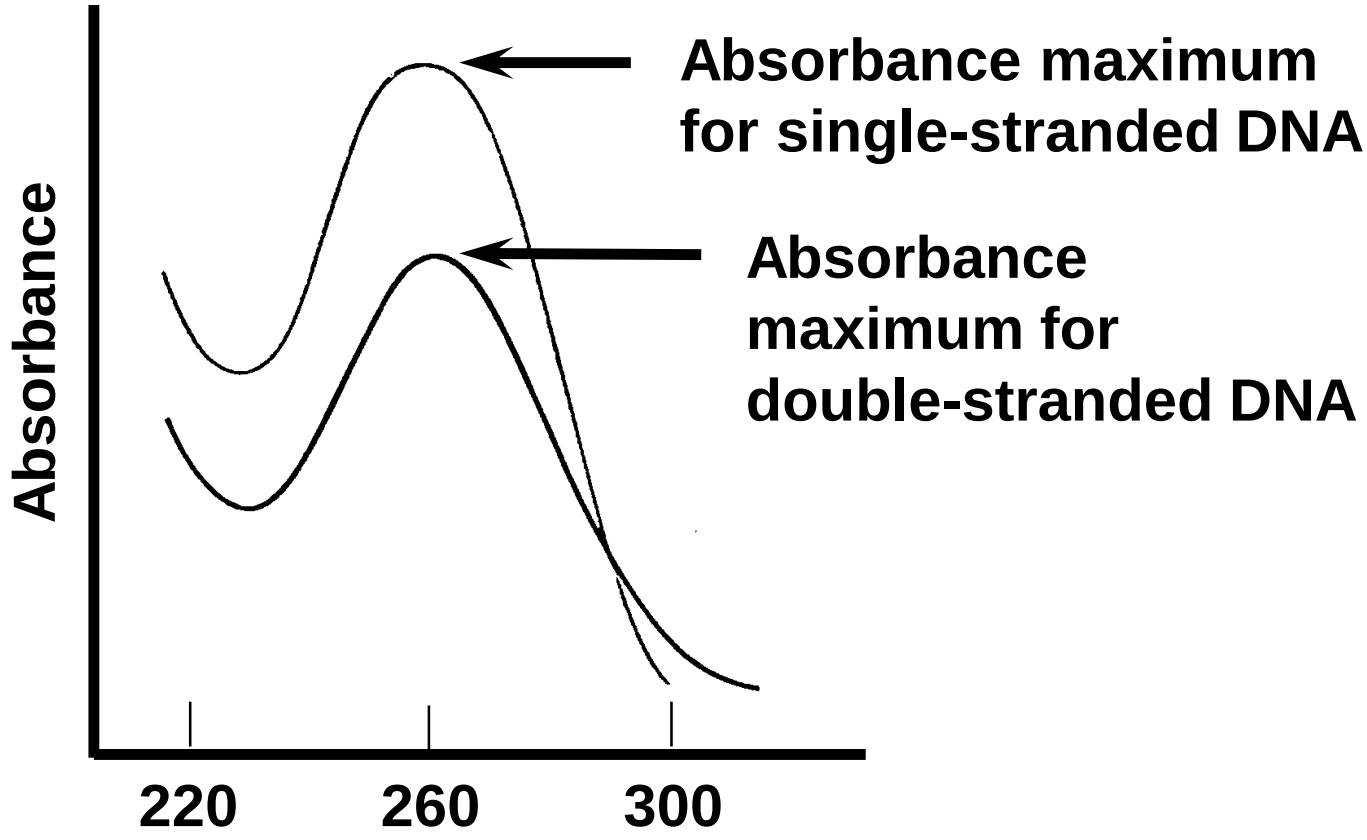
Hyperchromicity

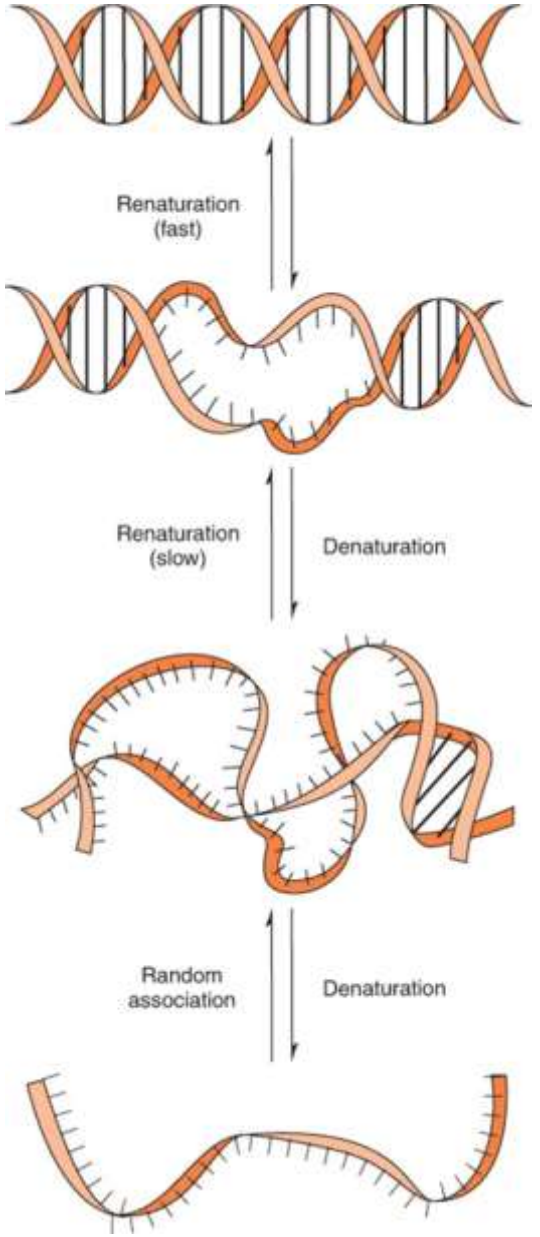


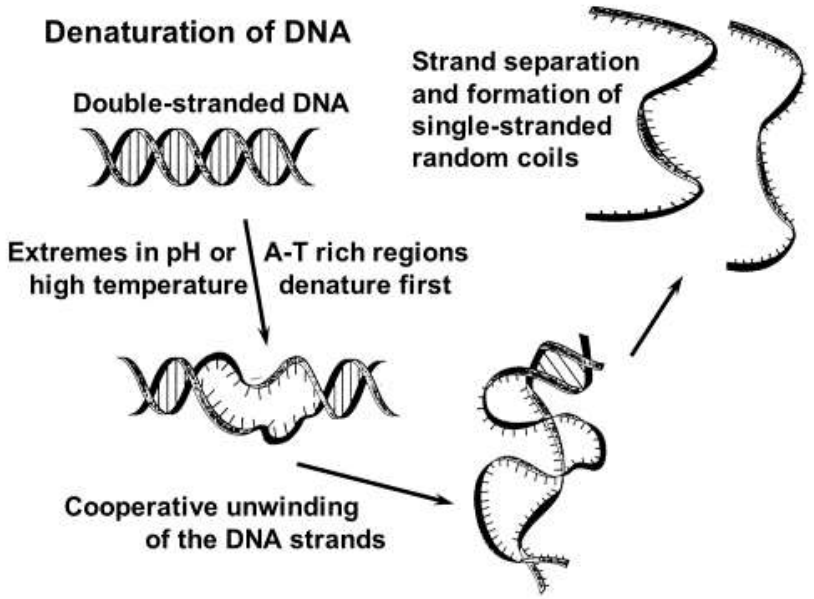
The absorbance at 260 nm of a DNA solution increases when the double helix is melted into single strands.

DNA Denaturation and Renaturation

- Can long strands of DNA unwind?
  - DNA solutions drop in viscosity when heated
    - Double helical structure collapses into single strands – **Denaturation**
- Denaturation can be recognized by U.V. 260 absorbance - for a 50μg/ml solution
  - Double stranded DNA A_{260}=1.00
  - Single stranded DNA A_{260}=1.37
  - Free Nucleotides A_{260}=1.60

DENATURATION AND RENATURATION




Denaturation of DNA
Double-stranded DNA
Strand separation and formation of single-stranded random coils
Extremes in pH or high temperature
A-T rich regions denature first
Cooperative unwinding of the DNA strands

