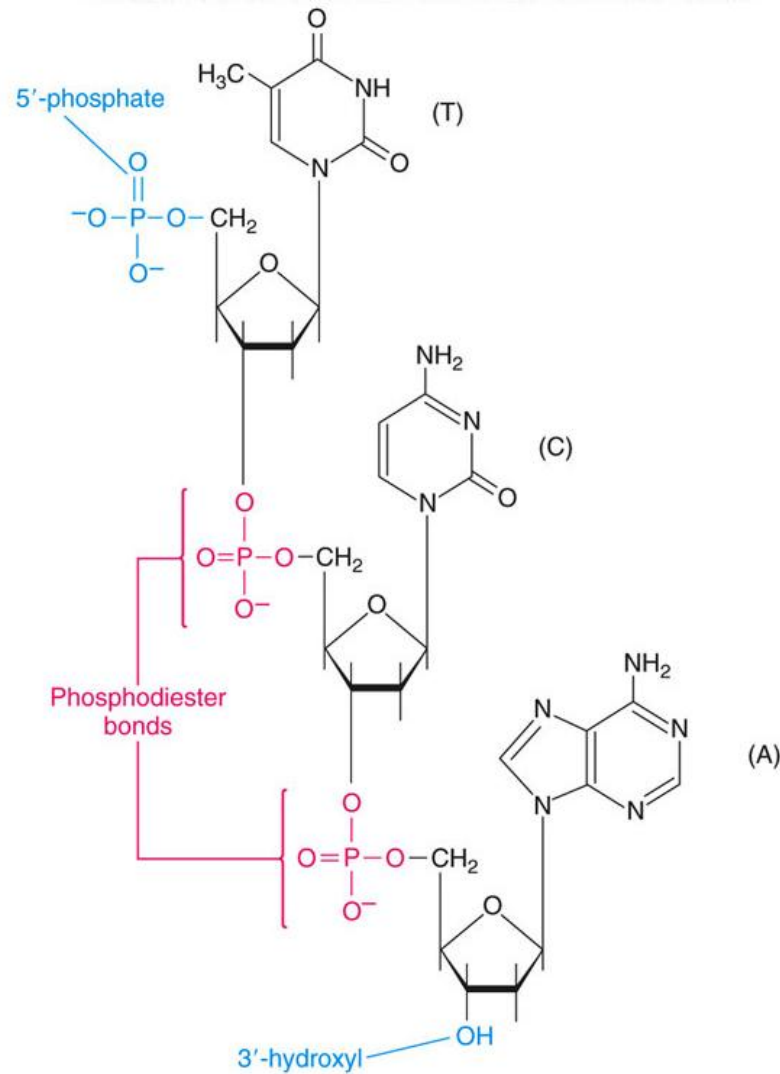


BCHEM 365  
Lecture 2  
Sept. 11, 2018

# Chemical composition of DNA and RNA

- DNA and RNA are chain-like molecules
- The basic subunit of DNA and RNA is a nucleotide
- A nucleotide contains a base linked to the 1'- position of a sugar and a phosphate
- The phosphate joins the sugars in a DNA or RNA chain through their 5'-OH group of one sugar and 3'-OH group of another sugar to create a phosphodiester bond
- Phosphodiester bond in a ribonucleic acid easily breaks down by alkaline hydrolysis owing to the presence of the -OH group on carbon 2 whereas that on deoxyribonucleic acid resists alkaline hydrolysis and is fairly stable

# A trinucleotide



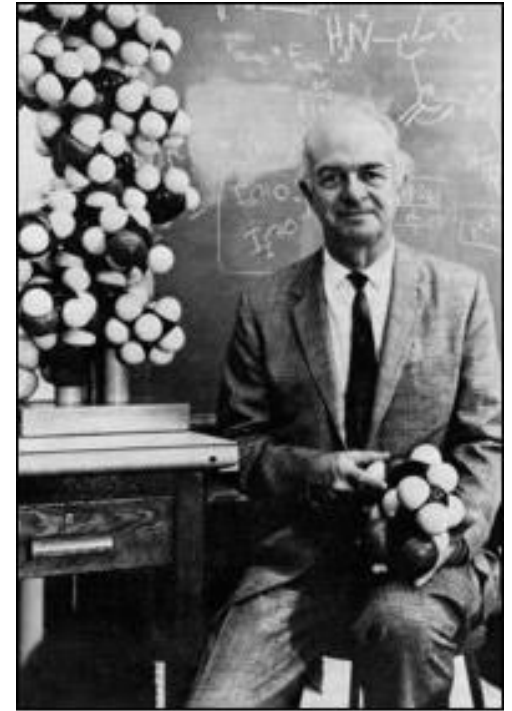
# Three-dimensional structure of DNA

# DNA structure

Determination of 3-D structure of DNA was preceded by elucidation of protein structure

**Linus Pauling (1953)**: a theoretical chemist at California Institute of Technology elucidated the  $\alpha$ -helix structure of proteins held together by hydrogen bonds

**Linus proposed the triple helix model of DNA in which the phosphates (predominantly negative) form the helical core, and the bases point outwards**



Linus Pauling

# DNA structure



Linus Pauling's triple helix  
DNA

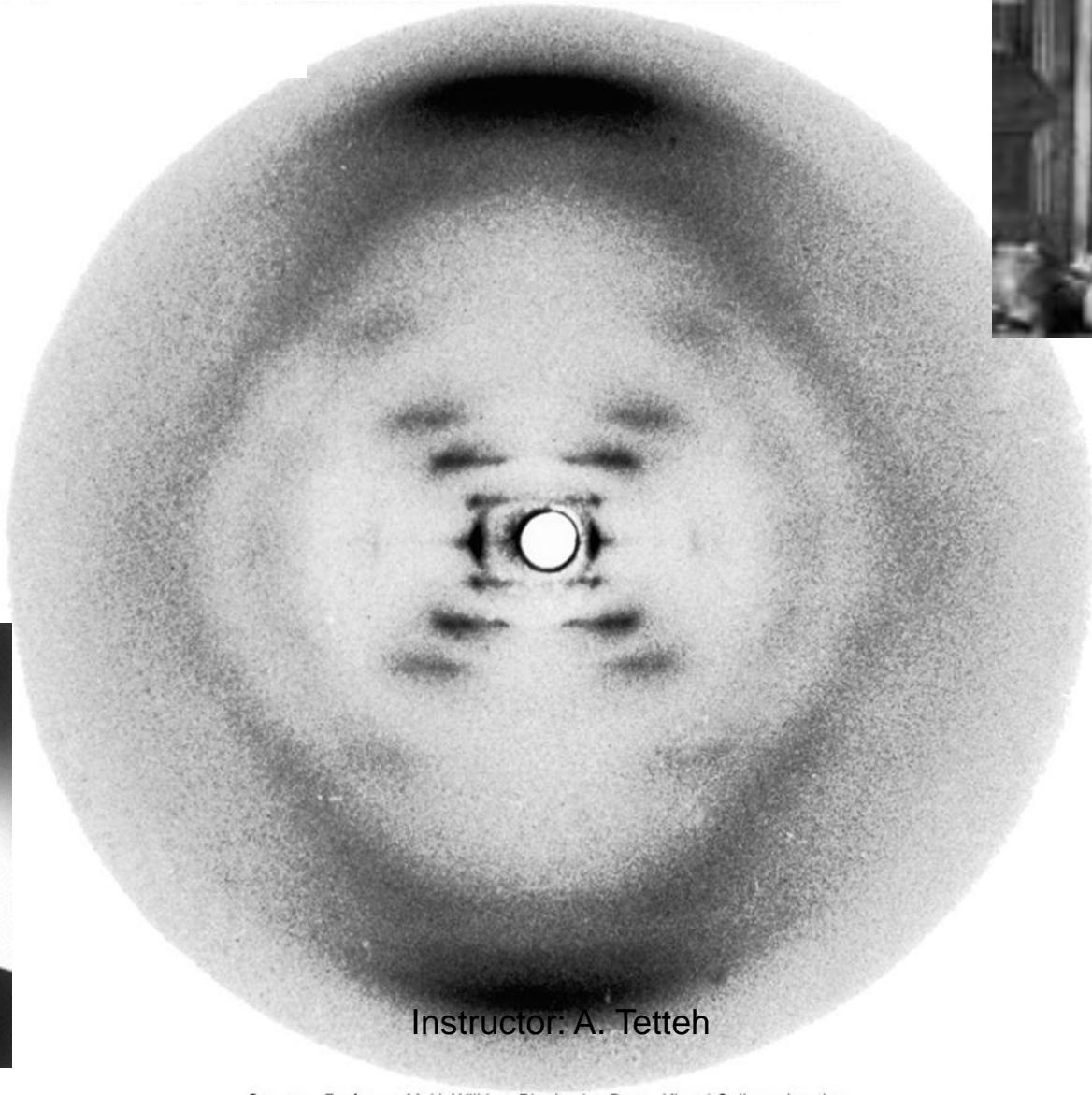
Linus Pauling's triple helix DNA would be impossible under normal cellular conditions as the many negative charges forced together within the core would repel each other and break down the structure

