MOLECULAR BIOLOGY

Basics and Concepts: What is what?

Part-II: DNA it's Structure & Properties

Based on:

(i) Molecular Biology **We a v e r R o b e r t F.** (5ed.,2012)

(ii) Biochemistry **Garrett R.H., Grisham C.M.**(2ed., 1999)

(iii) Molecular Biology of the Gene **Watson, J.D** (5ed)

-Atul Upreti

Watson (Left) &
Crick (right)

Upon solving the structure of DNA, Crick proclaimed in **"The Eagle"** a **pub** just across from Cavendish lab, **" We have discovered the secret of life"**.

Sourse: Biochemistry Garrat & Grisham

Deoxyribonucleic Acid: DNA

- A DNA molecule is characteristically made up of two polynucleotide strands bound together to form a long helical molecule.

- The strands are bound together by H bonds formed between nitrogenous bases (inter-chain hydrogen bonds). This is what we call base pairing.

Single stranded DNAs are found in some viruses though

Erwin Chargaff’s Finding: Chargaff’s rule (1951):

- He studied ratios of molar concentrations of different nitrogenous bases (A:G, T:C, A:T, G:C and purine: pyrimidinein) several different species.
- He found that in all the species A:T , G:C and purine : pyrimidene were constantly 1.
- He postulated that adenine and thymine are present in equimolar concentrations and so are guanine and cytosin.

$$[A] = [T]$$

$$\underline{[G] = [C]}$$

$$[A]+[G] = [C] + [T]$$

$$[\text{Purine}] = [\text{Pyrimidine}]$$

Watson and Crick's Double Helix

- James Watson and Francis Crick proposed the double helix model for DNA.

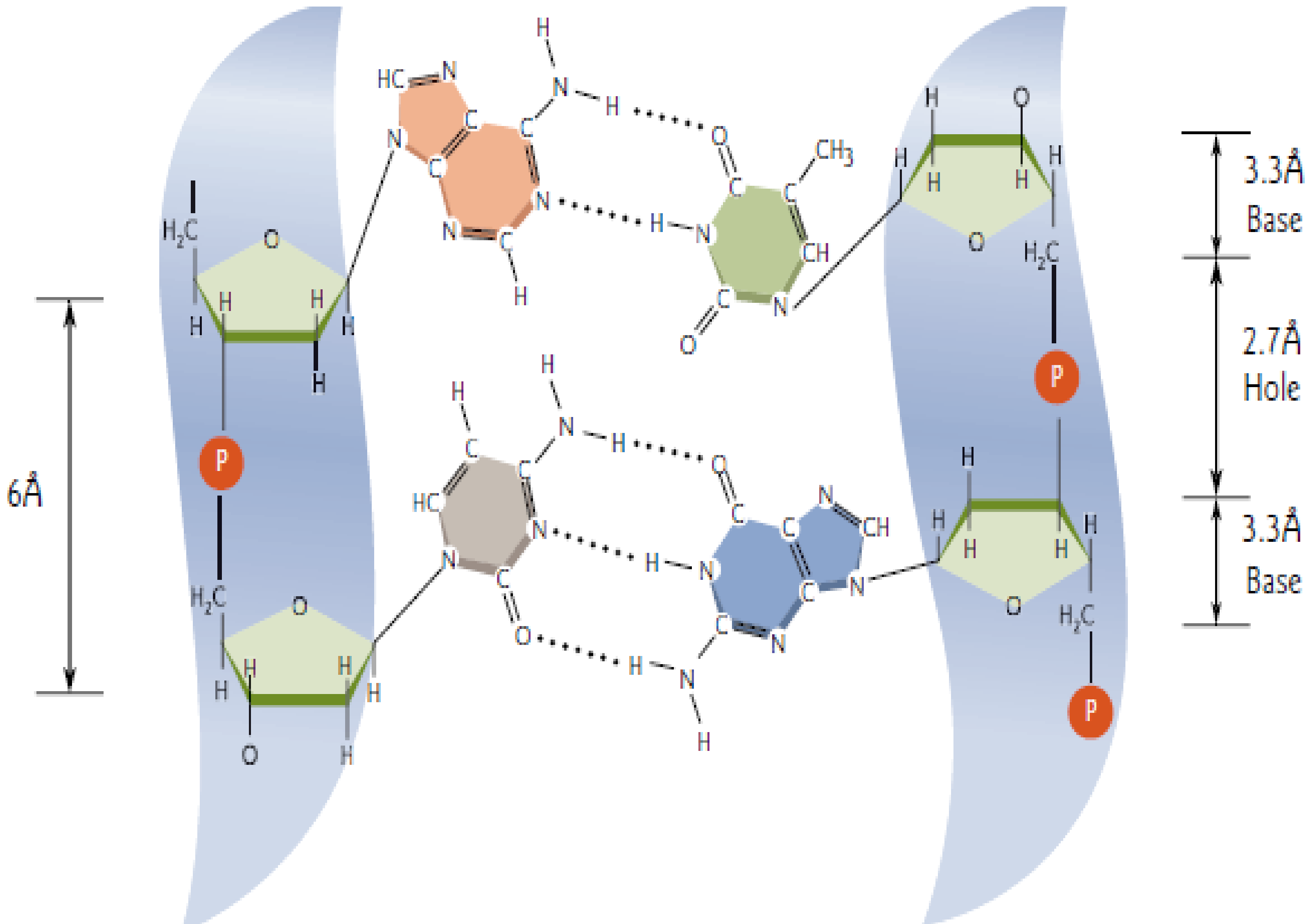
- They employed X ray crystallographic data of DNA obtained from **Rosalind Franklin** **and** Maurice Wilkins and Chargaff's observations.

