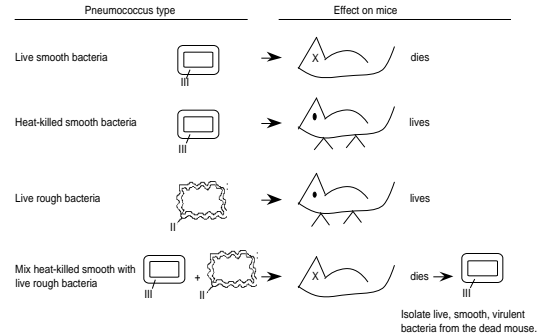


## Genes are composed of nucleic acids (usually DNA)

- Pneumococcus can be transformed from an avirulent to a virulent strain
- DNA is the transforming principle
- DNA in bacteriophage particles appears in the progeny, but very little protein does.

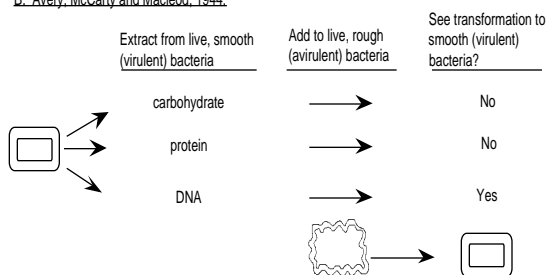
## DNA is the transforming principle

A. Griffith, 1928:



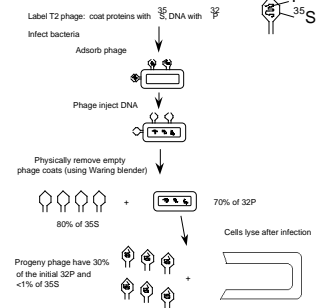
## DNA is the transforming principle

B. Avery, McCarty and Macleod, 1944:

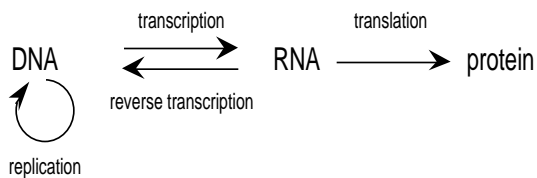


## DNA is passed on to progeny

Hershey & Chase, 1952



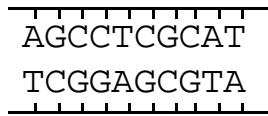
## Central Dogma of Molecular Biology



## Structures of nucleic acids

Nucleotides  
DNA structures  
Sedimentation and Electrophoresis

### A simple view of DNA

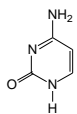


### Nucleotides

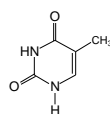
- 3 components to nucleotides:
  - Purine or pyrimidine base
  - Ribose (RNA) or 2-deoxyribose (DNA) sugar
  - Phosphate
- Base + sugar = **Nucleoside**
- Base + sugar + phosphate = **Nucleotide**

### Types of bases in nucleotides

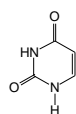
#### Pyrimidine



Cytosine



Thymine

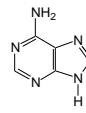


Uracil

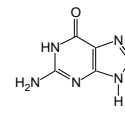
Amino-

Keto-

### Nucleotides: purine bases



Adenine

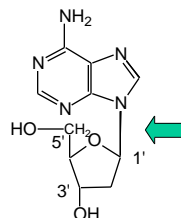


Guanine

6-aminopurine

A keto-purine

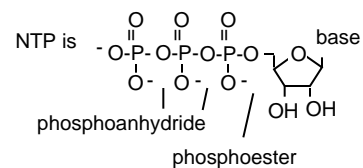
### Bases are attached to C1' of the sugar via an N-glycosidic bond



2'-deoxy- Adenosine , a nucleoside

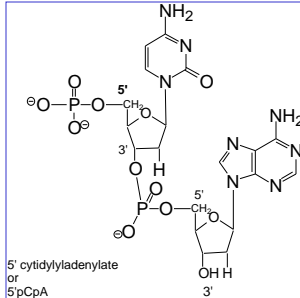
### Phosphate is attached to C5' of the sugar

1st phosphate is a phosphoester, others are attached as phosphoanhydrides.



### Structure of a dinucleotide

The 3' C of one nucleotide is linked to the 5' C of the next nucleotide in a phosphodiester linkage.