- The  $\alpha$ -helix structure of proteins laid the groundwork for the double-helix model of DNA proposed by Watson and Crick
- Maurice Wilkins and Rosalind Franklin at Kings College in London used X-ray diffraction studies to analyze 3D structure of DNA

# Maurice Wilkins and Rosalind Franklin experiment

- They made a highly concentrated, viscous solution of DNA
- Reached in with a needle and pulled out a fiber
- Fiber made of many molecules of DNA forced into side-by-side alignment by the pulling action
- The DNA fiber was exposed to X-ray. The fibers diffracted X-rays to produce a simple pattern
- Pattern was a series of spots arranged in an 'X' shape indicating that DNA structure must be very simple
- Regularity of pattern indicated DNA is a repeating molecule arranged in a corkscrew (helix) structure
- In contrast, protein gives a complex X-ray diffraction pattern

# Wilkins-Franklin's X-ray diffraction picture of DNA



Rosalind

Wilkins



Instructor: A. Tetteh

# How the repeating unit of DNA was determined

DNA thought to be a repeat of four nucleotides: **ACGT**

1950

**Erwin Chargaff**

Examined the base composition of different organisms. Found that different species have different base composition and content of purines was always roughly equal to content of pyrimidines

**A=T and C=G known as Chargaff's rules**

Instructor: A. Tetteh



# Chargaff's study: Base composition (expressed as G+C content) varies from one species to another

**Table 2.3 Relative G + C Contents of Various DNAs**

Sources of DNA	Percent (G + C)
<i>Dictyostelium</i> (slime mold)	22
<i>Streptococcus pyogenes</i>	34
Vaccinia virus	36
<i>Bacillus cereus</i>	37
<i>B. megaterium</i>	38
<i>Hemophilus influenzae</i>	39
<i>Saccharomyces cerevisiae</i>	39
Calf thymus	40
Rat liver	40
Bull sperm	41
<i>Streptococcus pneumoniae</i>	42
Wheat germ	43
Chicken liver	43
Mouse spleen	44
Salmon sperm	44
<i>B. subtilis</i>	44
T1 bacteriophage	46
<i>Escherichia coli</i>	51
T7 bacteriophage	51
T3 bacteriophage	53
<i>Neurospora crassa</i>	54
<i>Pseudomonas aeruginosa</i>	68
<i>Sarcina lutea</i>	72
<i>Micrococcus lysodeikticus</i>	72
Herpes simplex virus	72
<i>Mycobacterium phlei</i>	73

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Source: From Davidson, *The Biochemistry of the Nucleic Acids*, 8th edition revised by Adams et al. Lippencott.

# A=T and C=G

**Table 2.1 Composition of DNA in moles of base per mole of phosphate**

	Sperm		Human Thymus	Liver Carcinoma	Yeast		Avian Tubercle Bacilli	Bovine Thymus			Spleen	
	#1	#2			#1	#2		#1	#2	#3	#1	#2
A:	0.29	0.27	0.28	0.27	0.24	0.30	0.12	0.26	0.28	0.30	0.25	0.26
T:	0.31	0.30	0.28	0.27	0.25	0.29	0.11	0.25	0.24	0.25	0.24	0.24
G:	0.18	0.17	0.19	0.18	0.14	0.18	0.28	0.21	0.24	0.22	0.20	0.21
C:	0.18	0.18	0.16	0.15	0.13	0.15	0.26	0.16	0.18	0.17	0.15	0.17
Recovery:	0.96	0.92	0.91	0.87	0.76	0.92	0.77	0.88	0.94	0.94	0.84	0.88

Source: E. Chargaff "Chemical Specificity of Nucleic Acids and Mechanism of Their Enzymatic Degradation," *Experientia* 6:206, 1950.

## Watson and Crick - model builders

- performed no experiment but drew conclusions from:

The very simple X-ray diffraction pattern

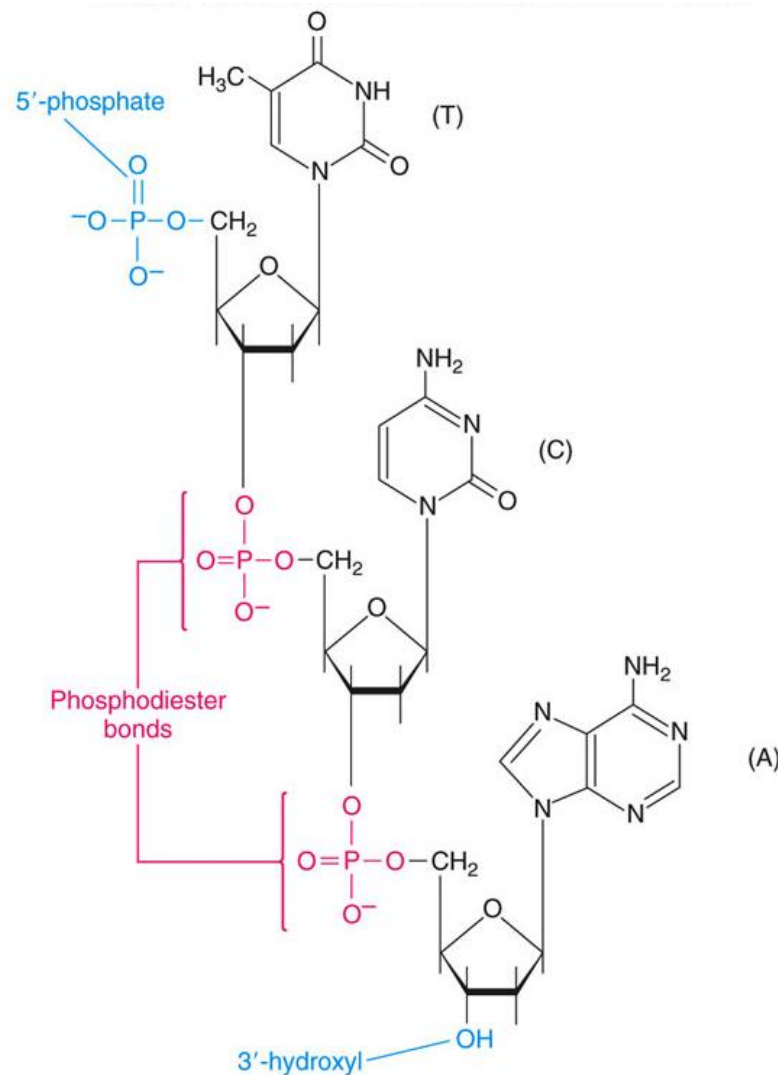
DNA structure must be simple, unlike proteins

