# Johns Hopkins Engineering

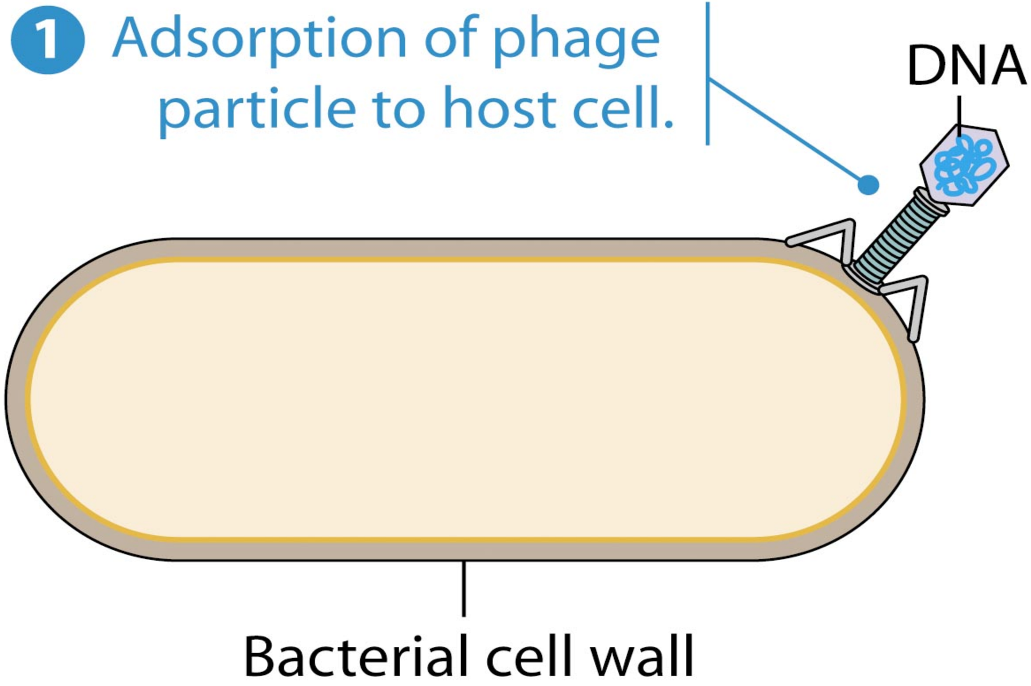
## Molecular Biology 585.407

DNA, Chromosomes, and the Nucleus," Part 2

# Information: DNA, Chromosomes, and the Nucleus
# Part 2

# Skepticism in the science community

- Though the experiments of Avery and his colleagues were rigorous, the assignment of a genetic role to DNA did not meet with immediate acceptance.
- Skepticism was due in part to the persistent, widespread conviction that DNA **lacked the necessary complexity** for such a role.
- Scientists questioned whether genetic information in bacteria had anything to do with heredity in other organisms.
- Most remaining doubts were alleviated eight years later when DNA was shown to be the genetic material of a virus, the bacteriophage T2.