- They concluded that

    - DNA is double helical.

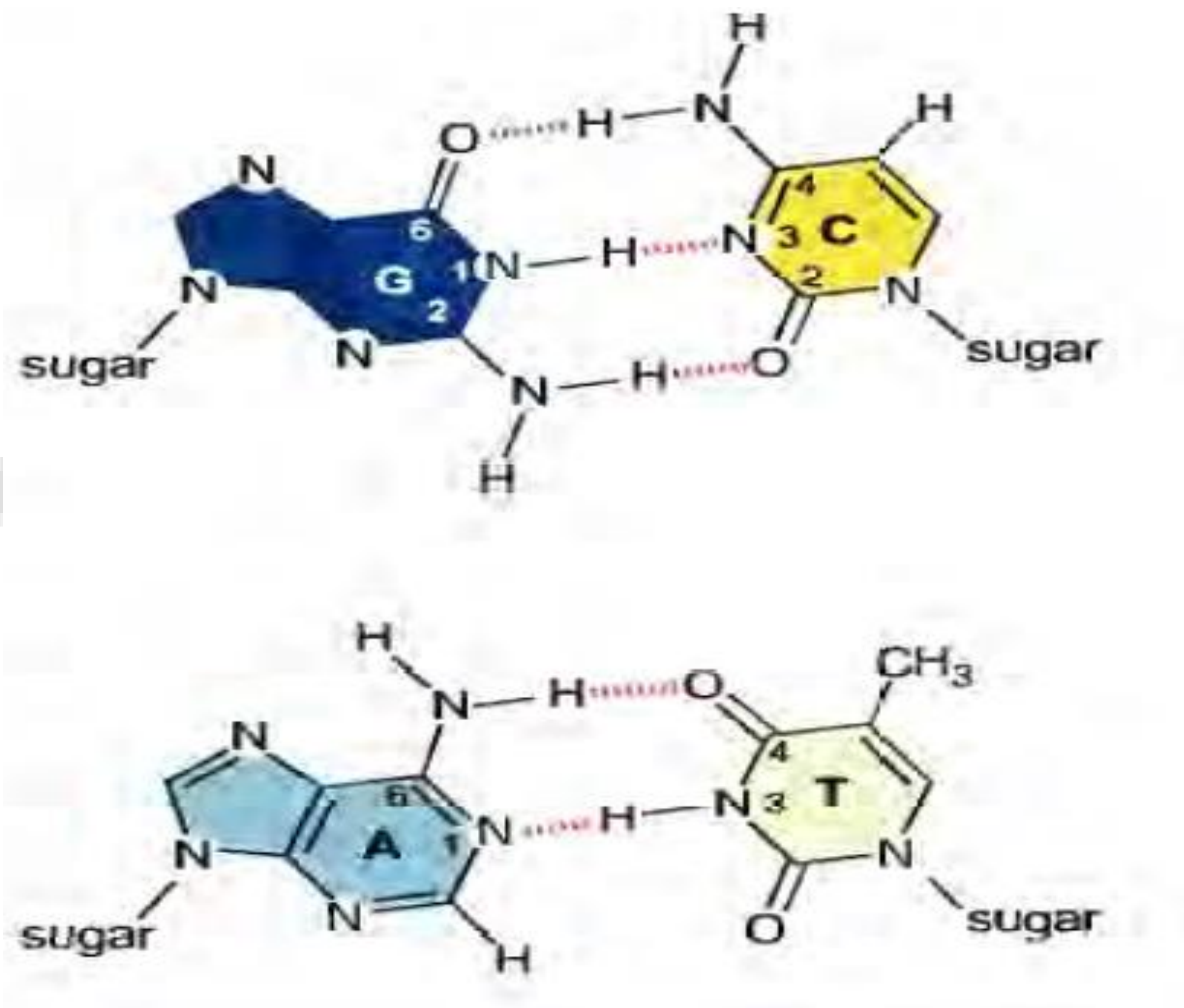
    - Two strands are anti-parallel.