### Simplest repeating structure:

that a long, thin molecule  
can take a corkscrew or helix  
structure



# A trinucleotide



The substituents, -OH, -NH<sub>2</sub>, C=O, PO<sub>4</sub><sup>3-</sup> participate in non-covalent interactions with each other to cause the formation of a helix

## Paradox:

Franklin: DNA was a helix with a **regular**, repeating structure

But, for DNA to serve its genetic function

it must have an **irregular sequence** of bases

Watson and Crick solved this paradox and satisfied Chargaff's rules at the same time

$A=T$  and  $C=G$

# DNA is a double helix

DNA must be a **double helix**:

sugar and phosphate on the outside  
and bases on the inside

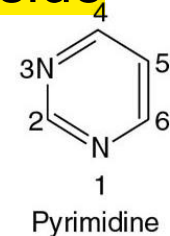
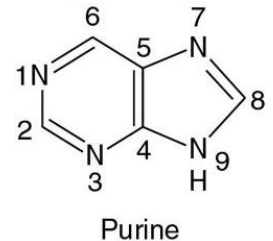
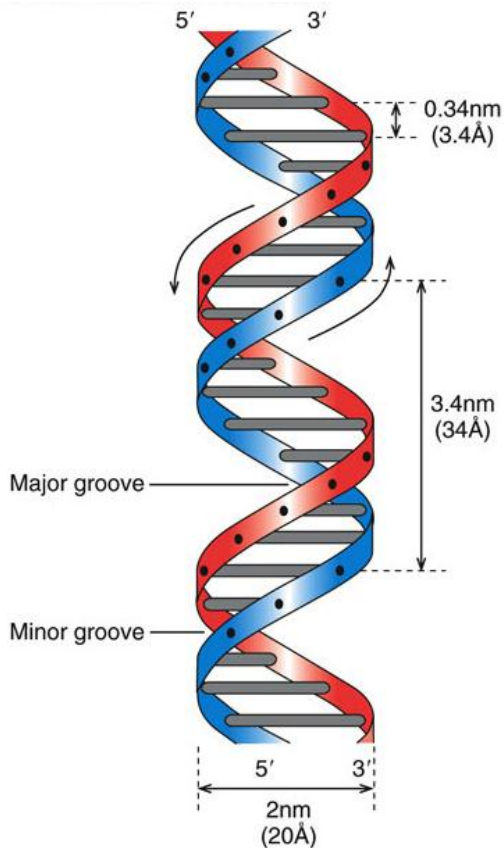
Bases must be paired :

purines in one strand pair with pyrimidines in  
the other strand

This would make the helix uniform

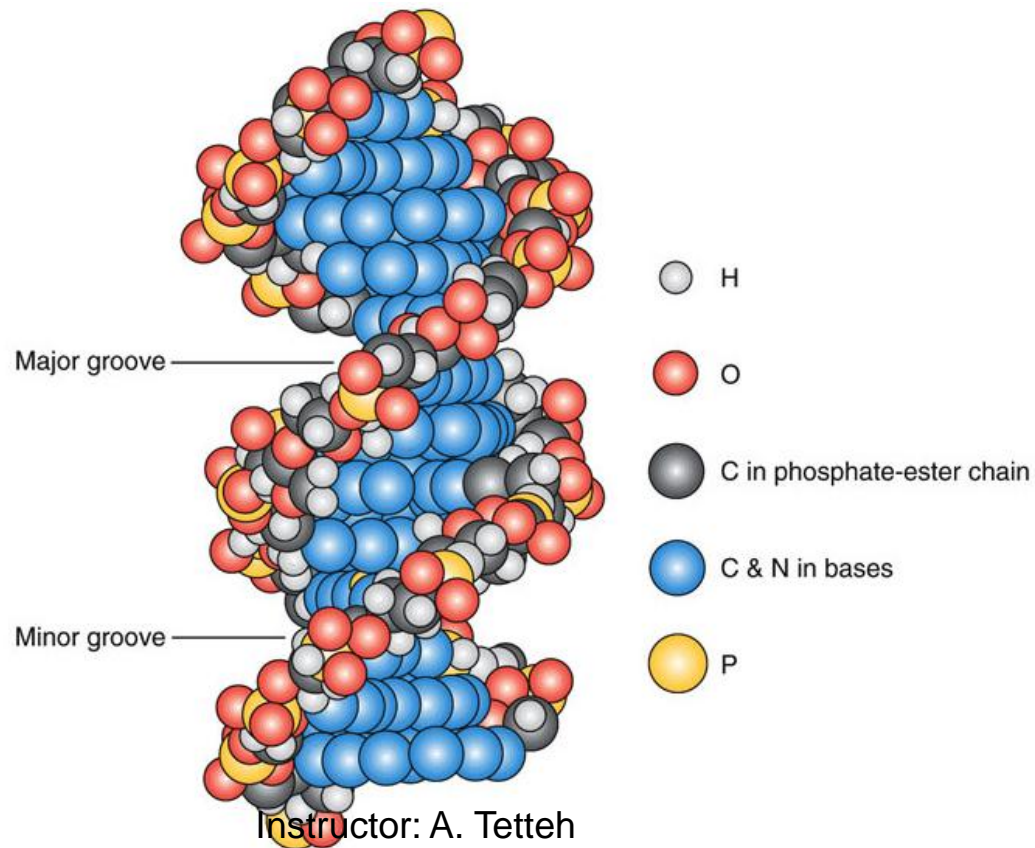
No bulges (purine-purine) and

No constrictions (pyrimidine-pyrimidine)