### Nucleic acids are linear chains of nucleotides

- The 3' C of one nucleotide is linked to the 5' C of the next nucleotide.
- The linkage is by a phosphoester.
- The chain has an orientation defined by the sugar-phosphate backbone.
- One terminal nucleotide has a "free" 5' end, and the other has a "free" 3' end.
- Thus we designate orientation by 5' to 3'.

### More on orientation of chains of nucleic acids

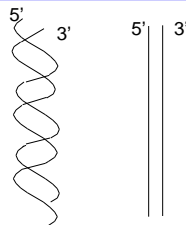
- 5' ACTG 3' is *different* from 3' ACTG 5'
- Unless specified otherwise, a chain is written with the 5' end on the left and the 3' end on the right.
- When complementary strands in DNA are written, usually the top strand is written 5' to 3', left to right, and the bottom strand is written 3' to 5', left to right.

5' GATTCGTACCG  
3' CTAAGCATGGC

### Basics of DNA structure

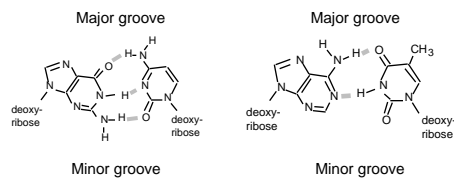
- 2 complementary strands of nucleic acids
- Complementarity is based on H-bonding between
  - Keto bases with amino bases
  - Pyrimidines with purines
- A pairs with T (or U)
- G pairs with C
- The complementary strands are antiparallel.
- The complementary strands are coiled around each other.

### Plectonemic coils, not paranemic junctions



In a plectonemic coil, the two strands wrap around each other.  
In a paranemic joint, the two strands align side-by-side.

### Base pairs in DNA



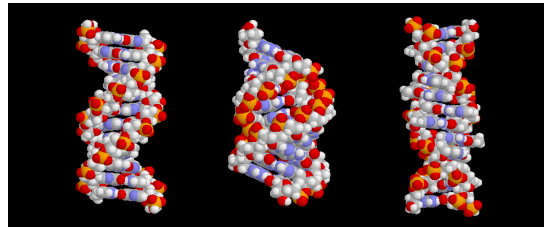
Guanine : Cytosine

Adenine : Thymine

### Major types of duplex nucleic acid structures

- B form
  - Most common form of DNA
  - Right handed DNA-DNA helix
  - Base pairs stack close to DNA central axis
- A form
  - right handed RNA-DNA and RNA-RNA helix
  - Base pairs stack away from the central axis
- Z form DNA
  - Repeating purines and pyrimidines
  - Left-handed helix
  - *May* serve as some regulatory signal in cells

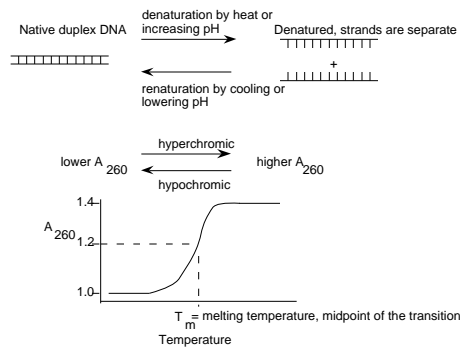
### Forms of nucleic acid duplexes



### Helical parameters for B, A and Z nucleic acids

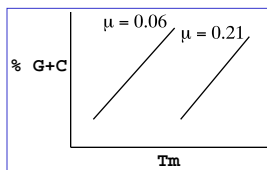
	<u>B</u>	<u>A</u>	<u>Z</u>
helix sense	RH	RH	LH
bp per turn	10	11	12
vertical rise per bp	3.4	2.56	3.7 Angstroms
rotation per bp	+36	+33	-30 degrees
helical diameter	19	23	18 Angstroms

### Hyperchromic shift when DNA is denatured



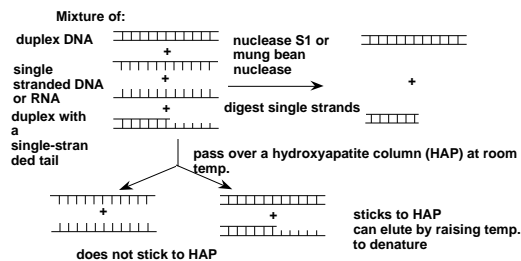
### Factors that affect melting temperature, p. 85