    - Base pairing between two strands is specific *ie.* A base pairs with T and G base pairs with C and thus two strands are reverse complimentary



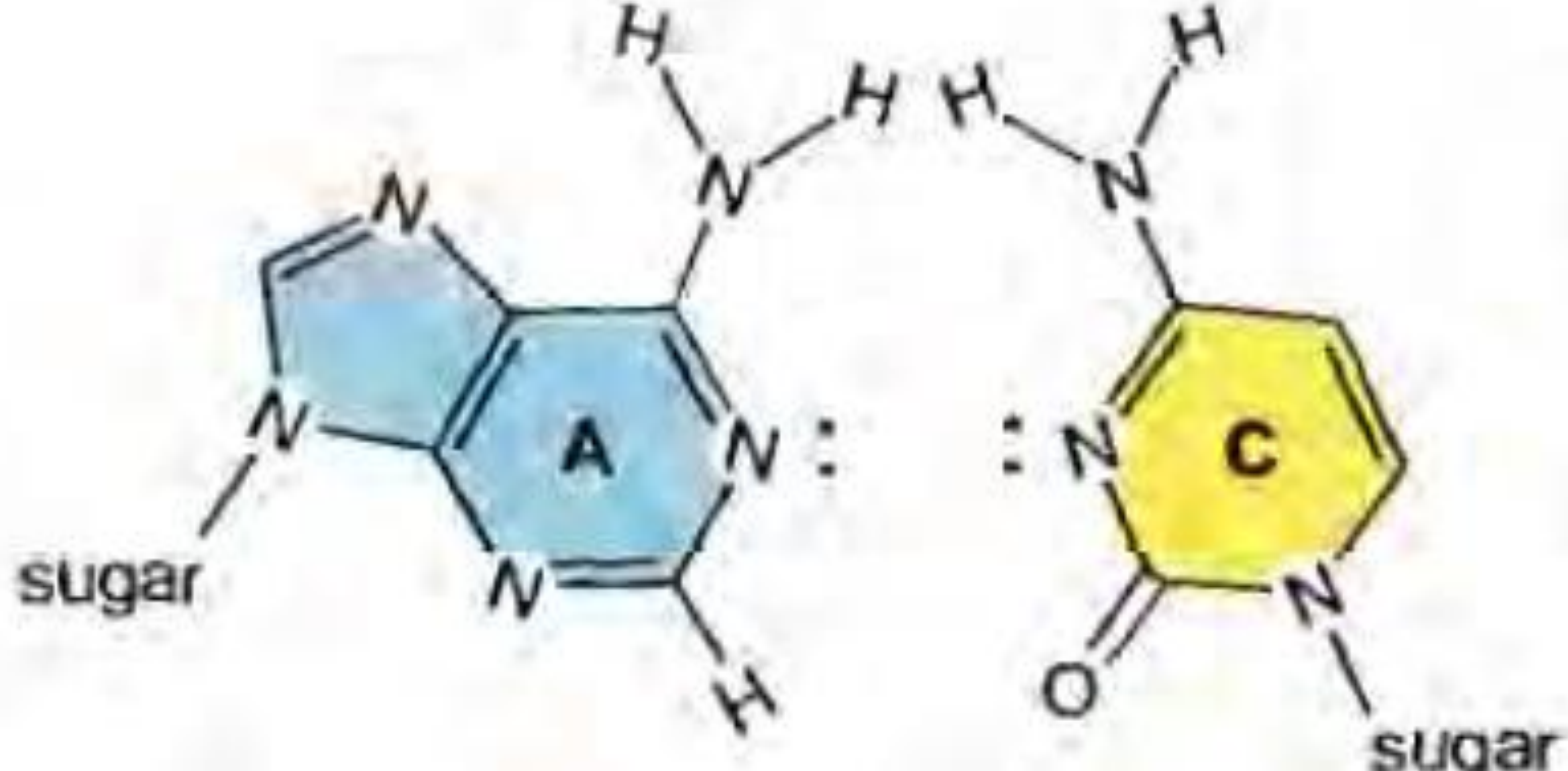
Two strands of DNA.

