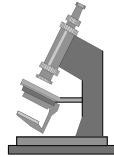


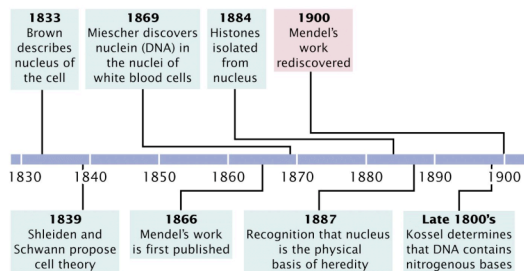
## DNA structure



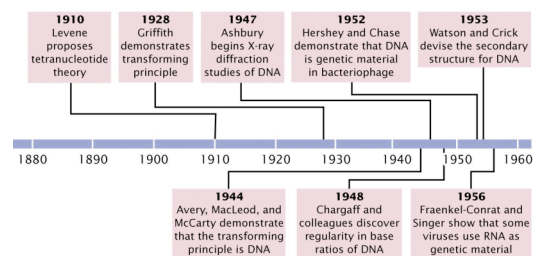
## Iceman DNA (Sept. 19, 1991)



## History



## History



## History

- ✓ 1869 Miescher Discovers nuclein
  - ⇒ Isolated nuclei from pus
  - ⊃ High in phosphates and slightly acidic
  - ⊃ Later renamed nucleic acid
- ✓ 1887 Nucleus contains the heredity material
  - ⇒ Protein or nucleic acid?
  - ⇒ Kossel (late 1800s)
    - ⊃ DNA contains four nitrogenous bases
      - ⇒ Adenine, cytosine, guanine, and thymine

## History

- ✓ 1910 Levene
  - ⇒ DNA consists of linked nucleotides
  - ⊃ Each consists of sugar, phosphate, and a base
  - ⊃ Proposed DNA consisted of chain of the four nucleotides in a fixed order
  - ⊃ This did not allow for variability - Many thought it must be protein because there were 20 amino acids
- ✓ 1948 Chargoff
  - ⇒ Discovered the base ratios of DNA

## Ratio of DNA bases?

### ✓ Tetranucleotide hypothesis

⇒ DNA is a tetramer of equal quantities of bases - 1:1:1:1 ratio

### ✓ Chargaff's rule

⇒ A 1:1 correspondence exists between the purine and pyrimidine bases

Species	Adenine	Thymine	Guanine	Cytosine
Human being (liver)	30.3%	30.3%	19.5%	19.9%
Mycobacterium tuberculosis	15.1%	14.6%	34.9%	35.4%

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Table 14.1 Chargaff's Analysis of DNA Nucleotide Base Compositions

Organism	Base Composition (Mole Percent)			
	A	T	G	C
<i>Escherichia coli</i> (K12)	26.0	23.9	24.9	25.2
<i>Mycobacterium tuberculosis</i>	15.1	14.6	34.9	35.4
Yeast	31.3	32.9	18.7	17.1
Herring	27.8	27.5	22.2	22.6
Rat	28.6	28.4	21.4	21.5
Human	30.9	29.4	19.9	19.8

Table 10.1 Base composition of DNA from different sources and ratios of bases

Source of DNA	A	T	G	C	Ratio		
					A/T	G/C	A + G/T + C
<i>E. coli</i>	26.0	23.9	24.9	25.2	1.09	0.99	1.04
Yeast	31.3	32.9	18.7	17.1	.95	1.09	1.00
Sea urchin	32.8	32.1	17.7	18.4	1.02	.96	1.00
Rat	28.6	28.4	21.4	21.5	1.01	1.00	1.00
Human	30.3	30.3	19.5	19.9	1.00	0.98	0.99

## Nature of DNA

### ✓ Protein?

⇒ Before 1944

- ↳ abundant & explained variability
- ↳ Tetranucleotide hypothesis
  - ⇒ Little variability and to simple
- ↳ Active research at time

### ✓ Nucleic acid

⇒ Transforming principle

- ↳ Avery, Macleod, and McCarty that followed the work of Griffith's experiments

## Smooth versus rough colonies of *Streptococcus (Diplococcus) pneumoniae*

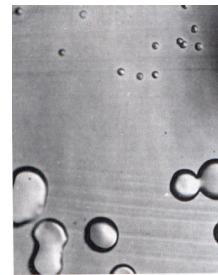


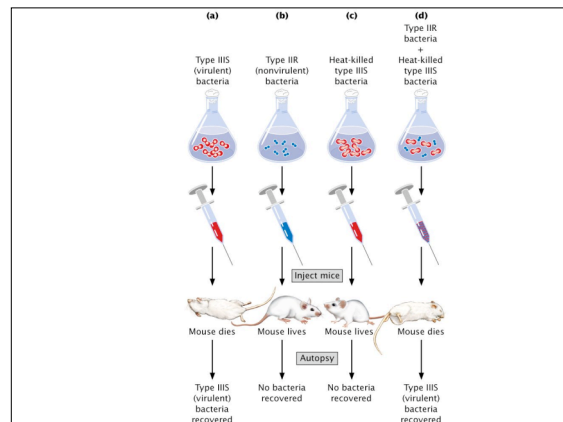
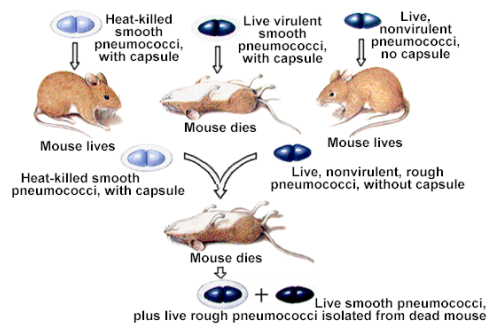
TABLE 10.1