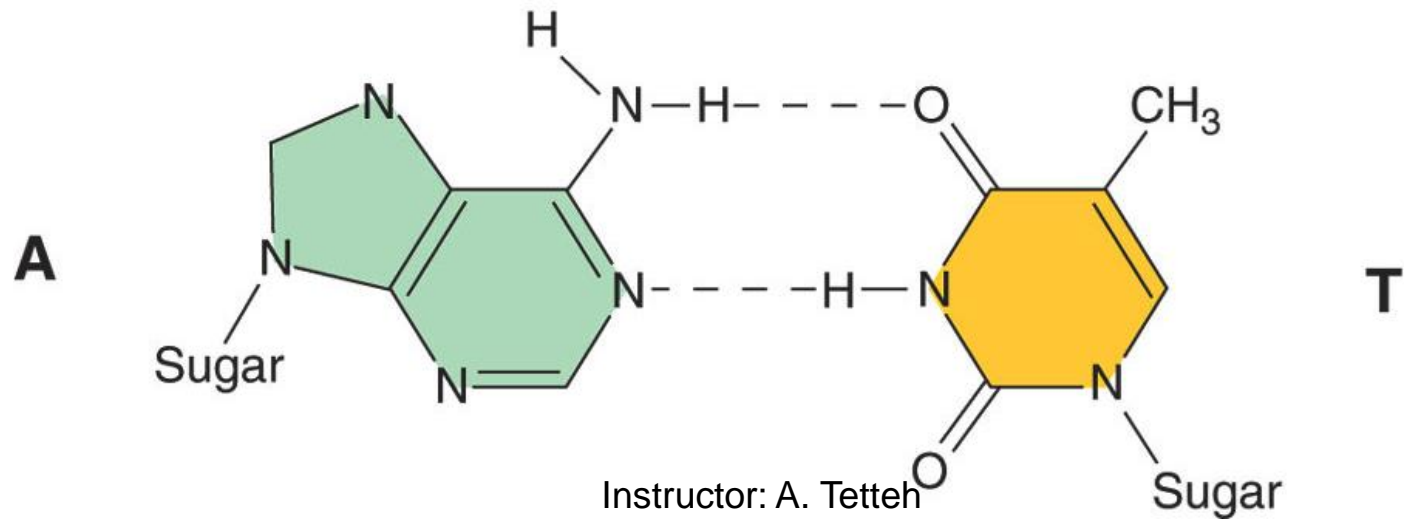
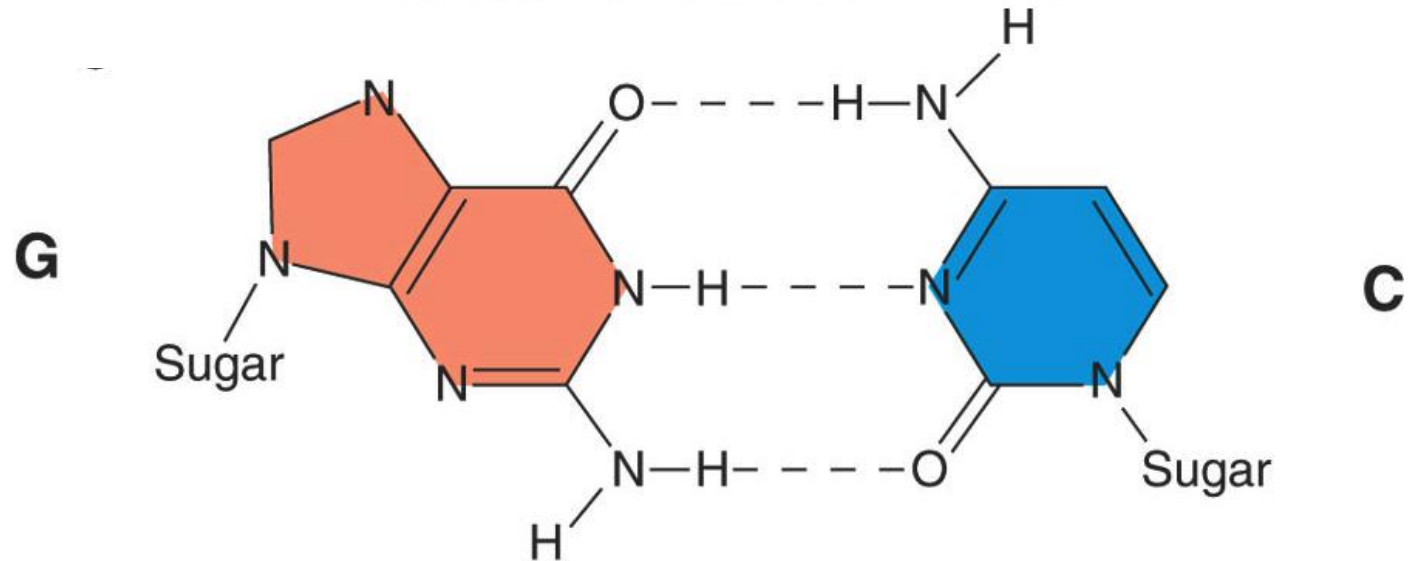


# DNA is a double helix

with sugar and phosphate on the outside  
and bases on the inside



# Purines pair with pyrimidines



# Nature of purine-pyrimidine pair

- Guanine-cytosine pair held together by three hydrogen bonds
- This pair has the same shape as an adenine-thymine pair held together by two hydrogen bonds
- Similarity in shape makes DNA regular molecule
- Differences in bases make DNA irregular and functional

## Watson and Crick (Nature 1953)

“It has not escaped our notice that the specific base pairing we have proposed, immediately suggests a possible copying mechanism for the genetic material.”

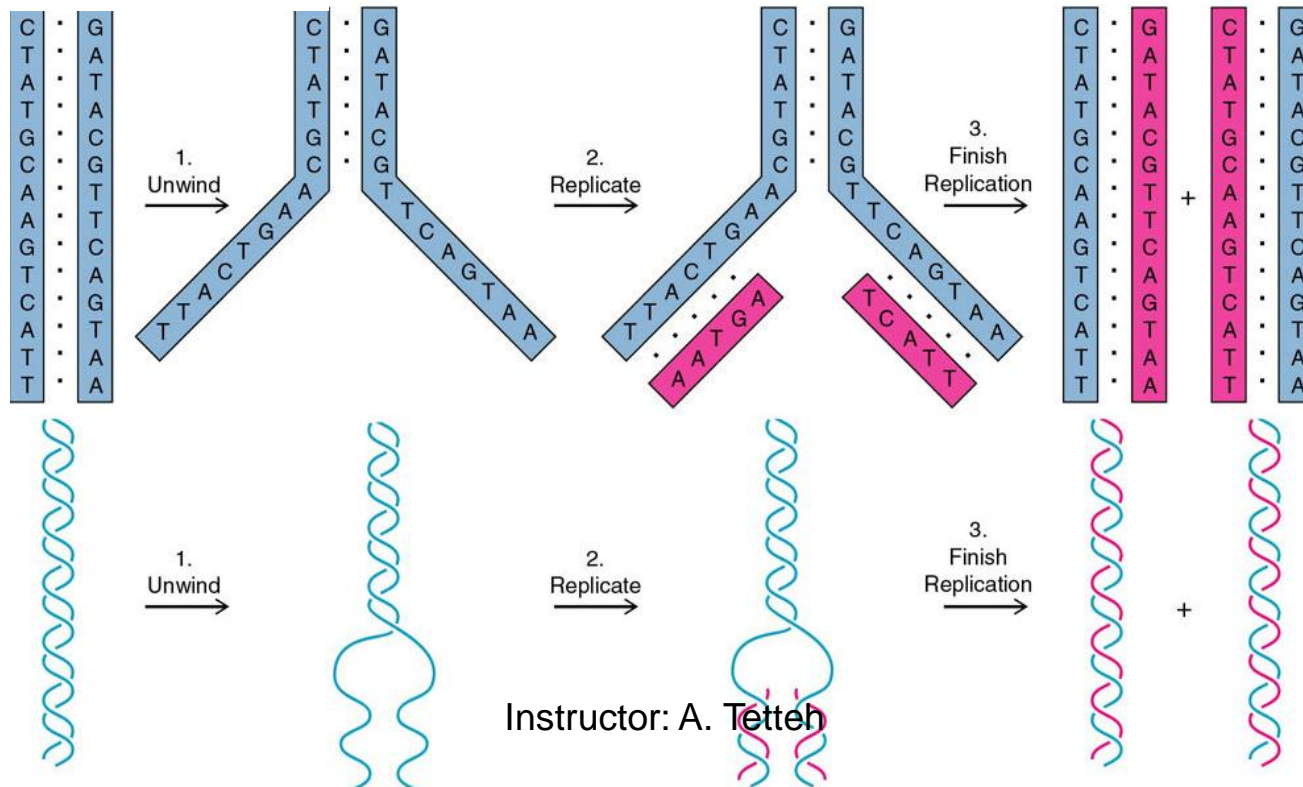
One strand is the complement of the other- **complementary strands**