DNA Denaturation and Renaturation

Slowly heated DNA solution in 0.15 M NaCl

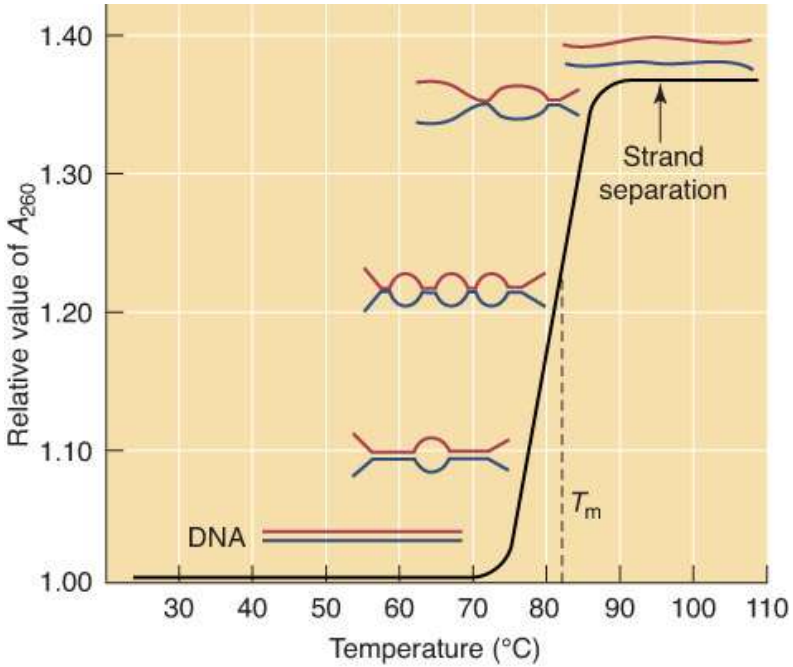


Figure 03.04: DNA melting curve. A melting curve of DNA showing Tm (the melting temperature) and possible molecular conformations for various degrees of melting.

DNA Denaturation and Renaturation

- The temperature at which the rise in A_{260} is half complete is the T_m- the melting temperature
- Hydrogen bonds stabilize double stranded DNA
  - T_m increases with increasing G+C content

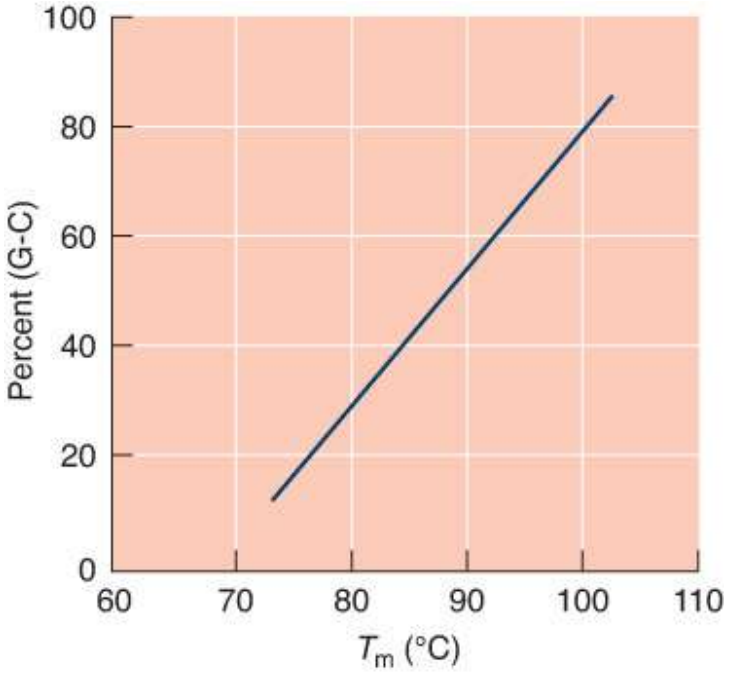


Figure 03.05: Effect of G-C content on DNA melting temperature. Tm increases with increasing percent of G + C.