STRAINS OF *DIPLOCOCCUS PNEUMONIAE* USED BY FREDERICK GRIFFITH IN HIS ORIGINAL TRANSFORMATION EXPERIMENTS

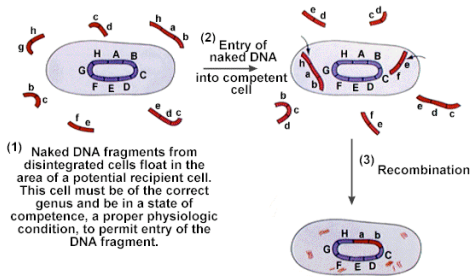
Serotype	Colony Morphology	Capsule	Virulence
IIR	Rough	Absent	Avirulent
IIIS	Smooth	Present	Virulent

FIGURE 14.5 Transformation of *Streptococcus*. This photo, from the original publication of Avery and coworkers, shows the transformation of nonpathogenic R *Streptococcus* (the small colonies) to pathogenic S *Streptococcus* (the large colonies) in an extract prepared from heat-killed S *Streptococcus*.

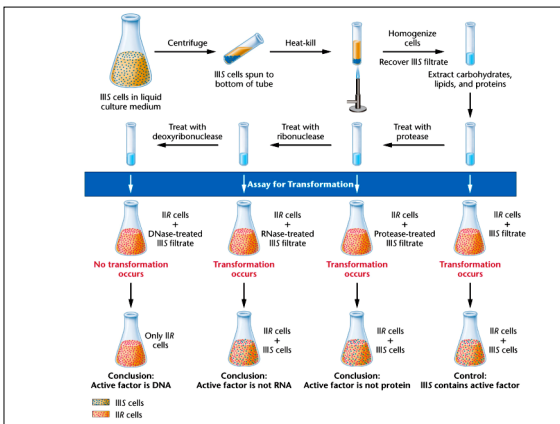
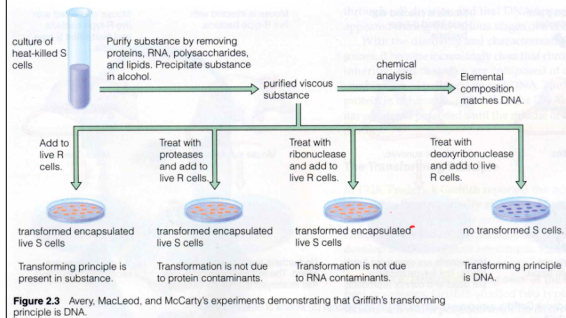
## Griffith's experiment (1928)



## Transformation with recombination



## Avery, Macleod, and McCarty's experiments



## DNA is the Genetic material in bacteriophages

✓ 1952 Hershey and Chase

⇒ Used T2 phage

⇒ Infects *Escherichia coli*

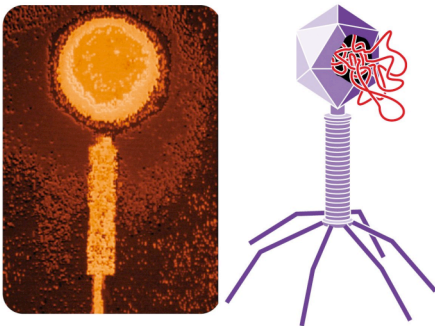
↳ Radioactive isotopes

↳  $^{35}\text{S}$  found in proteins

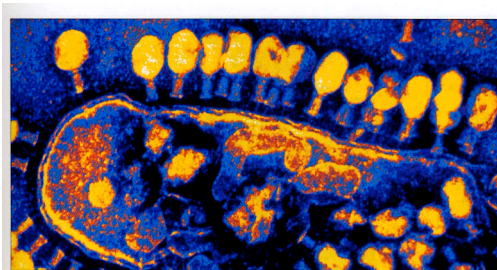
↳  $^{32}\text{P}$  found in DNA

## T even phage

(a)

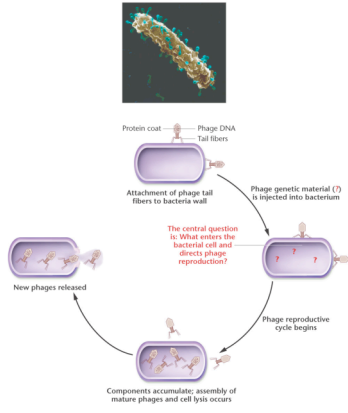


## T2 phages

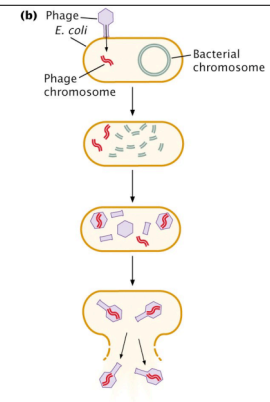


a The micrograph shows T2 bacteriophages infecting an *E. coli* cell.