The strands can be separated and each serve as the template for the other

**Semiconservative replication** ensures two daughter DNA duplexes are exactly the same as the parent

# Watson and Crick 1953

## Semiconservative replication



# The double helix

- Spacing between base pairs is 3.4 Ångstrom units
- Overall helix repeat distance is about 34 Ångstrom units
- It means there are about 10 bp per turn of the helix
- The arrows indicate that the 2 strands are antiparallel: 5'→3' polarity from top to bottom
- The other must have 5'→3' polarity from bottom to top

‘Take home message’

- DNA is double helix; the two strands are complementary and anti-parallel

# Physical structure of DNA

- DNA and RNA molecules can assume several different structures and behave differently under certain conditions
- Can assume certain conformations
- Can be denatured – i.e., the two strands can be separated
- Can anneal - the two separated strands can come together

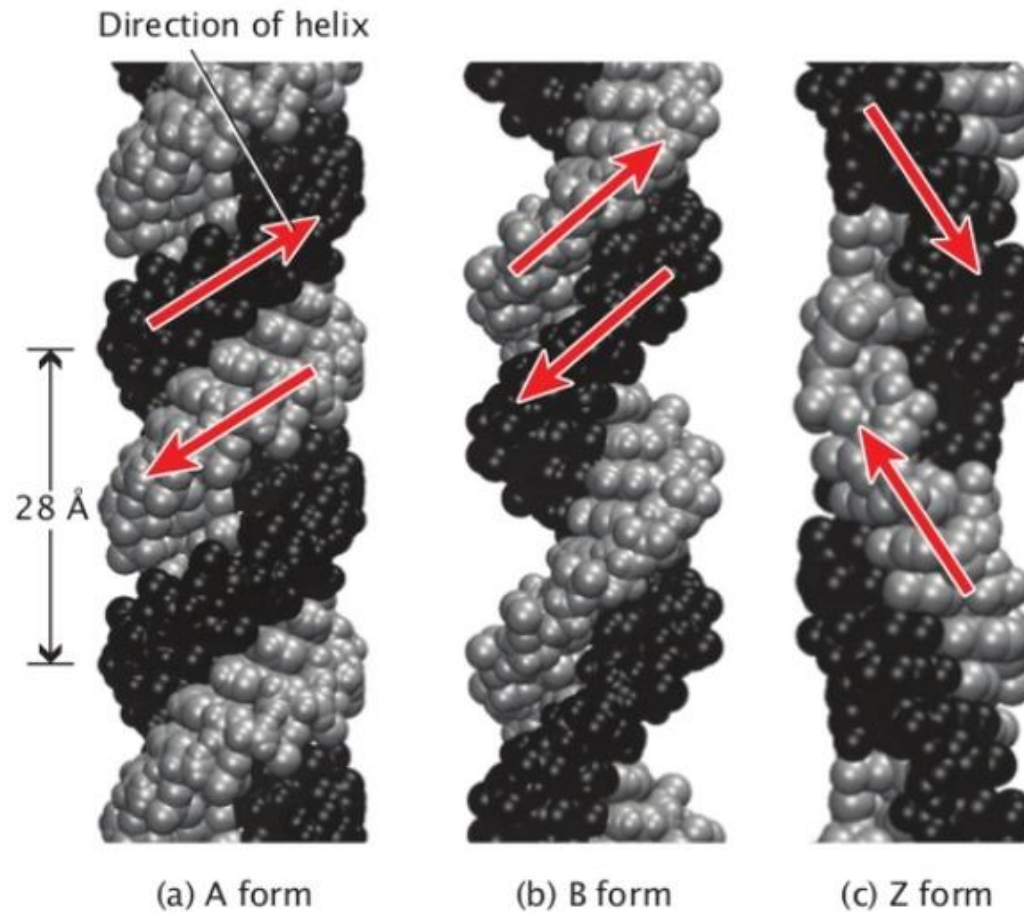
# DNA conformations

- Three DNA conformations have been found, *A*, *B*, and *Z* forms
- The sodium salt of DNA fiber at high relative humidity (92% RH) assumes the ***B*** form: the structure proposed by Watson and Crick, the most common conformation in most living cells. *B*-DNA is a right-handed helix This has 10 bp per turn as specified in Watson and Crick structure. A turn occurs after every 34 Ångstrom units
- The sodium salt of DNA fiber at relative humidity (75% RH) has ***A*** form with 11 bp per turn. *A*-DNA is short, wide and right-handed helix. Each turn occurs in 25 Ångstrom units. Found rarely under normal physiological conditions. Present in RNA-DNA hybrids
- Right-handed: helix turns clockwise away from you

# DNA conformations

- **Z-** form - first discovered in 1979
- **a left-handed helix**, i.e., the double helix winds to the left in a zig-zag pattern. It is much elongated
- A transient form of DNA which exists occasionally in response to certain types of biological activity
- Scientists have since discovered that certain proteins bind very strongly to Z-DNA, suggesting that **Z-DNA plays an important biological role in protection against viral disease**