Forces affecting the stability of the DNA double helix

- **hydrophobic interactions - stabilize**
  - **hydrophobic inside and hydrophilic outside**
- **stacking interactions - stabilize**
  - **relatively weak but additive van der Waals forces**
- **hydrogen bonding - stabilize**
  - **relatively weak but additive and facilitates stacking**
- **electrostatic interactions - destabilize**
  - **contributed primarily by the (negative) phosphates**
  - **affect intrastrand and interstrand interactions**
  - **repulsion can be neutralized with positive charges (e.g., positively charged Na^+ ions or proteins)**

DNA Denaturation and Renaturation

- More energy is required to disrupt the 3 hydrogen bonds in a G-C base pair than the 2 in A-T base pairs
- Denaturing agents lower T_m by disrupting hydrogen bonds between base pairs
  - Supports the role of hydrogen bonds in double stranded DNA structure

DNA Denaturation and Renaturation

- Base stacking stabilizes double stranded DNA
  - Base stacking is maintained by van der Waals forces
  - Base stacking helps stabilize hydrogen bonds between base pairs

DNA Denaturation and Renaturation

Ionic strength influences DNA structure

- In the absence of salt (NaCl) DNA strands repel each other through the negatively charged phosphates
- As salt is added Na^+ shields the phosphates from each other
- T_m rises with NaCl concentration

DNA Denaturation and Renaturation

The DNA molecule is in a dynamic state

- Evidence that DNA bases continually un-pair and pair
  - Called DNA breathing
  - Transient melting occurs more often in A-T rich regions
    - A-T base pairs with 2 hydrogen bonds
    - G-C base pairs with 3 hydrogen bonds

DNA Denaturation and Renaturation

Alkali denatures DNA strands without breaking phosphodiester bonds

- High temperature can break phosphodiester backbone
  - Degradation can be avoided by using a base (NaOH) for denaturing DNA
  - Acid causes de-purination

DNA Denaturation and Renaturation

DNA renaturation or re-anealing is a critical tool in molecular genetics

- Two requirements must be met
  - Salt concentration high enough to eliminate electrostatic repulsion
    - Usually 0.15 to 0.5 M
  - Temperature high enough to disrupt random hydrogen bonds but low enough to allow for stable inter-strand base pairing
    - 20˚-25˚ C below the T_m

Topoisomers

Bacterial DNA usually exists as a covalently closed circular double stranded DNA

- The bacterial chromosome is a very large circular molecule
- Circular DNA is more conveniently studied in plasmids
  - Plasmid: Autonomously replicating small circular DNA molecule

Topoisomers

- Closed circular molecules often have superhelical structures
  - Unwind linear DNA by two turns
  - Connect ends to recircularize

Topoisomers

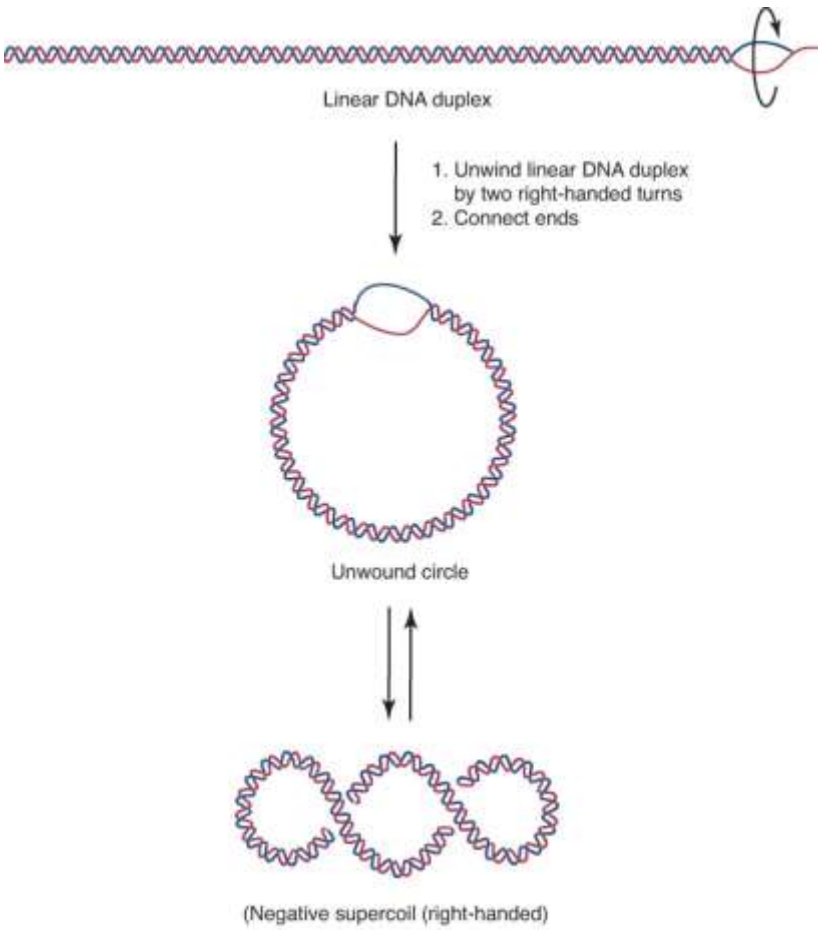


Figure 03.12: Formation of a negative supercoil.