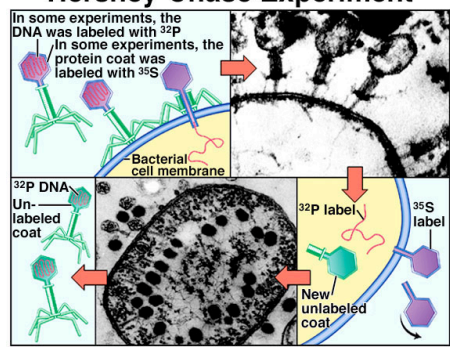
What was the genetic material that directed the synthesis of new viruses



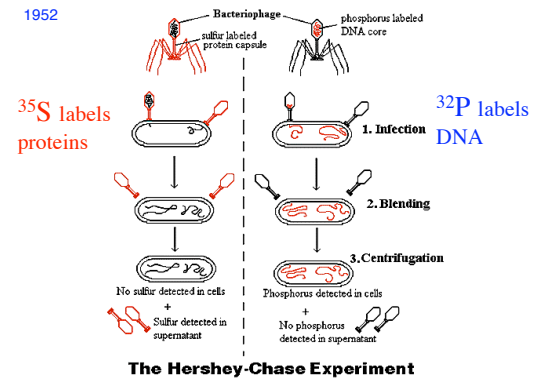
Lytic phage life cycle



Hershey-Chase Experiment



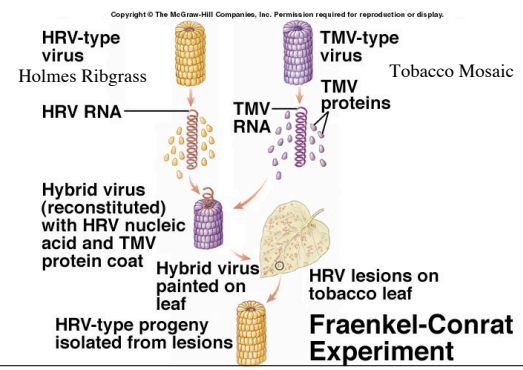
1952



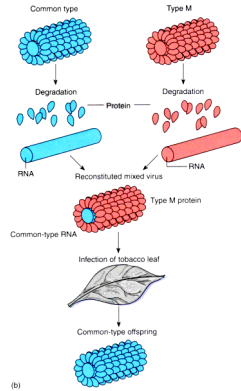
RNA as a Genetic material

- ✓ 1956 Fraenkel-Conrat and Singer
- ⇒ Mixed two different types of viruses
- ⇒ RNA, not protein determined the virus structure

RNA determined lesions



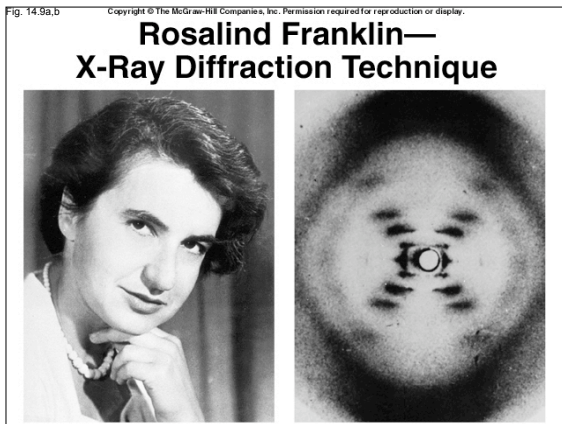
RNA is the genomic material of the TMV, RNA determined type of lesions



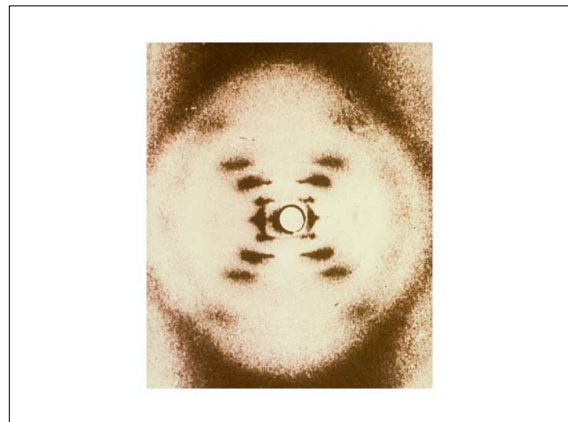
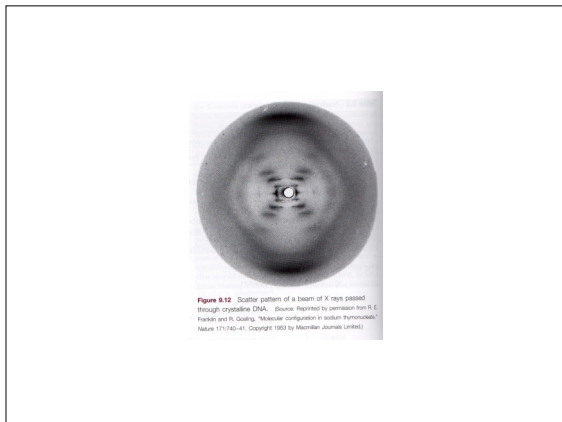
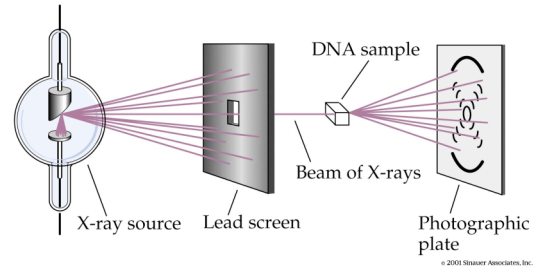
## Other evidence that DNA is the Genetic material

### ✓ Indirect

- ⇒ DNA is found primarily where genetic function is found
  - DNA and protein (protein found elsewhere)
  - DNA in mitochondria and chloroplasts
  - Correlation of ploidy and DNA (not protein)
- ⇒ UV mutagenesis
  - DNA absorbs best at 260 nm and most mutagenic
  - Protein absorbs best at 280 nm and not significantly mutagenic



## X-ray diffraction



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### DNA Double-Helix Model (1)

Rosalin Franklin

DNA - double helix  
Repeating structure  
1-Turn every 3.4 nm

The diagram illustrates a right-handed DNA double helix. The two strands are antiparallel, with one running 5' to 3' and the other 3' to 5'. The distance between two stacked nitrogenous bases is 3.4 nm. The helix has a major groove and a minor groove. The overall width of the double helix is 2 nm.