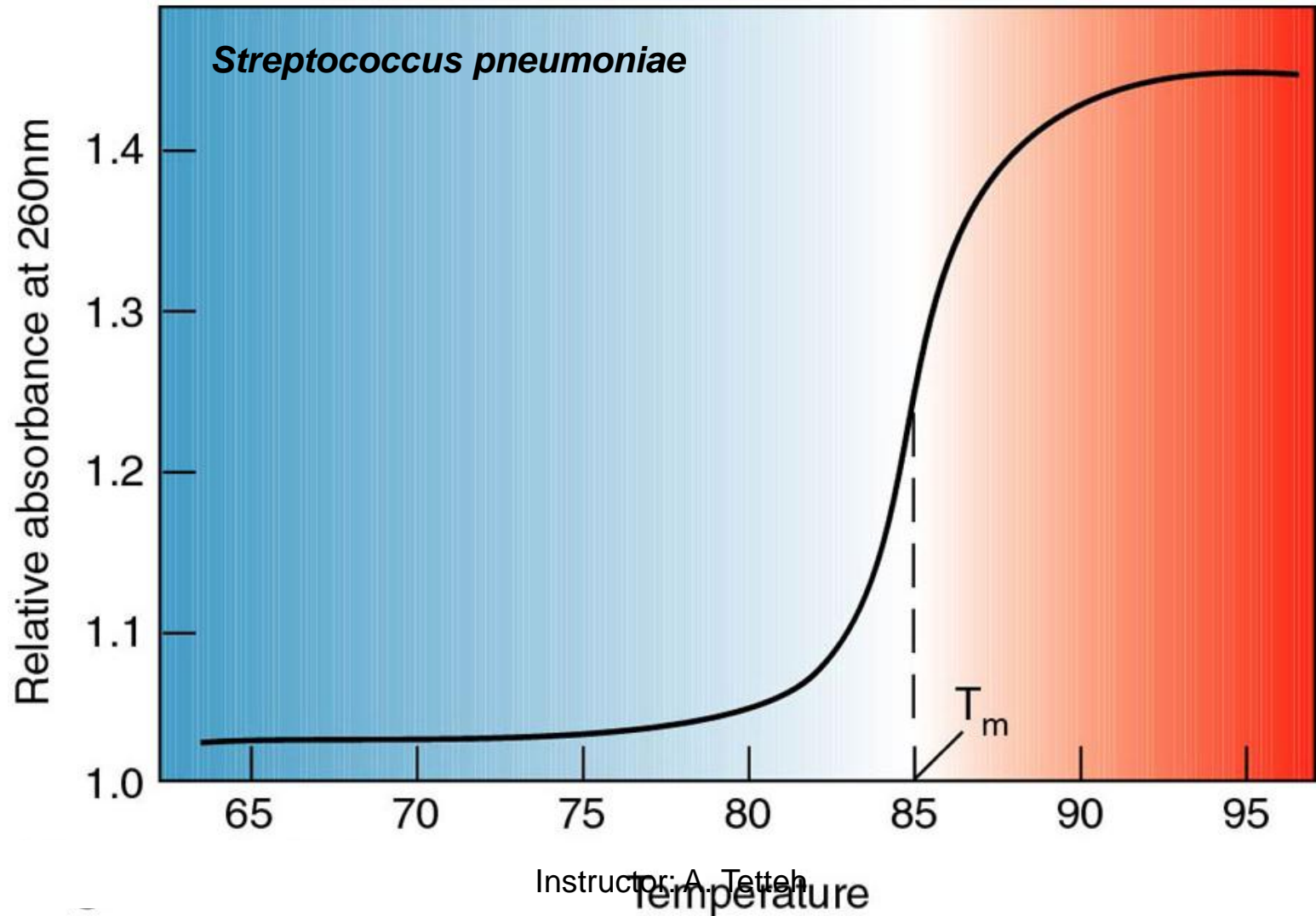
# DNA conformations



# Denaturation of DNA- by heating

- Heat DNA solution
- the noncovalent forces (H-bonds) holding the 2 strands together weaken and break
- The two strands come apart in a process known as **DNA denaturation/melting**
- The amount of strand separation or melting is measured by absorbance at  $\lambda_{260}$  against temperature
- Plot a graph of absorbance against temperature to follow DNA melting

# Melting curve of DNA



$T_m$

$T_m$  or melting temperature is the temperature at which  
 $\frac{1}{2}$  of the DNA strands are melted

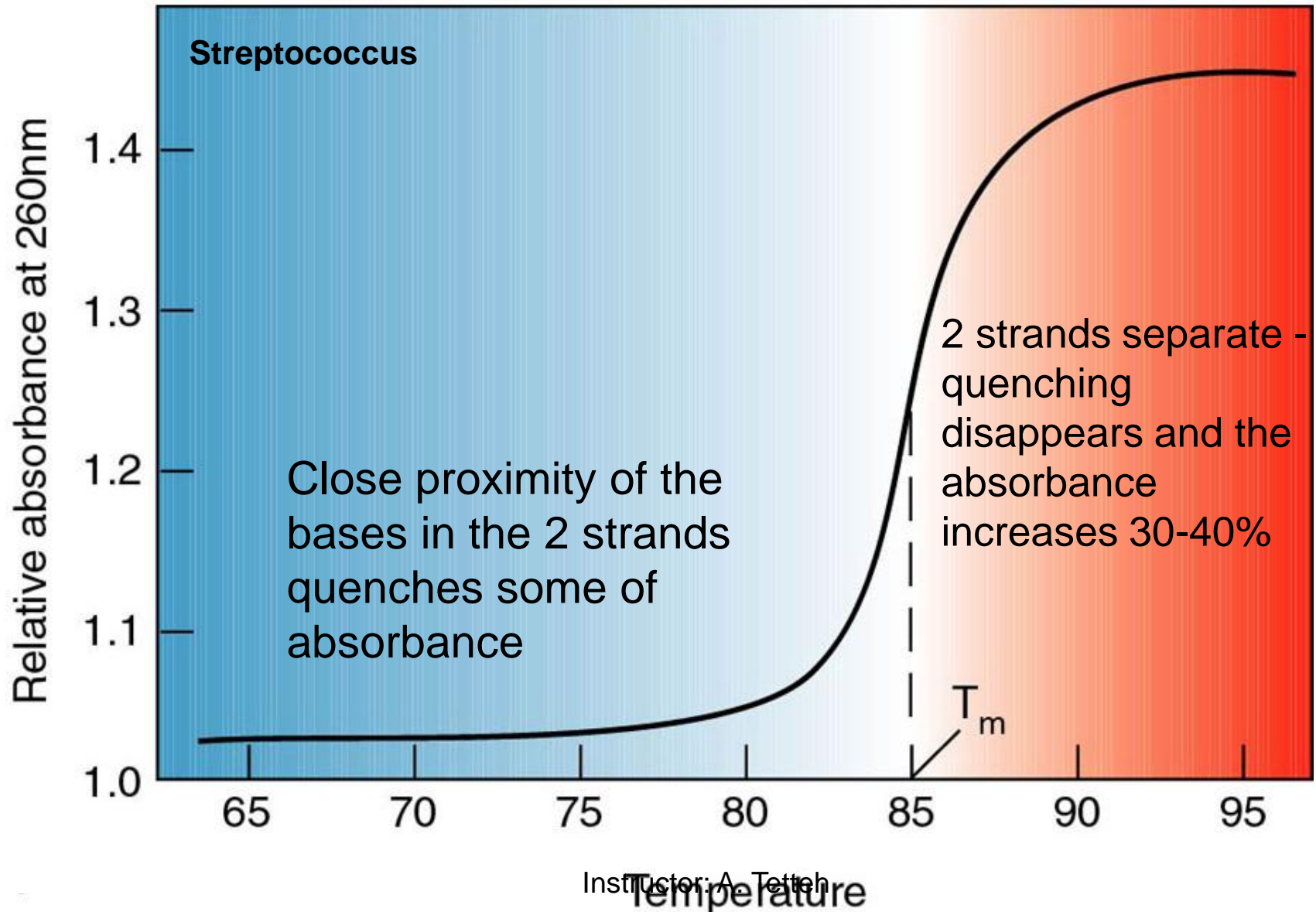
or

temperature at which melting is half complete

## Hyperchromic shift

- The initial slow rise in the curve shows that the two strands hold fast at low temperature, hence low absorbance
- When temperature increases and approaches  $T_m$ , the strands separate and absorbance increases sharply until a plateau is reached when there is no more separation

# Hyperchromic shift



## Hyperchromic shift

Nucleic acids absorb light at wavelength of 260 nm because of the electrical nature of the bases