Topoisomers

- Relaxed circle has no crossover points (nodes)
- Circular DNA with nodes is said to writhe
- Two identical circular DNA's with different degrees of supercoiling:
  - topological isomers or topisomers

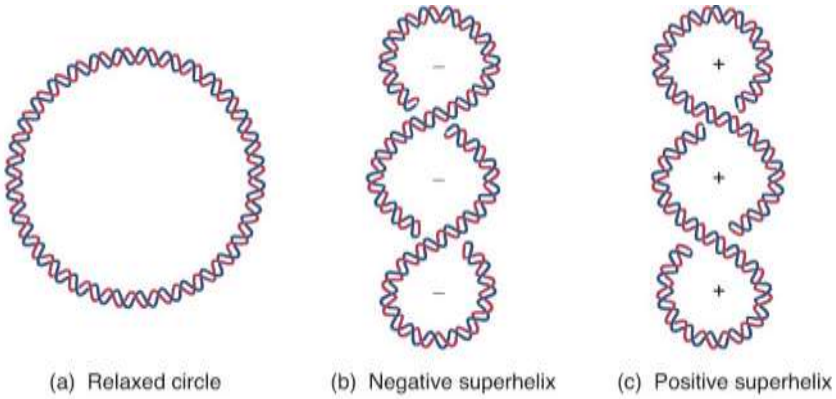


Figure 03.13: Topoismers. (a) Relaxed circle. (b) Negative superhelix. (c) Positive superhelix.

Topoisomers

- Most bacterial DNA molecules are slightly underwound
- Underwinds introduced by DNA gyrase
  - Introduces strain
  - Results in greater amounts of single stranded bubbles compared to relaxed circle DNA
    - Tend to be A-T rich sequences
    - Critical for transcription and replication

Non-B DNA Conformations

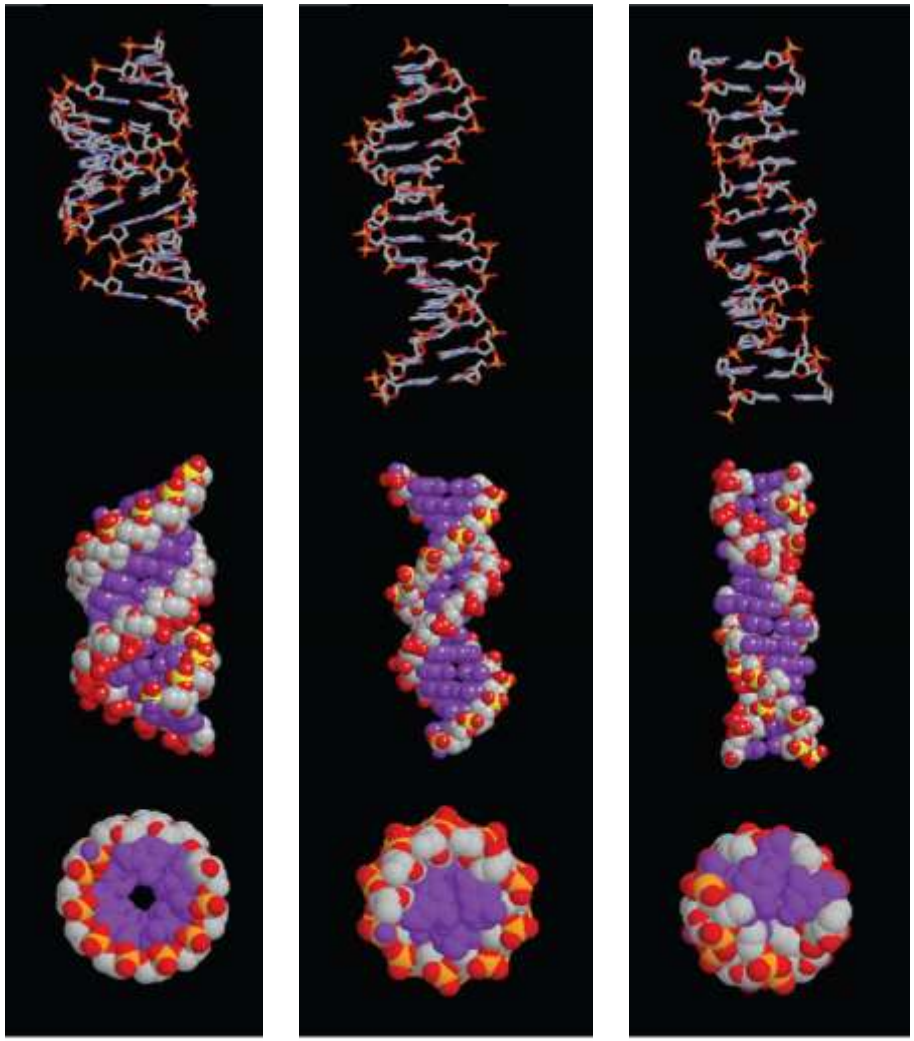
- B-DNA is predominant form in living systems
  - Right hand double helix
- A-DNA
  - Right hand double helix
- Z-DNA
  - Left hand double helix