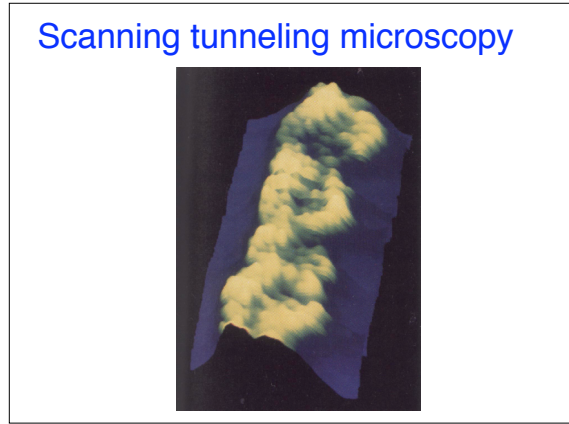


Fig. 14.10b Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.

### Watson-Crick DNA Structure

### Watson & Crick DNA structure

Right-handed double helix  
Antiparallel  
Bases are stacked - 3.4 Å  
Nitrogenous bases are H-bonded  
One complete turn every 10 bases or 34 Å  
Major groove and minor groove  
Double helix measures 20 Å in diameter

The diagram shows a right-handed DNA double helix. The major groove is the wider of the two grooves, and the minor groove is the narrower. The distance between stacked bases is 3.4 Å. One full rotation of the helix occurs every 10 base pairs, which is 34 Å. The overall diameter of the double helix is 20 Å.

Fig. 14.7 Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.

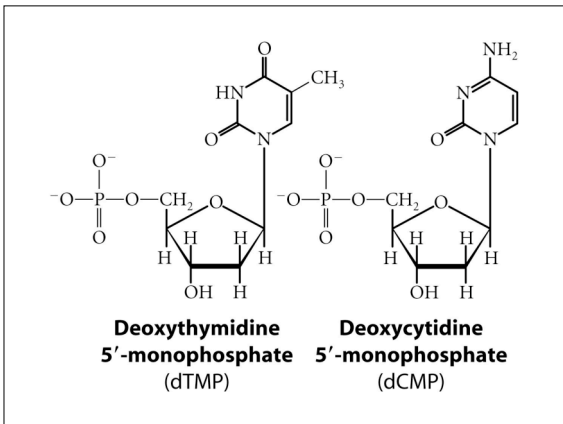
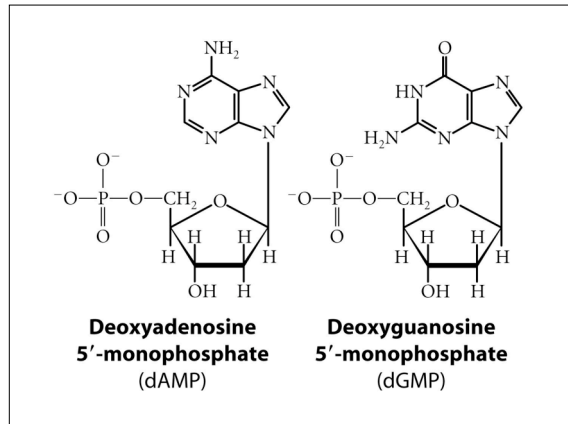
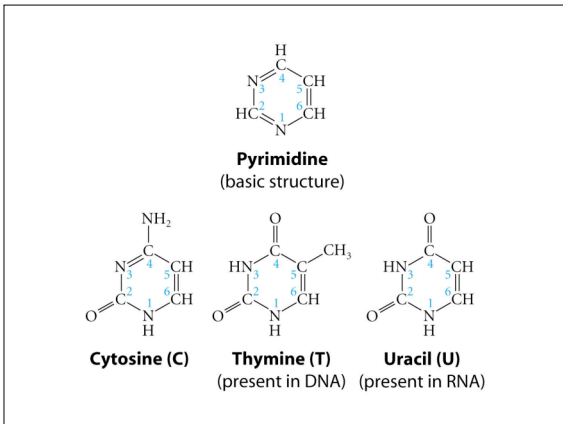
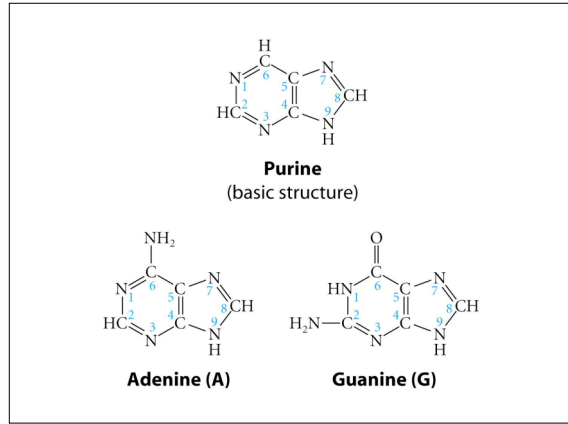
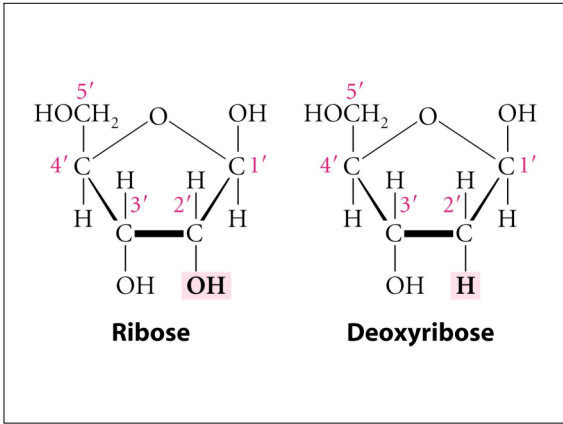
### Numbering Carbon Atoms in a Nucleotide