(a) Phage replication



Figure 18A-2a

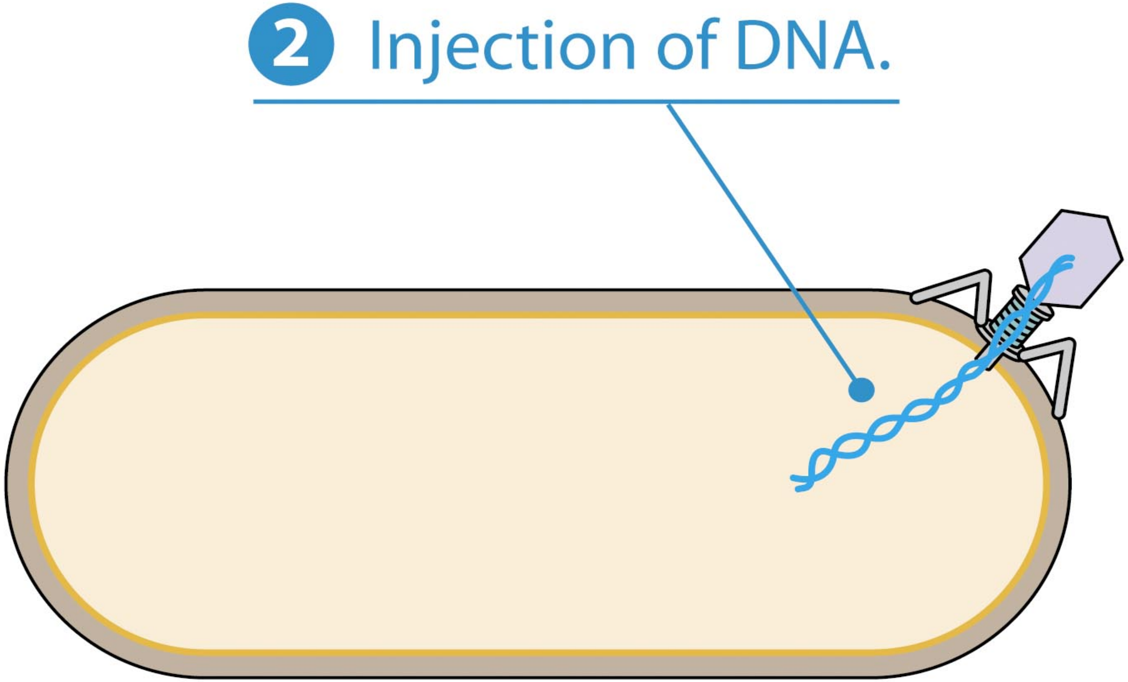







Figure 18A-2b

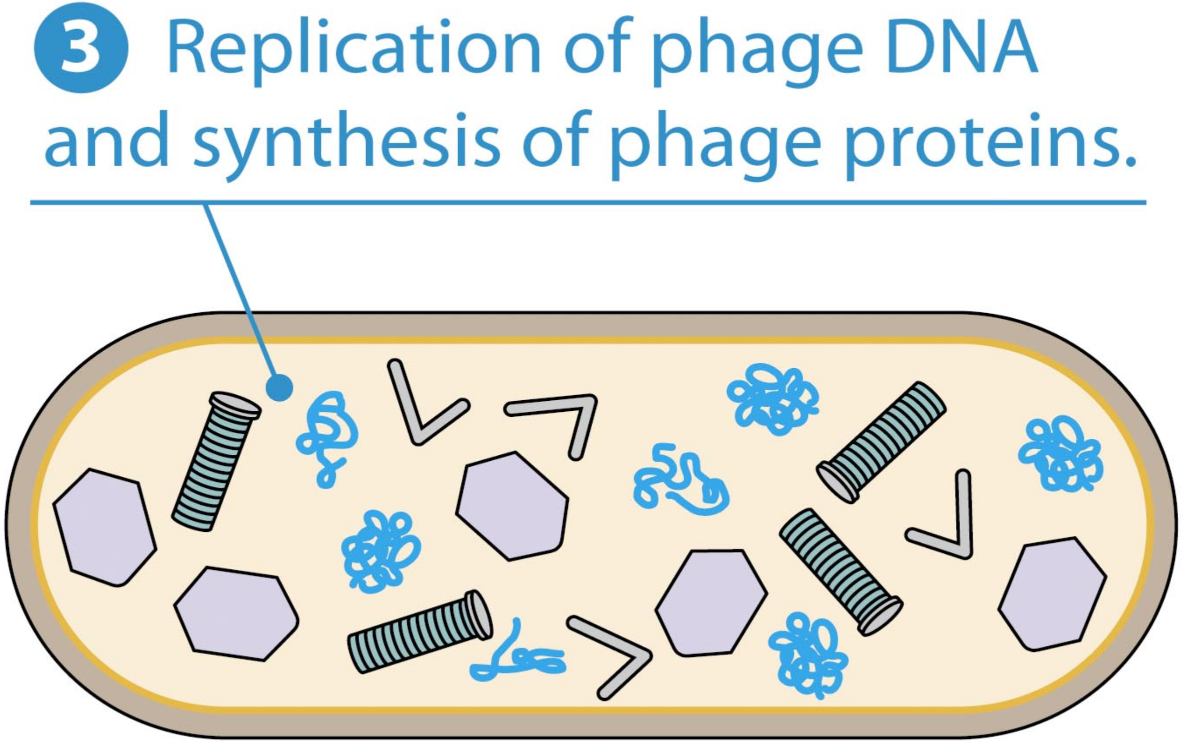





Figure 18A-2c

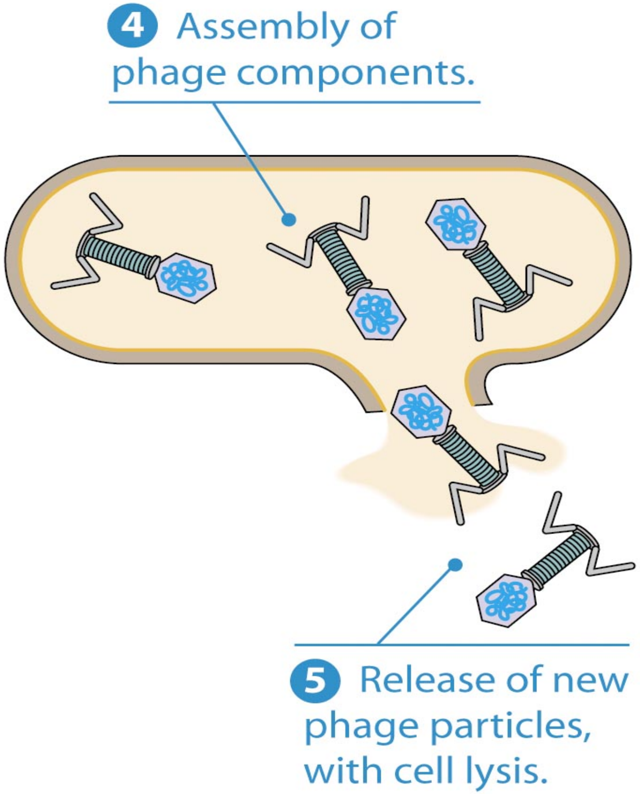





Figure 18A-2d

# Hershey and Chase showed that DNA is the genetic material of viruses

- **Bacteriophages or phages** are viruses that infect bacteria. They have been objects of scientific study since the 1930s, and much of our early understanding of molecular genetics came from experiments involving these viruses. Box 18A describes the anatomy and replication cycle of some phages and highlights their advantages for genetic studies.