- The melting temperature ( $T_m$ ) increases as
  - Increase G+C
  - Increase ionic strength (or  $\mu$ )
- $T_m$  decreases as
  - Increase denaturants
  - Increase number of mismatches



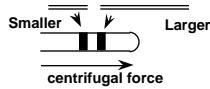
$$T_m = 0.41 (\% \text{ G+C}) + 16.6 \log M + 81.5 - 0.7 (\% \text{ formamide}) - 1^\circ (\% \text{ mismatch})$$

### Distinguishing between duplexes and single strands



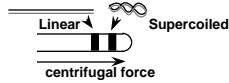
### Sedimentation velocity to measure SIZE

For a set of molecules of the same shape, large molecules will sediment faster.



In dilute solutions,  $\log M$  is proportional to  $\log d$ , where  $M$  is molecular weight and  $d$  is distance sedimented.

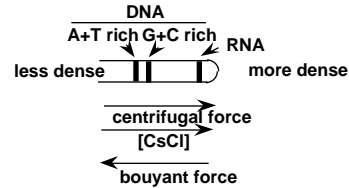
For a set of molecules of the same size, a more compact form will sediment faster.



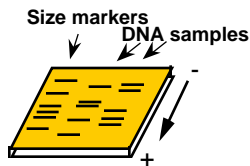
### Sedimentation equilibrium to measure DENSITY

Use a gradient of CsCl so that the molecules will band at the [CsCl] corresponding to their density.

The position at which the molecule bands is independent of its size.



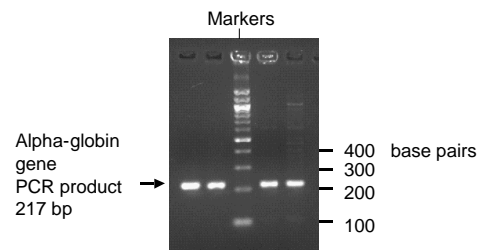
### Electrophoresis to measure SIZE



For molecules of the same shape,  $\log M$  is inversely proportional to  $d$ .

For molecules of the same size, more compact forms, such as supercoiled DNA, moves faster than more extended forms, such as linear DNA.

### Example of gel electrophoresis



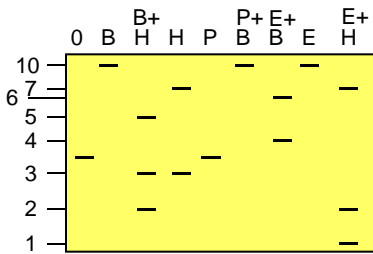
### Constructing restriction maps of DNA

- Restriction endonucleases cleave DNA at specific sites
- Examples:
  - EcoRI G'AATT-C HindIII A'AGCT-T
  - C-TTAA'G T-TCGA'A
- An ordered array of restriction endonuclease cleavage sites is a restriction map.

### Restriction maps: Double digests

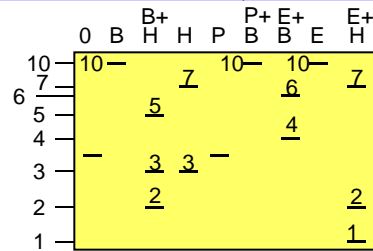
- The DNA to be mapped is cleaved with restriction endonucleases singly and in pairwise combinations.
- The sizes of the resulting DNA fragments allows them to be assembled in an order.

Restriction maps and blots: prob. 1.18



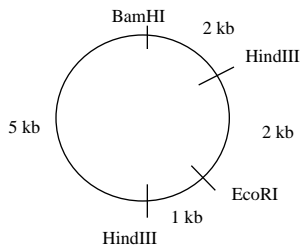
DNA was digested with the indicated enzymes, singly and in combination, and the resulting fragments were resolved on an agarose gel.

Probl. 1.18, cont'd



Which nucleases cut? Which do not?  
Is the DNA circular or linear?  
What is the map of cleavage sites?

Problem 1.18, answer



Restriction maps: Partial digests

- The size of incompletely digested DNA fragments reveals which products of complete digestion are adjacent.
- e.g. a 9 kb partial digestion product is explained by a 5 kb and 4 kb DNA fragments being adjacent.
- Introduction of a label (e.g. radioactive isotope) at one end of the duplex DNA, followed by partial digestion and resolution on gels, allows the distribution of cleavage sites to be determined.