Close proximity of the bases in the 2 strands (double helix) quenches some of absorbance

When the 2 strands separate upon heating, quenching disappears and the absorbance increases by 30-40%. This is hyperchromic shift

Hyperchromic shift occurs when nucleic acids change from double stranded to single stranded structure

## Denaturation of DNA – separating the two strands of the double helix

Differences in GC content produce differences in physical properties of DNA

GC (G + C) content can vary from one DNA to the other

Values range from 22-73%

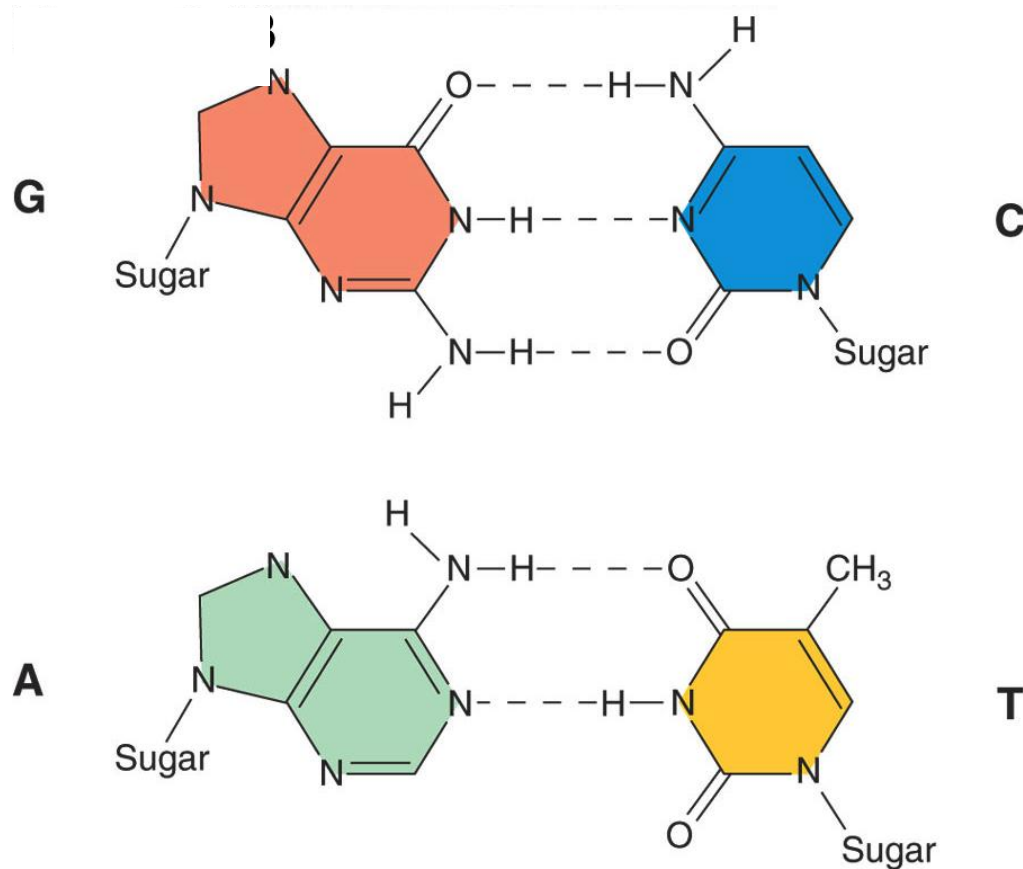
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Source: From Davidson, *The Biochemistry of the Nucleic Acids*, 8th ed., revised by Adams et al. Lippencott.

Instructor: A. Tetteh

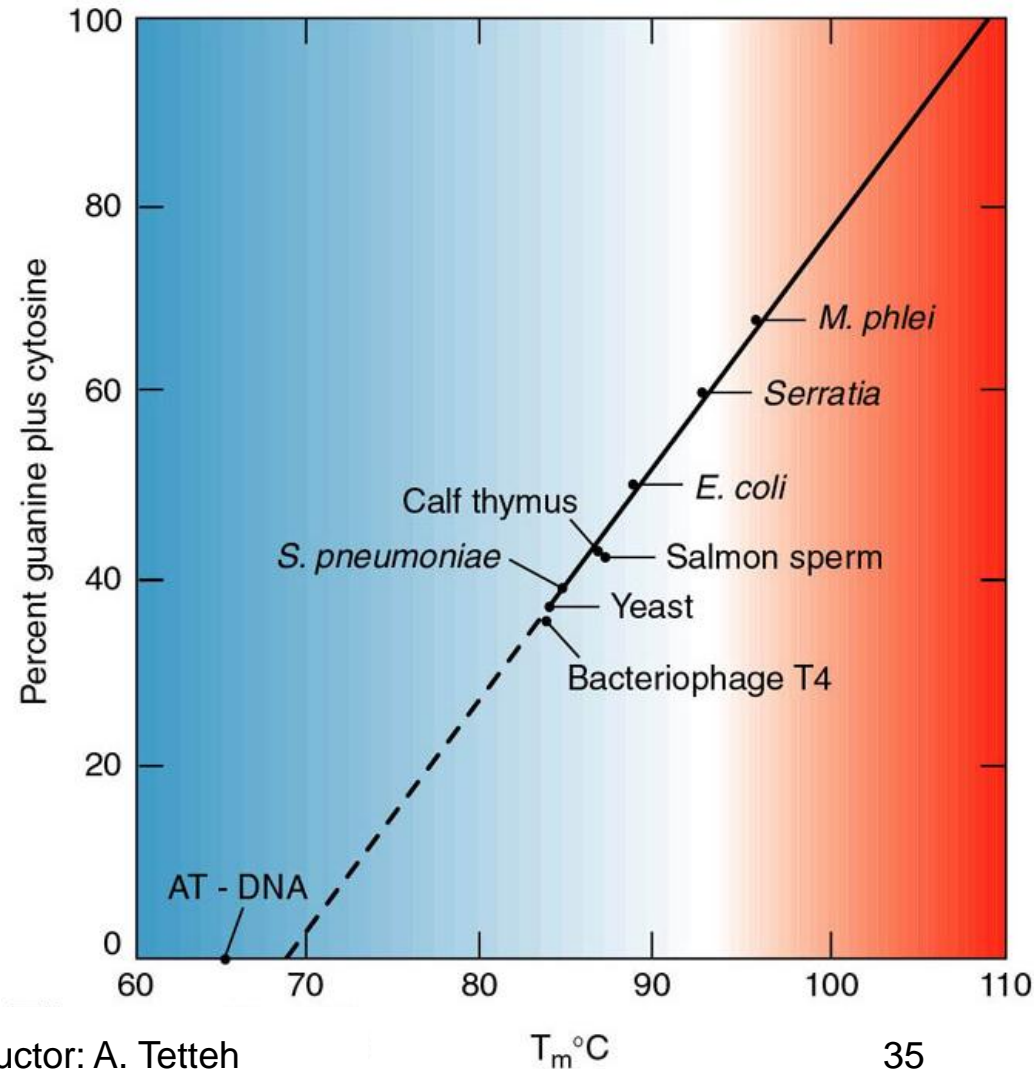
**Higher the GC content - higher the  $T_m$**