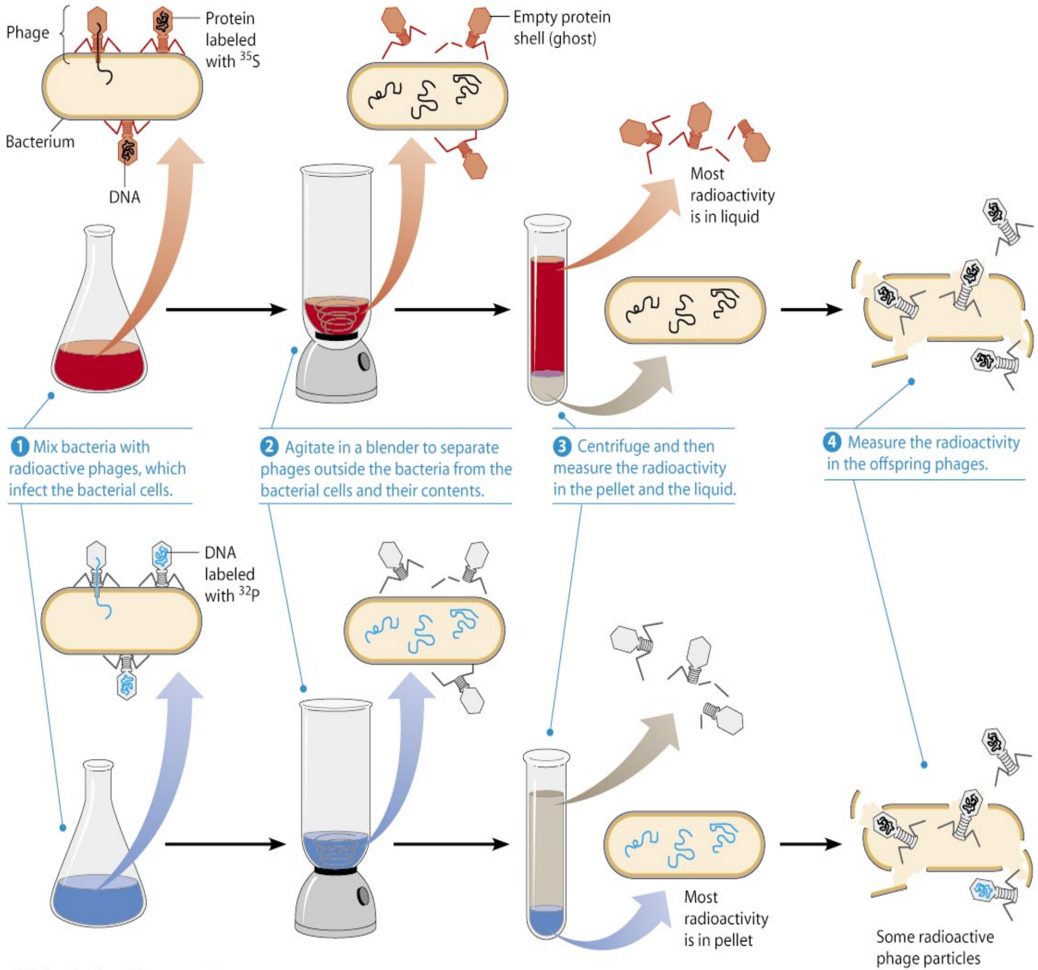
- One of the most thoroughly studied of the phages that infect bacterium *Estherichia coli is* bacteriophage T2. During infection, this virus attaches to the bacterial cell surface and injects material into the cell. Shortly thereafter, the bacterial cell begins to produce thousands of new copies of the virus.

- This scenario suggests that material injected into the bacterial cell carries the genetic information that guides the production of the virus. What is the chemical nature of the injected material?

- In 1952 Alfred Hershey and Martha Chase designed an experiment to address this question. Only two possibilities exist because the T2 virus is constructed from only two kinds of molecules: DNA and protein.

- To distinguish between these two alternatives, Hershey and Chase took advantage of the fact that the proteins of the T2 virus, like most proteins, contain the element sulfur (in the amino acids methionine and cysteine) but not phosphorus, while the viral DNA contains phosphorus (in its sugarphosphate backbone) but not sulfur.

- Hershey and Chase prepared two batches of T2 phage particles (as intact phages are called) with different kinds of radioactive labeling. In one batch, t phage proteins were labeled with the radioactive isotope $^{35}S$; in the other batch, the phage DNA was labeled with the isotope $^{32}P$.




(a) The Hershey-Chase experiment



Figure 18-3a

# Chargaff's rules

- Comparison of such data revealed to Chargaff that DNA preparations from closely related species have similar base compositions, whereas those from very different species tend to exhibit quite different base compositions. Again, this is what would be expected of a molecule that stores genetic information.

- But Chargaff's **most striking observation** was his discovery that for all DNA samples examined, the **number of adenines is equal to the number of thymines** (A = T), and the **number of guanines is equal to the number of cytosines** (G = C).

- This meant that the number of purines is equal to the number *of* pyrimidines (A + T = C + G).

- The significance of these equivalencies, known as **Chargaff's rules,** was an enigma and remained so until the double-helical model of DNA was established by Watson and Crick in 1953.

**Table 18-1** DNA Base Composition Data That Led to Chargaff's Rules

| | Number of Each Type of Nucleotide* | | | | Nucleotide Ratios** | | |
|---|---|---|---|---|---|---|---|
| **Source of DNA** | **A** | **T** | **G** | **C** | **A/T** | **G/C** | **(A + T)/(G + C)** |
| Bovine thymus | 28.4 | 28.4 | 21.1 | 22.1 | 1.00 | 0.95 | 1.31 |
| Bovine liver | 28.1 | 28.4 | 22.5 | 21.0 | 0.99 | 1.07 | 1.30 |
| Bovine kidney | 28.3 | 28.2 | 22.6 | 20.9 | 1.00 | 1.08 | 1.30 |
| Bovine brain | 28.0 | 28.1 | 22.3 | 21.6 | 1.00 | 1.03 | 1.28 |
| Human liver | 30.3 | 30.3 | 19.5 | 19.9 | 1.00 | 0.98 | 1.53 |
| Locust | 29.3 | 29.3 | 20.5 | 20.7 | 1.00 | 1.00 | 1.41 |
| Sea urchin | 32.8 | 32.1 | 17.7 | 17.3 | 1.02 | 1.02 | 1.85 |
| Wheat germ | 27.3 | 27.1 | 22.7 | 22.8 | 1.01 | 1.00 | 1.19 |
| Marine crab | 47.3 | 47.3 | 2.7 | 2.7 | 1.00 | 1.00 | 17.50 |
| *Aspergillus* (mold) | 25.0 | 24.9 | 25.1 | 25.0 | 1.00 | 1.00 | 1.00 |
| *Saccharomyces cerevisiae* (yeast) | 31.3 | 32.9 | 18.7 | 17.1 | 0.95 | 1.09 | 1.79 |
| *Clostridium* (bacterium) | 36.9 | 36.3 | 14.0 | 12.8 | 1.02 | 1.09 | 2.73 |

*The values in these four columns are the average number of each type of nucleotide found per 100 nucleotides in DNA.
**The A/T and G/C ratios are not all exactly 1.00 because of experimental error.