The diagram shows a nucleotide consisting of a phosphate group (PO<sub>4</sub>), a five-carbon sugar, and a nitrogenous base. The carbon atoms in the sugar are numbered 1' through 5'. The phosphate group is attached to the 5' carbon. The base is attached to the 1' carbon. The 2' and 3' carbons of the sugar are also shown, with a hydroxyl group (OH) at the 3' position.

### Nucleosides vs. nucleotides

The diagram shows the chemical structures of two nucleosides and their corresponding nucleotides. Uridine is a nucleoside consisting of a pyrimidine base and a ribose sugar. Deoxyadenylic acid is a nucleoside consisting of a purine base and a deoxyribose sugar. The nucleotides are formed by the addition of a phosphate group to the 5' carbon of the sugar.

Ribonucleosides	Ribonucleotides
Adenosine	Adenylic acid
Cytidine	Cytidylic acid
Guanosine	Guanylic acid
Uridine	Uridylic acid
Deoxyribonucleosides	Deoxyribonucleotides
Deoxyadenosine	Deoxyadenylic acid
Deoxycytidine	Deoxycytidylic acid
Deoxyguanosine	Deoxyguanylic acid
Deoxythymidine	Deoxythymidylic acid



**Building blocks of DNA**

**Sugar**  
Ribose                      Deoxyribose

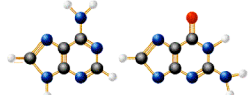
**Base**  
Adenine                      Guanine

**Purines**  
Cytosine                      Thymine                      Uracil

## Bases of nucleotides

### Nucleotides

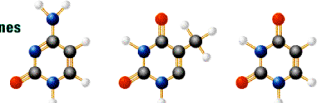
#### Purines



adenine (A)

guanine (G)

#### Pyrimidines



cytosine (C)

thymine (T)  
(only in DNA)

uracil (U)  
(only in RNA)

## H-bonding -- Base pairing

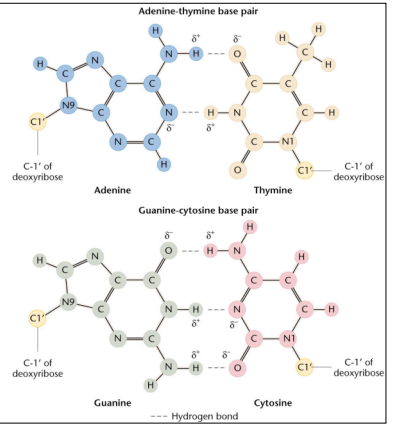
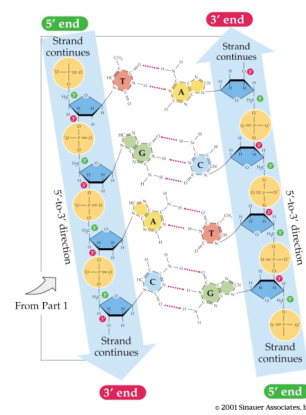
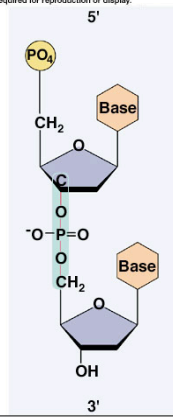
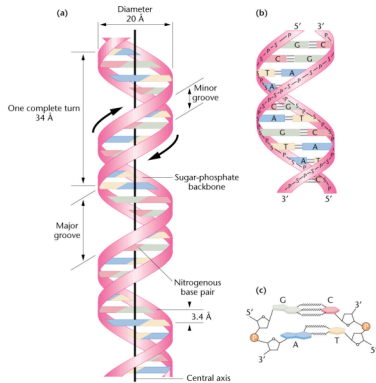


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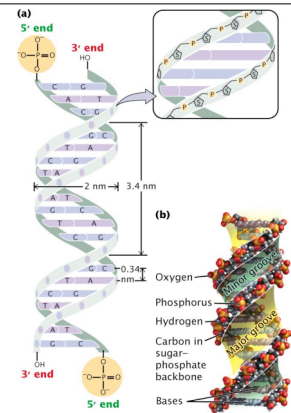
## Phosphodiester Bond