Non-B DNA Conformations

TABLE 3.2 Comparison of Major Features in A-, B-, and Z-Forms of DNA

Parameter	A-DNA	B-DNA	Z-DNA
Helix sense	Right	Right	Left
Base pairs per turn	11	10.5	12
Axial rise per base pair (nm)	0.26	0.34	0.45
Base pair tilt (°)	20°	–6°	7°
Diameter of helix (nm)	2.3	2.0	1.8

Non-B DNA Conformations



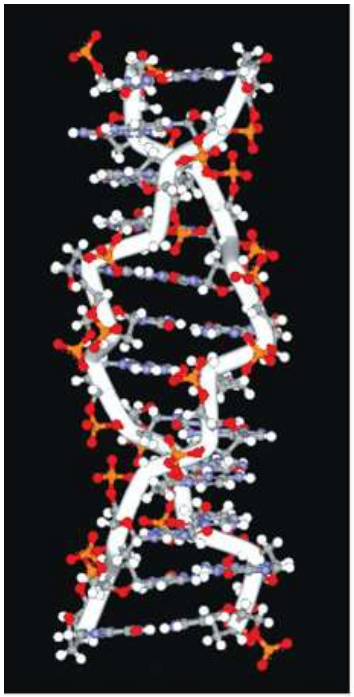
(a) A-DNA (b) B-DNA (c) Z-DNA

Figure 03.17: DNA conformations. (a) A-DNA, (b) B-DNA, and (c) Z-DNA.

(Top structures from Protein Data Bank 213D. B. Ramakrishnan and M. Sundaralingam, Biophys. J. 69 [1995]: 553–558. Prepared by B. E. Tropp; Middle structures from Protein Data Bank IBNA. H. R. Drew, et al., Proc. Natl. Acad. Sci. USA 78 [1981]: 2179–2183. Prepared by B. E. Tropp; Bottom structures from Protein Data Bank 2ZNA. A. H. -J. Wang, et al., Left-handed double helical DNA . . . Prepared by B. E. Tropp.)

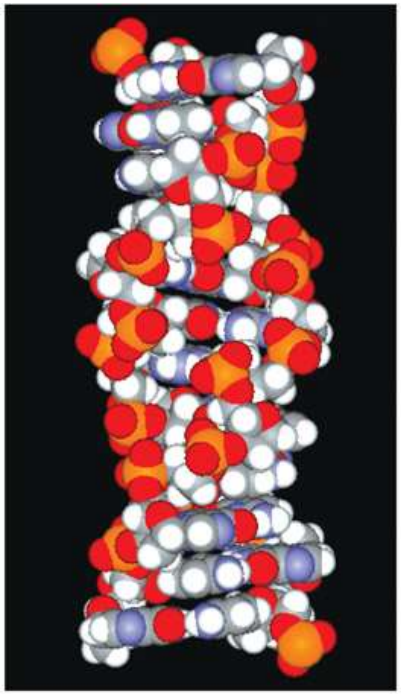
Non-B DNA Conformations

- Z-DNA
  - Formed with CA or TG repeats under high salt conditions (>2M)



(a) Z-DNA with zig-zag sugar phosphate backbone shown in white

Figure 03.18A: Z-DNA. Z-DNA with its zigzag backbones shown as white tubes. The bases, sugars, and phosphates are shown as ball and stick structures.