Table 18-1

# Chargaff's rules

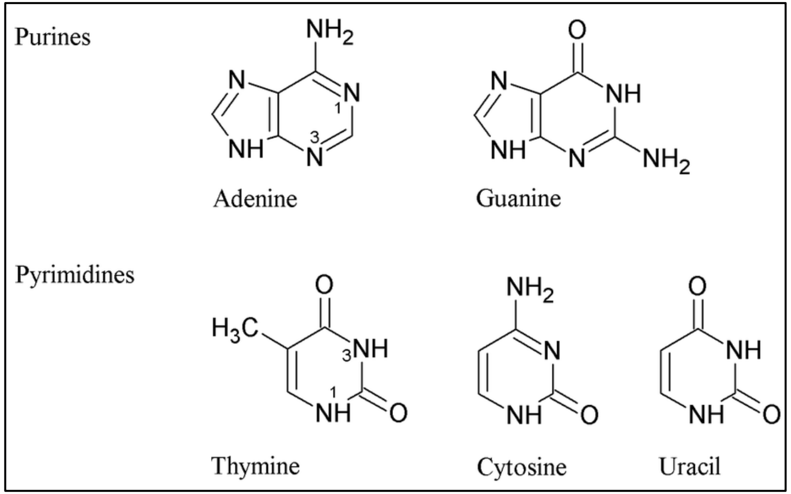
**First rule**

- A double-stranded DNA molecule globally has percentage base pair equality: %A = %T and %G = %C. Validation of the rule constitutes the basis of Watson-Crick pairs in the DNA double helix model.