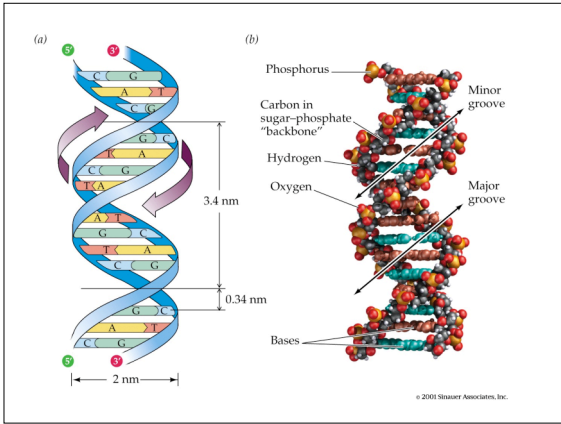


## Structure of DNA



## 2° structure of DNA

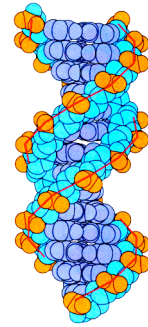




## B-DNA

Right handed helix  
 Diameter - 20 Å  
 Base pairs/turn - 10  
 Helix pitch - 34 Å  
 Helix rise/bp - 3.4 Å  
 36° twist

Most DNA



■ B-DNA model with a line superimposed to show the smoothness of the double helix.

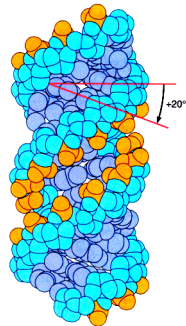
## A-DNA

Right handed helix  
 Diameter - 26 Å  
 bp/turn - 11  
 Helix pitch - 28 Å  
 Helix rise/bp - 2.6 Å  
 32.7° twist

Not normally found in living cells

Less water - High humidity

RNA-DNA hybrid and RNA-RNA helices



■ A-DNA model. The bases tilt at a greater angle than in B-DNA, and the right handed double helix is wound less tightly.

## Z-DNA

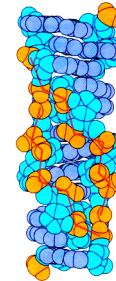
Left handed helix  
 Diameter - 18 Å  
 bp/turn - 12  
 Helix pitch - 45 Å  
 Helix rise/bp - 3.7 Å  
 -30° twist

Limited regions of Z-DNA in living cells

Alternating purine-pyrimidine (Gs & Cs)

Bases form a zig-zag

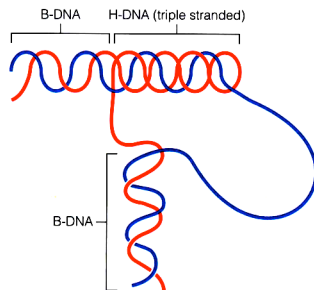
Gene regulation



■ Z-DNA model. The double helix is left-handed, and the molecule is narrower than B-DNA. A superimposed line shows that the sugar-phosphate backbones of Z-DNA form a helix that zigzags, in contrast to the smooth helix of B-DNA.

## H-DNA

Test tube  
 Recombination?  
 DNA repair?



■ Rearrangement of B-DNA to form the triple helix of H-DNA.

Fig. Nc.

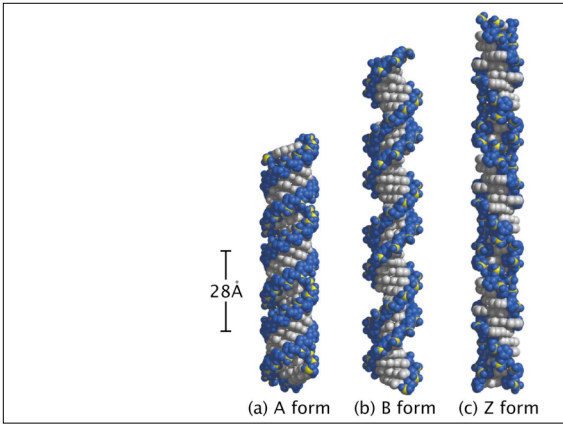
## P-DNA

✓ Longer and narrower than B-DNA  
 ✓ Phosphate groups are on the inside  
 ✓ 2.62 bases per turn

**Table 10.2** Characteristics of DNA secondary structures

Characteristic	A-DNA	B-DNA	Z-DNA
Conditions required to produce structure	75% H <sub>2</sub> O	92% H <sub>2</sub> O	Alternating purine and pyrimidine bases
Helix direction	Right-handed	Right-handed	Left-handed
Average base pairs per turn	11	10	12
Rotation per base pair	32.7°	36°	-30°
Distance between adjacent bases	0.26 nm	0.34 nm	0.37 nm
Diameter	2.3 nm	1.9 nm	1.8 nm
Overall shape	Short and wide	Long and narrow	Elongated and narrow

Note: Within each structure, the parameters may vary somewhat owing to local variation and method of analysis.



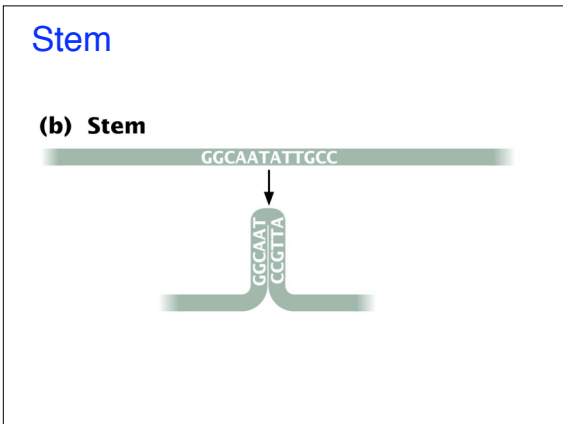
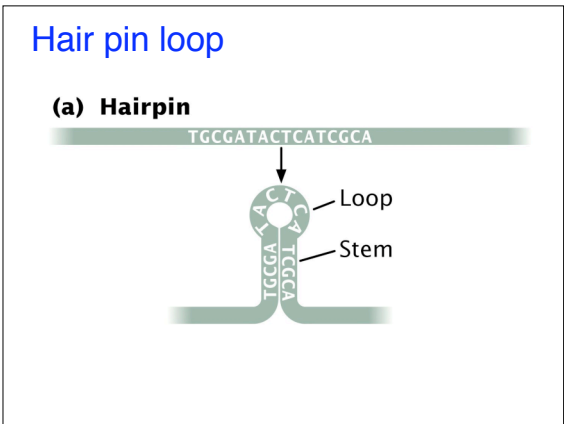
### Structure of RNA