# Higher the GC content - higher the $T_m$

**Table 2.3** Relative G + C Contents of Various DNAs

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Wheat germ	43
Chicken liver	43
Mouse spleen	44
Salmon sperm	44
<i>B. subtilis</i>	44
T1 bacteriophage	46
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Instructor: A. Tetteh

$T_m$  °C

35

## G-C content and Tm

- G-C pairs form three H-bonds , whereas A-T pairs form two H bonds
- So the two strands of DNA rich in G+C will hold to each other more tightly than those of A-T-rich DNA
- Harder to separate GC-rich than AT-rich DNA

## Conclusions

DNA is the genetic material

DNA consists of nucleotides that contain

bases

sugars

phosphate groups

The structure of DNA is a double helix

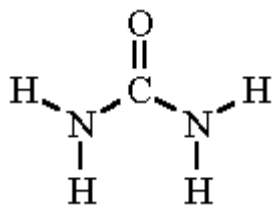
The strands are complementary and antiparallel

The double helix is denatured at high temperatures

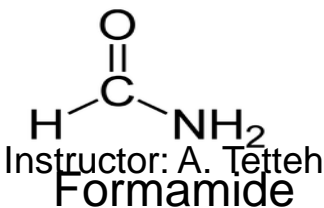
Other ways to **denature DNA**:

organic solvents such as dimethyl sulfoxide (DMSO), **urea** and **formamide** (denaturants)

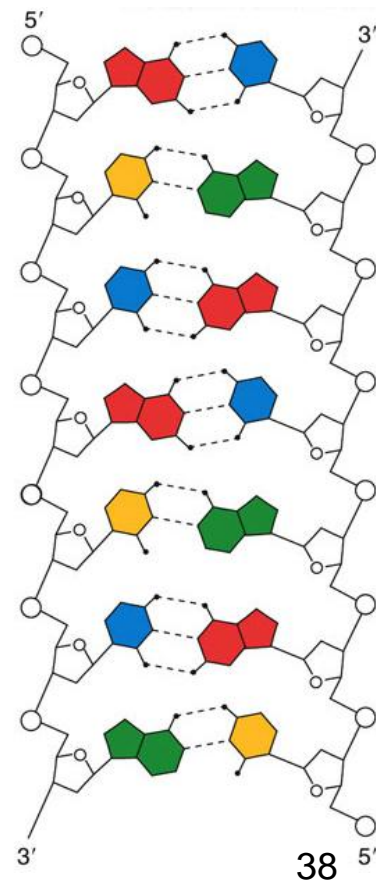
Urea and formamide contain functional groups that can form H-bonds with the electronegative centers of the N-bases. High concentrations of urea (8M) or formamide (70%) compete for H-bonds favoring interactions between the denaturant and the N-bases rather than between complementary bases. As a result, the two strands separate.



Urea



Formamide



## Other ways to **denature DNA**

### **High pH**

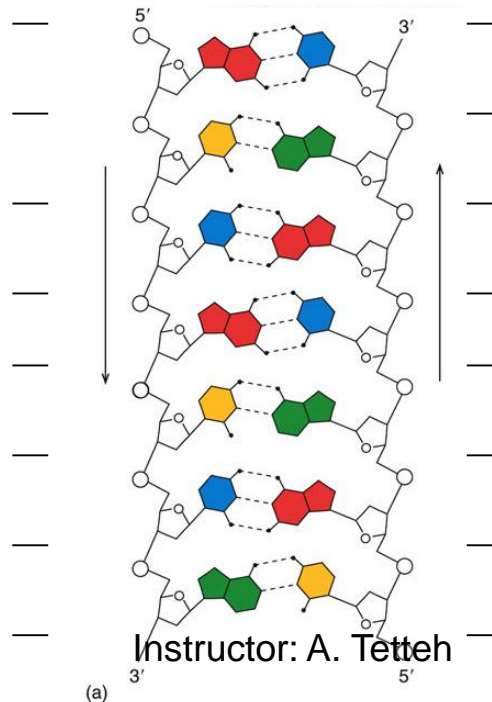
Disrupts hydrogen bonds between DNA strands, and promote denaturation. Bases such as NaOH raise the pH until the  $H^+$  shared between the N-base electronegative centers ( N-H and O= ) is stripped from the H-bond.

**Low salt -** removes ions that shield the negative charges on the two strands from each other

# Denaturation of DNA

At low ionic strength

mutually repulsive forces of the negative charges  
are strong enough to denature the DNA  
at relatively low temperatures



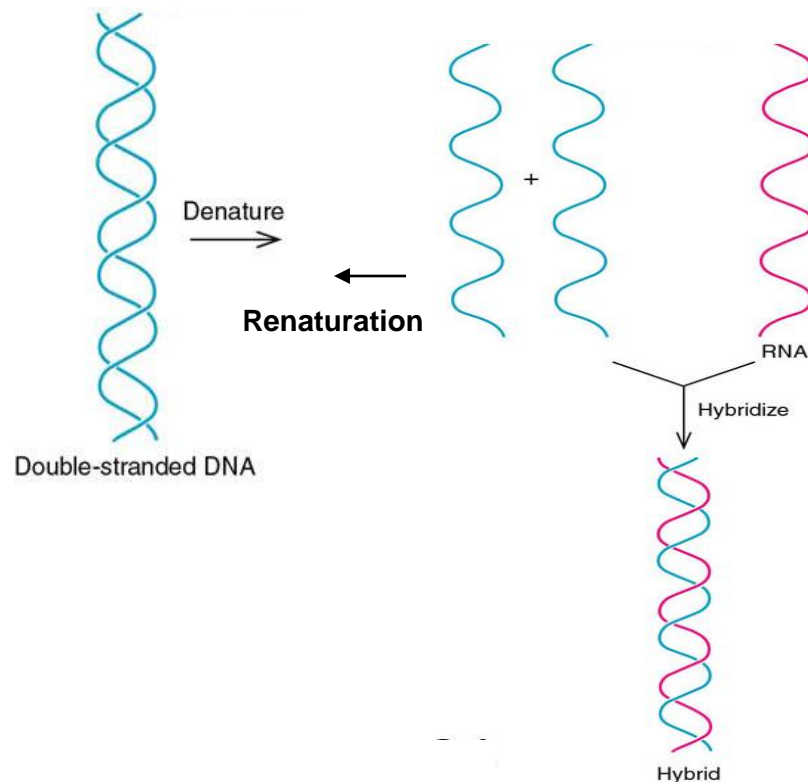
## Summary

**These conditions promote DNA denaturation**

1. High temperature
2. Organic solvents
3. High pH
4. Low salt

# Reuniting the separated DNA strands

- Under proper conditions, the separated strands can come back together
- Known as **annealing or renaturation**



# Renaturation

## Depends on

### 1. Temperature

the best temperature for renaturation is about 25 °C below  $T_m$

This temperature is low enough

-Does not promote further denaturation

This temperature high enough to

- allow rapid diffusion of DNA molecules
- weaken transient bonding between mismatched sequences and intrastrand base-pairing

Rapid cooling prevents renaturation by slowing down diffusion

### 2. DNA concentration

### 3. Time

### 4. Complexity

# Renaturation

## DNA concentration

the higher the concentration, the more likely two complementary strands will encounter each other within a given time and participate in H-bond formation

The higher the concentration, the faster the annealing

## Renaturation time

- the longer the time allowed for annealing, the more annealing will occur

## 4. Complexity

-Cot curves