**Second rule**

- Both %A = %T and %G = %C are valid for each of the two DNA strands.

# DNA structure



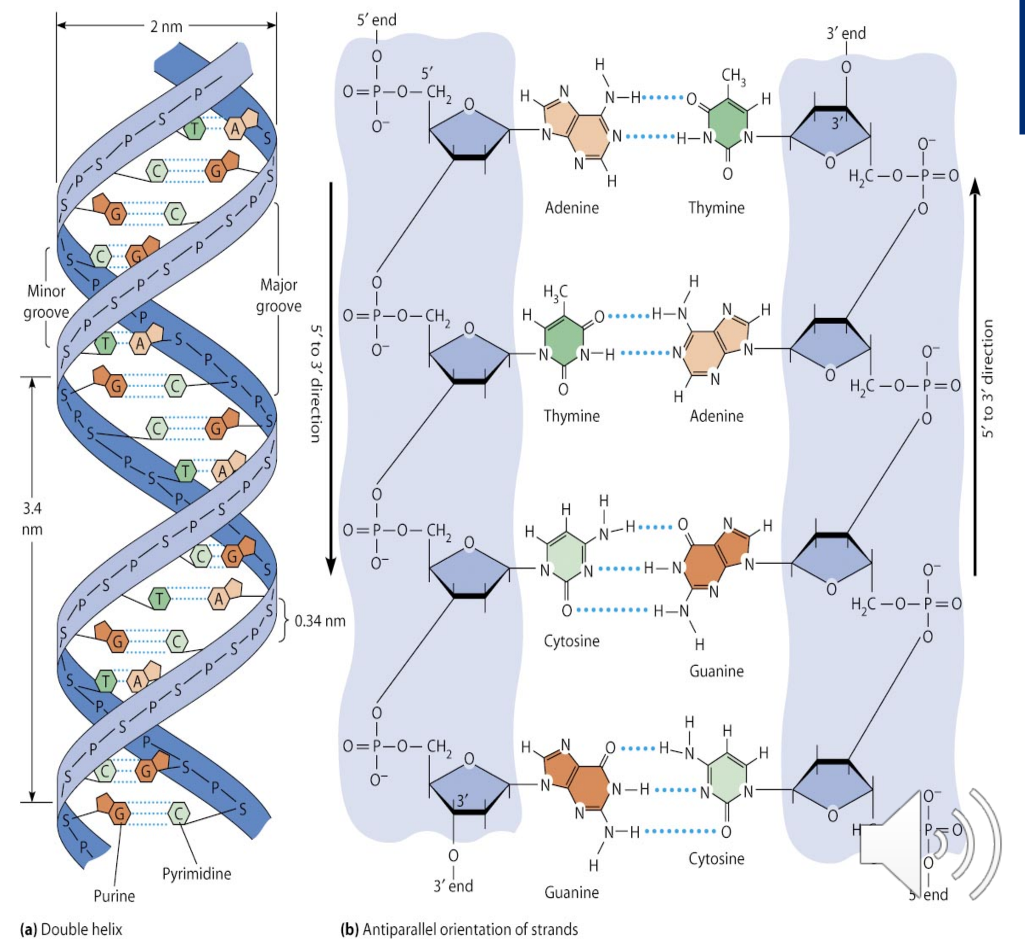
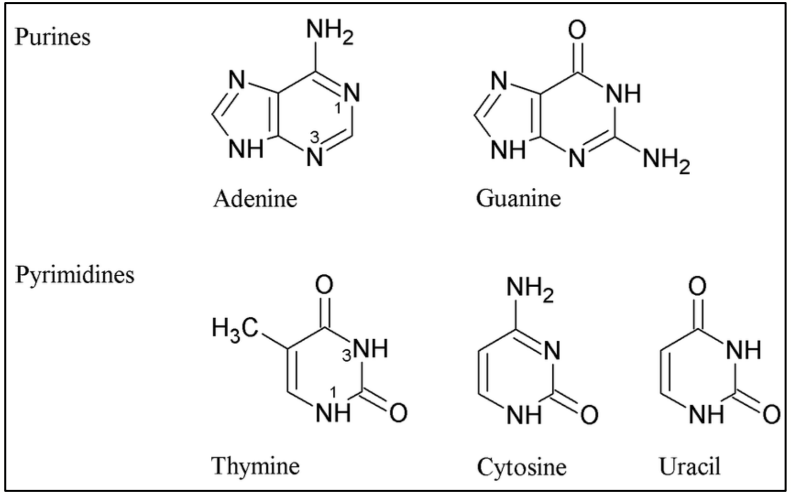
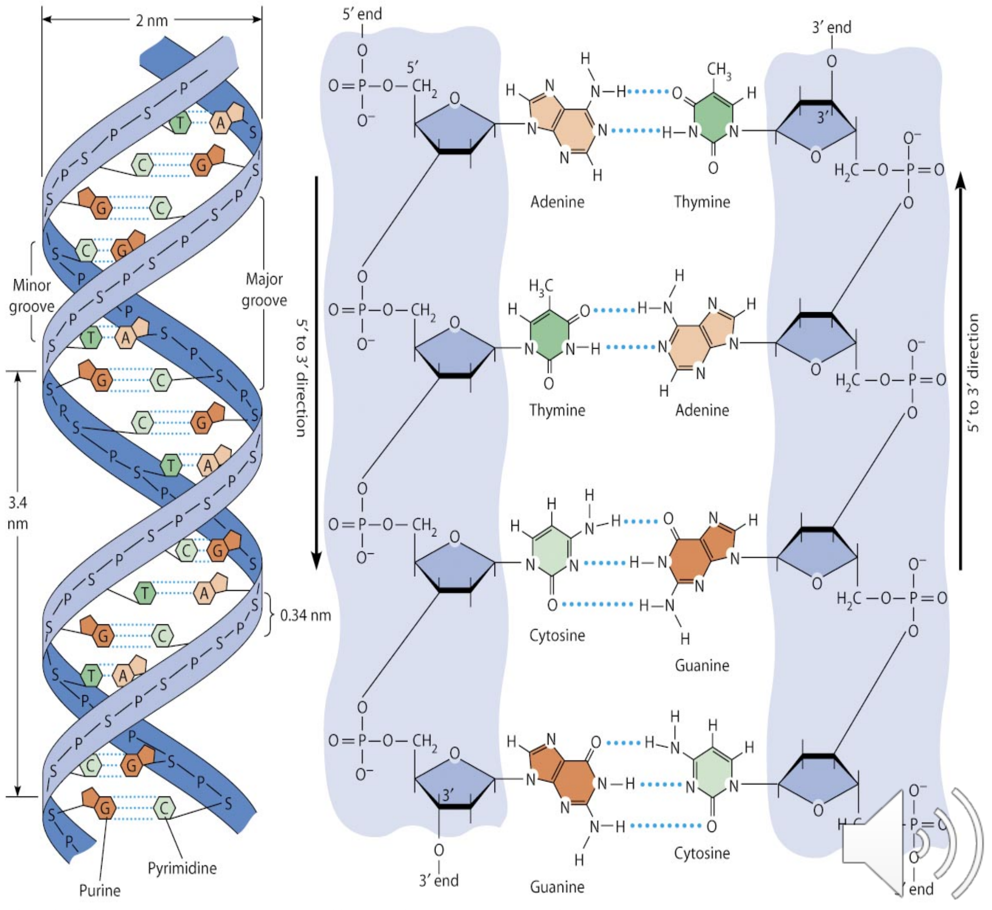