RNA is “single stranded”  
 Ribose replaces Deoxyribose  
 Uracil replace thymine

**TABLE 10.4** RNA CHARACTERIZATION

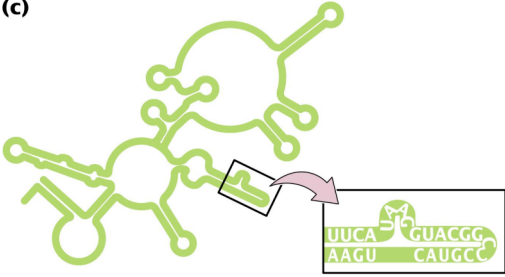
RNA Class	% Total RNA*	Components (Svedberg Coefficient)	Eukaryotic (E) or Prokaryotic (P)	Number of Nucleotides
Ribosomal (rRNA)	80	5S	P and E	120
		5.8S	E	160
		16S	P	1542
		18S	E	1874
		23S	P	2904
Transfer (tRNA)	15	28S	E	4718
		4S	P and E	75-90
Messenger (mRNA)	5	varies	P and E	100-10,000

\*In *E. coli*



## 2° structures of RNA

(c)

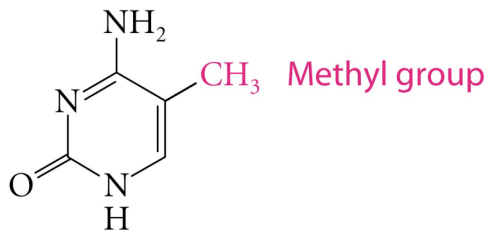


## Cruciform

(d) Cruciform

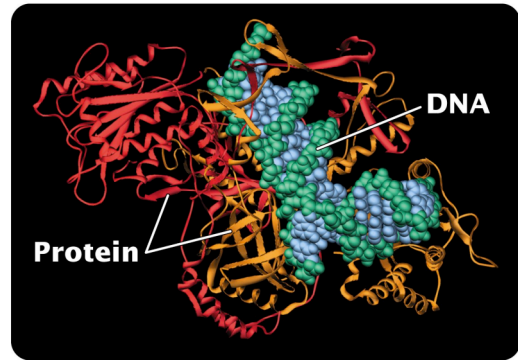


## Base modification

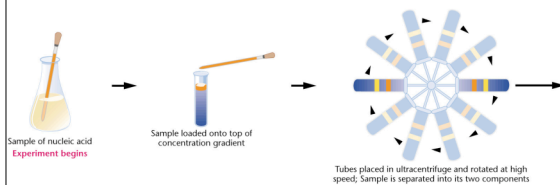


## 5-Methylcytosine

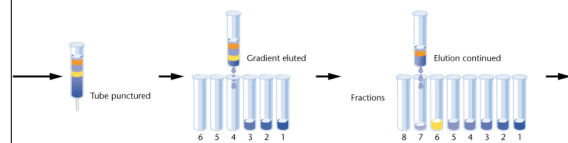
## DNA protein binding



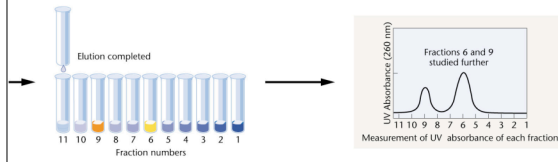
## Separating Nucleic Acids



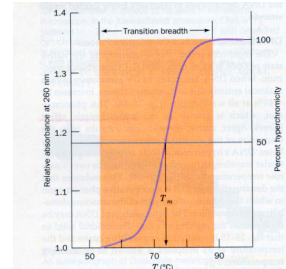
## Eluting the gradient



## Studying the fractions

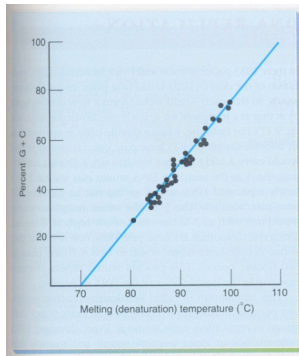


## DNA melting curve



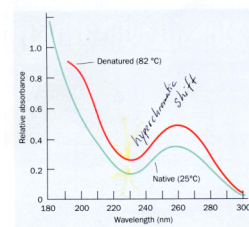
**Figure 28-15**  
An example of a DNA melting curve. The relative absorbance is the ratio of the absorbance (customarily measured at 260 nm) at the indicated temperature to that at 25°C. The melting temperature,  $T_m$ , is the temperature at which one half of the maximum absorbance increase is attained.