Note aniti-parallala orientation and specific base pairing



A:T and C:G base pairing

Molecular Biology of the Gene **Watson, J.D** (5ed)



A and C are incompatible to form H bonds

In the same manner G and T are incompatible

Molecular Biology of the Gene **Watson, J.D** (5ed)

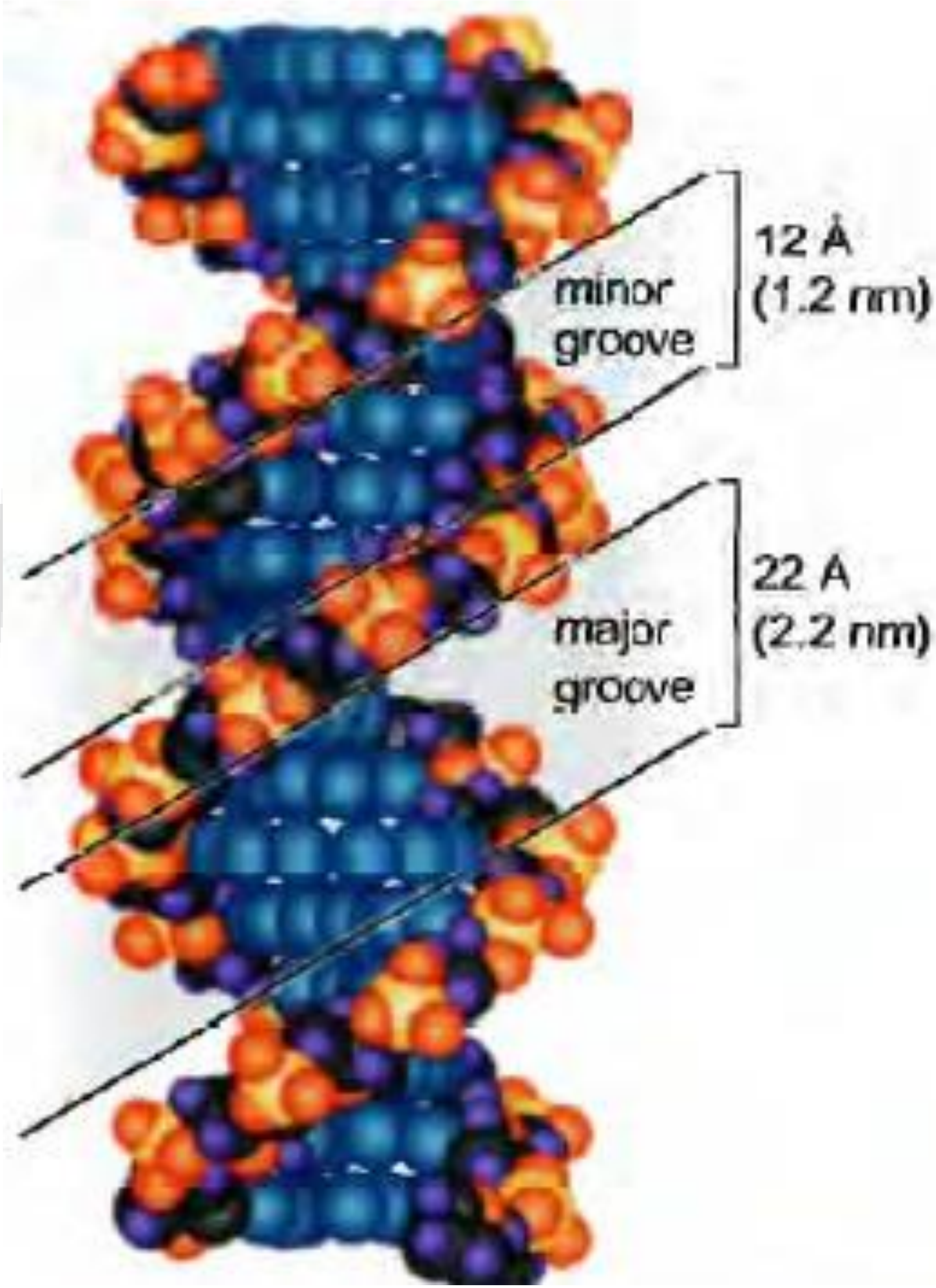
Importance of H Bonding