Figure 18-4

# DNA structure



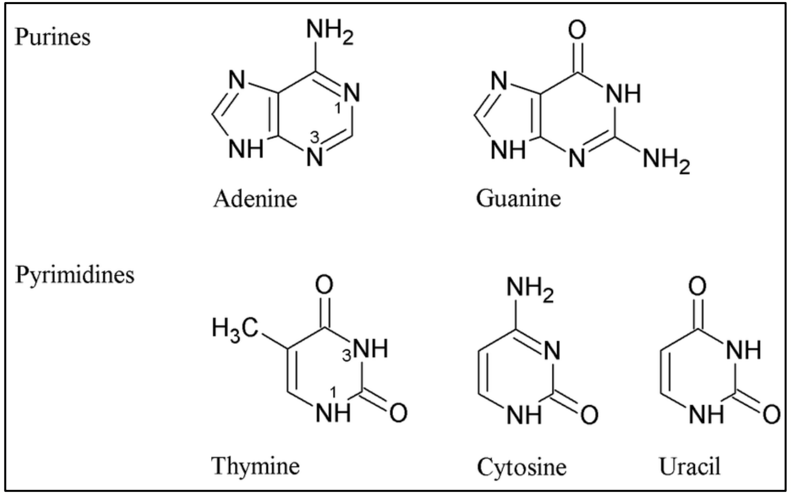




(a) Double helix
(b) Antiparallel orientation of strands



Figure 18-4

# DNA structure



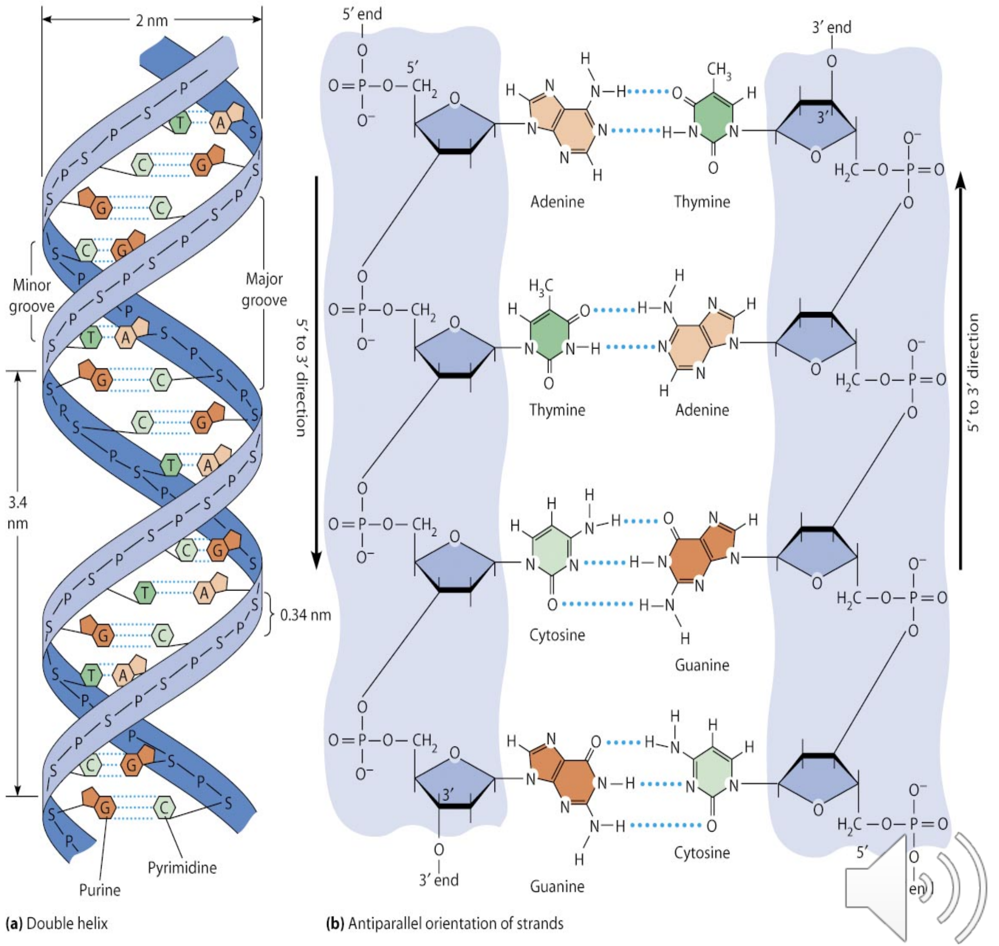






Figure 18-4

# DNA structure

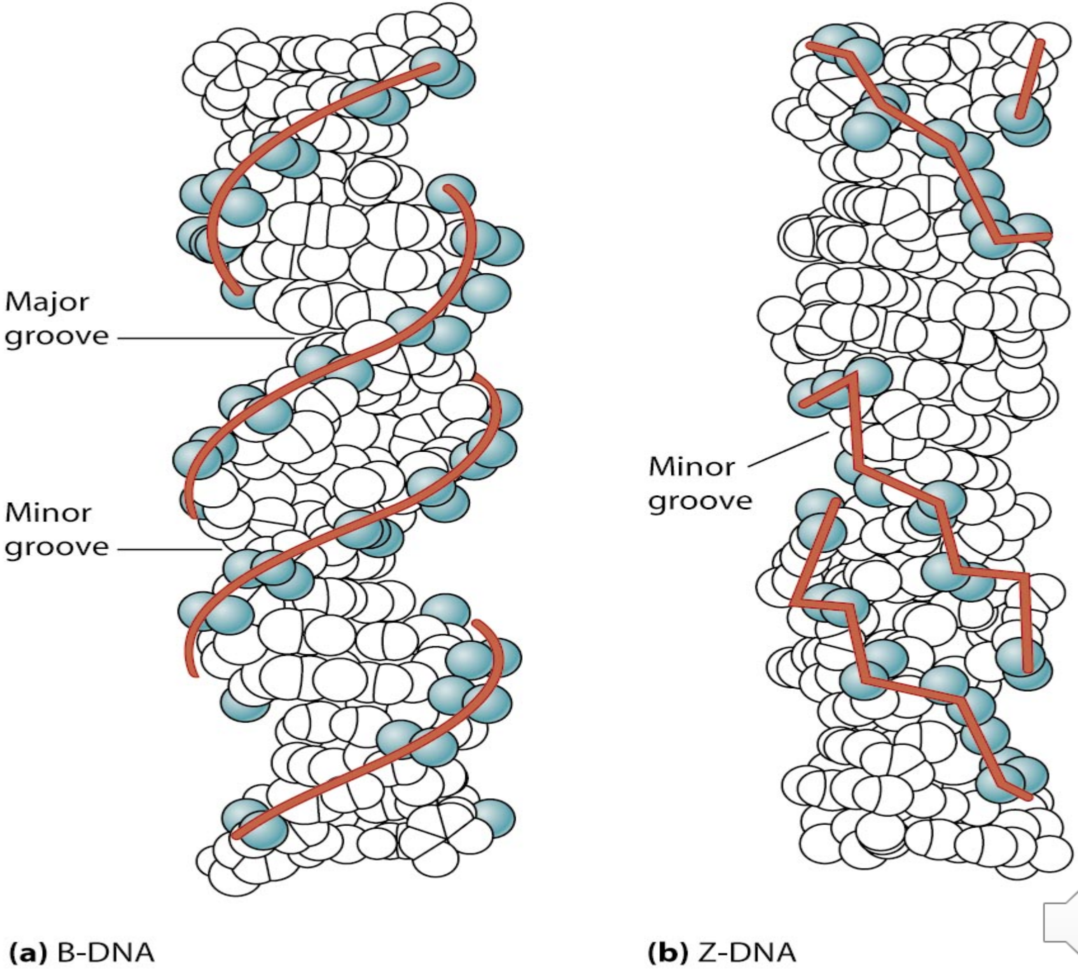





Figure 18-5

# DNA can be interconverted between relaxed and supercoiled forms

- DNA double helix can be twisted upon itself to form **supercoiled DNA**.
- Circular DNA molecules are also found in viruses, bacteria, mitochondria, and chloroplasts.