## Percent G+C versus melting temperature

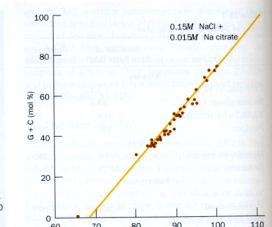


## Hyperchromatic shift & GC content

806 Section 28-3. Forces Stabilizing Nucleic Acid Structures

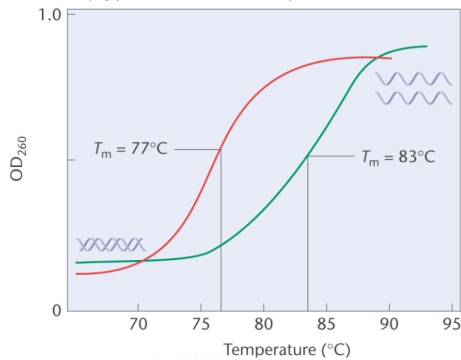


**Figure 28-14**  
The UV absorbance spectra of native and heat-denatured *E. coli* DNA. Note that denaturation does not change the general shape of the absorbance curve but only increases its intensity. [After Voet, D., Gatzert, W. B., Cox, R. A., and Doty, P., *Biopolymers* 1, 205 (1963).]

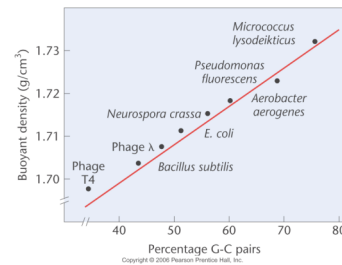


**Figure 28-16**  
The variation of the melting temperatures,  $T_m$ , of various DNAs with their G + C content. The DNAs were dissolved in a solution containing 0.15M NaCl and 0.015M Na citrate. [After Marmur, J. and Doty, P., *J. Mol. Biol.* 5, 113 (1962).]

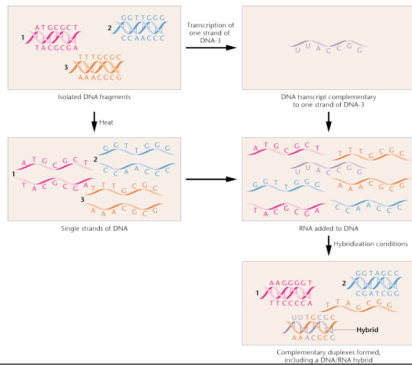
## Increase in UV absorption as temperature increases (hyperchromatic shift)



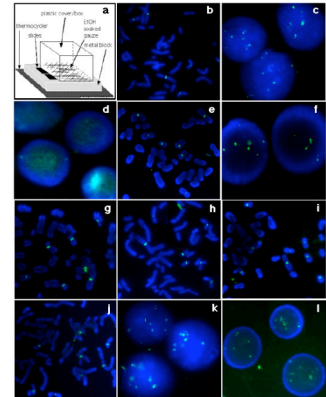
## GC versus buoyant density



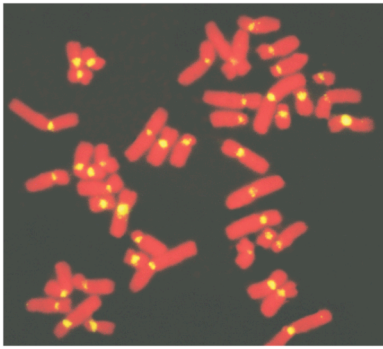
## DNA hybridization



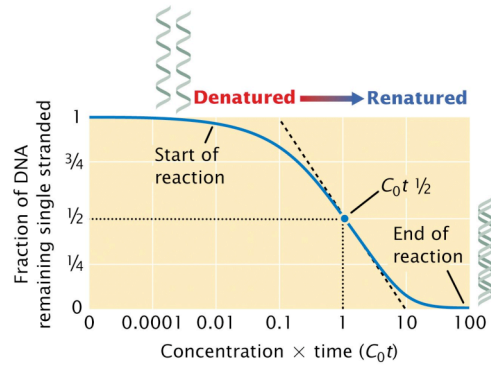
## FISH - Fluorescent *in situ* hybridization



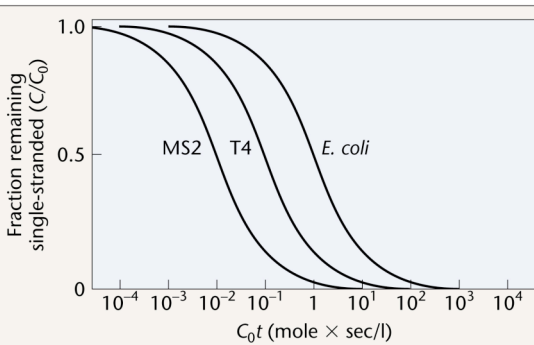
## FISH probe to centromeric sequences



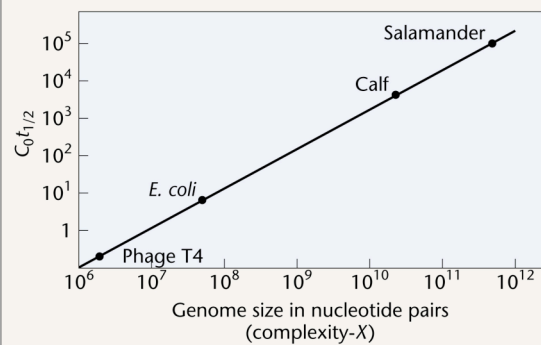
## Cot curves or reassociation kinetics



## Annealing rates



## Cot 1/2 complexities



## Cot curves