- H bond is the most important factor for thermodynamic stability of double helical structure.

- It also determine the specificity .

Grooves: Major and Minor

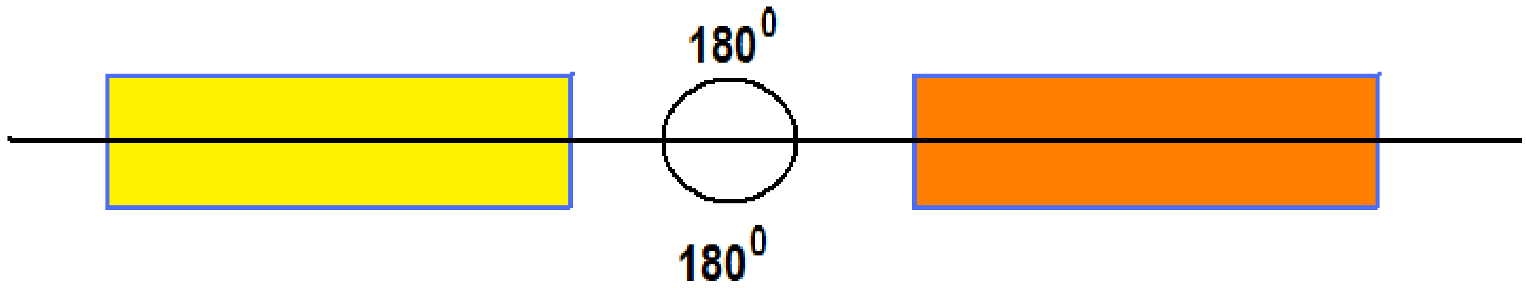
- As any double helical structures would have , DNA also has two grooves side by side.
- The two grooves are not equal.
- The wide groove is called the major groove and the narrow is called minor.