# Supercoiling helps to make the DNA more compact

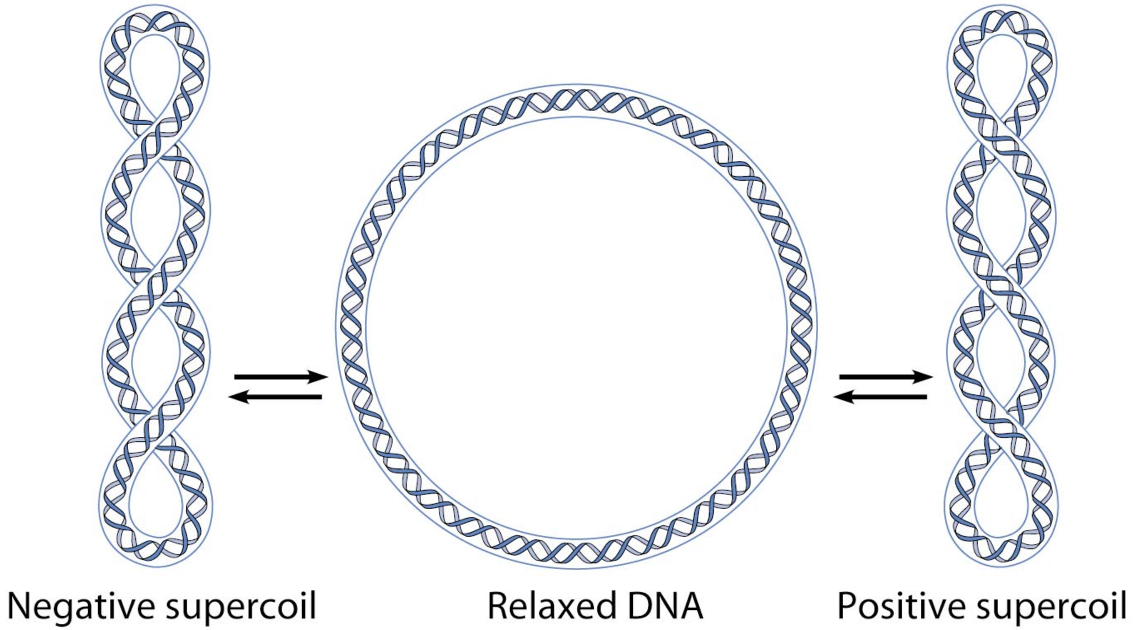
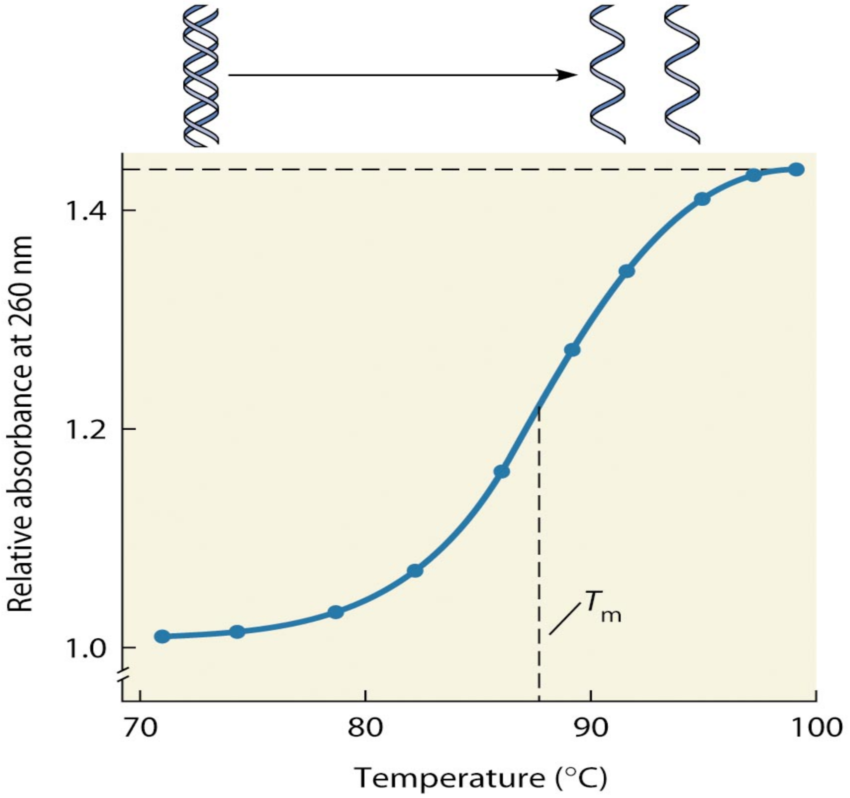





Figure 18-6a



1.4
1.2
1.0
Relative absorbance at 260 nm
$T_m$
70
80
90
100
Temperature (°C)