(b) The same Z-DNA with the zigzag sugar phosphate backbone shown in space filling display

Figure 03.18B: Z-DNA. The same Z-DNA shown in a space filling display.

(Structure from Protein Data Bank ID: 2ZNA. Wang, A. H. J., et al. Science.)

Non-B DNA Conformations

- Conditions for Z-DNA are never approached *in vivo* but:
  - Z-DNA can exist in localized chromosomal regions
    - Z-DNA binding proteins exist
      - Possible role in gene regulation
  - Potential hot spots for DNA double stranded breaks

RNA Structure

- Like protein structure
  - Primary RNA structure
    - base sequence
  - Secondary RNA structure
    - Watson-Crick base pairing

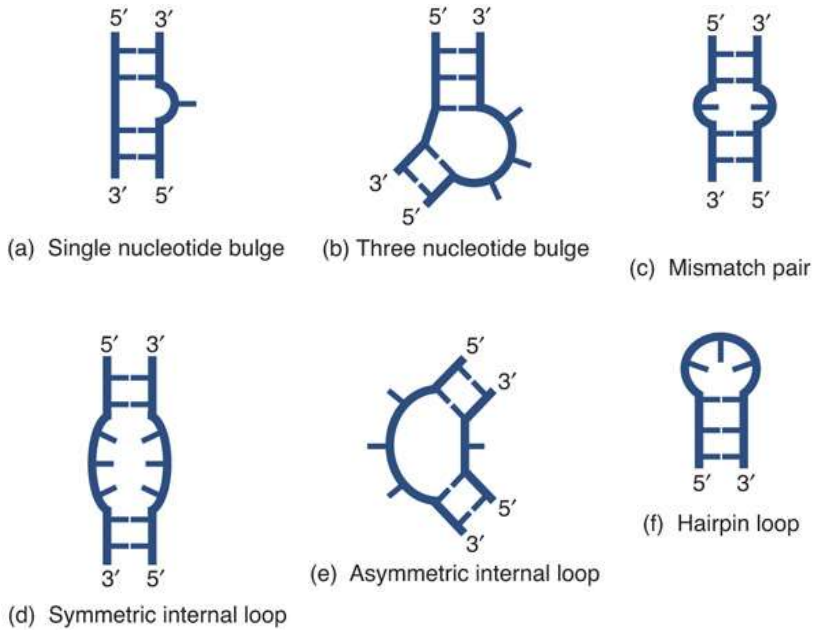


Figure 03.22: RNA loop and bulge secondary elements. (a) single nucleotide bulge, (b) three nucleotide bulge, (c) Mismatch pair, (d) symmetric internal look, (e) asymmetric internal loop, (f) Hairpin loop.

(Slightly modified from Nowakowski, J., and Tinoco, I. Jr. 1997. RNA structure and stability. Semin Virol 8:153–165.)

RNA Structure

- RNA tertiary structure
  - Three dimensional structure between 2 or more secondary structures
    - Pseudoknot
    - Kissing hairpins

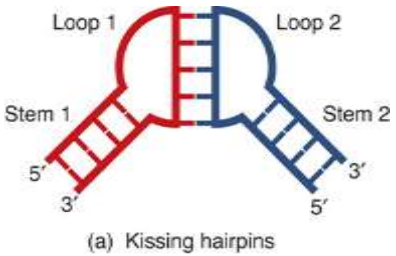


Figure 03.25A: Interactions that bring distant RNA segments together. (a) Kissing hairpin interaction.

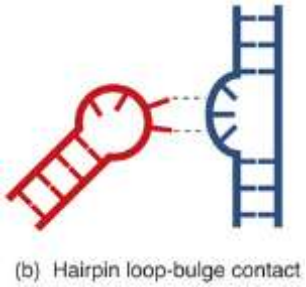


Figure 03.25B: Interactions that bring distant RNA segments together. (b) Hairpin loopbulge interaction.

(Slightly modified from Nowakowski, J., and Tinoco, I. Jr. 1997. RNA structure and stability. Semin Virol 8:153–165.)