**Figure 18-8**

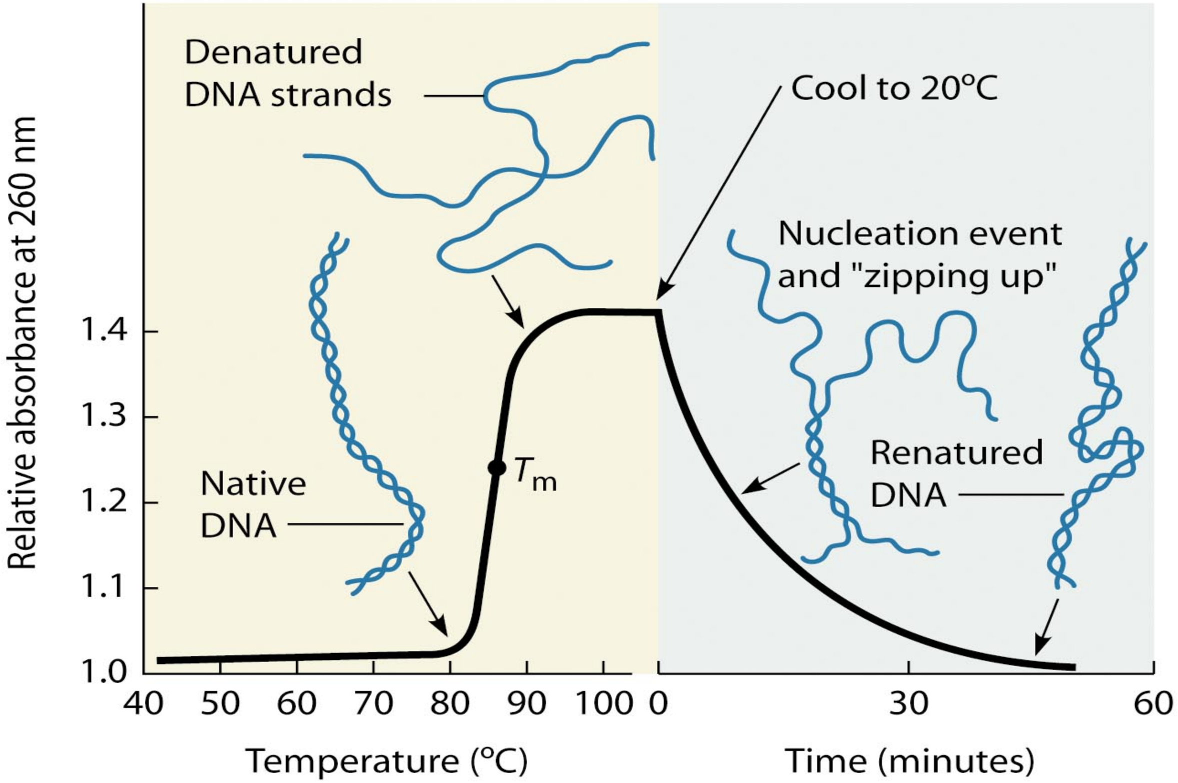

Denatured
DNA strands
Cool to 20°C
Nucleation event
and "zipping up"
Native
DNA
$T_m$
Renatured
DNA
Relative absorbance at 260 nm
1.4
1.3
1.2
1.1
1.0
40 50 60 70 80 90 100 0 30 60
Temperature (°C)
Time (minutes)




**Figure 18-10**

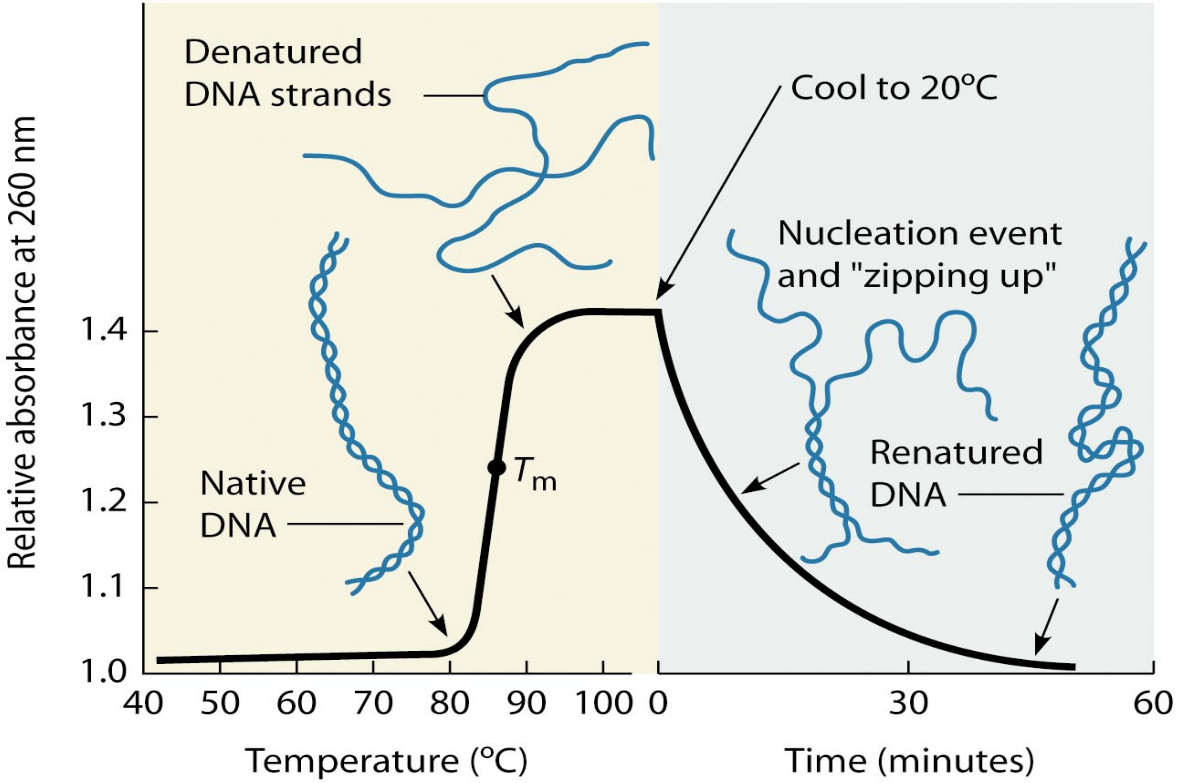

Denatured
DNA strands
Cool to 20°C
Nucleation event
and "zipping up"
Native
DNA
Renatured
DNA
$T_m$
Relative absorbance at 260 nm
1.4
1.3
1.2
1.1
1.0
40 50 60 70 80 90 100 0 30 60
Temperature (°C)
Time (minutes)



**Figure 18-10**