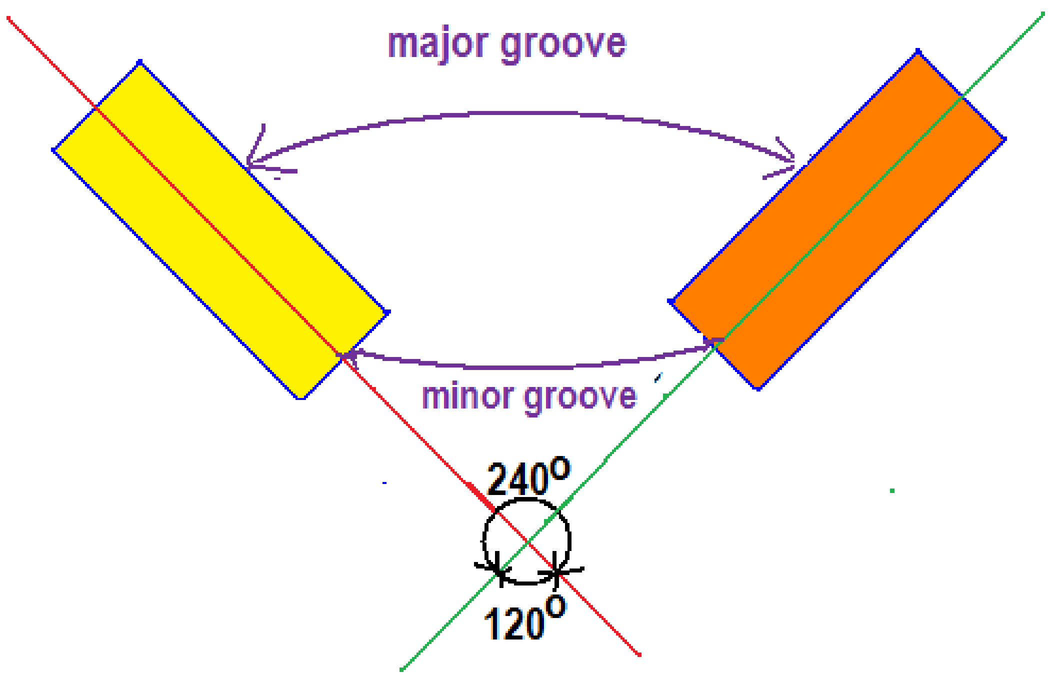

12 Å
(1.2 nm)
minor
groove
22 Å
(2.2 nm)
major
groove

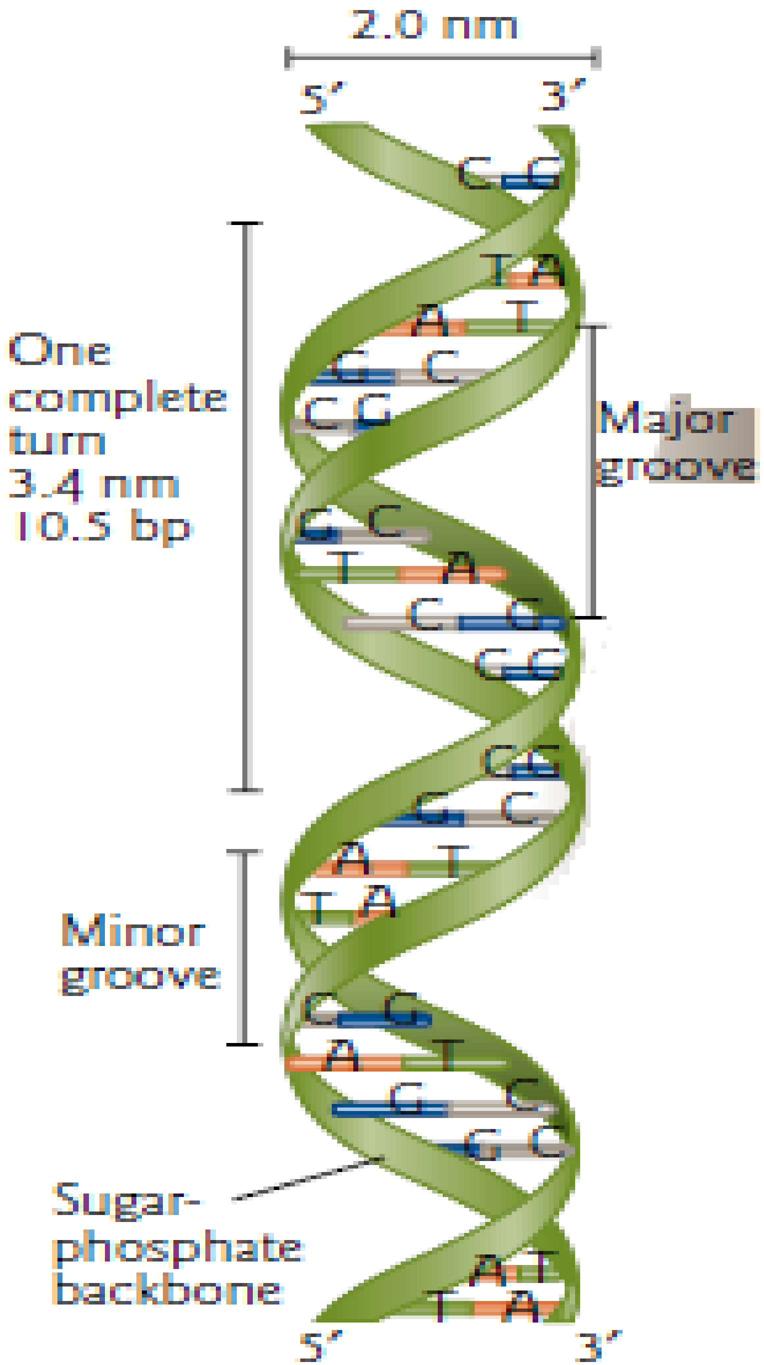
WHY MAJOR AND MINOR GROOVES ?

- Major and minor grooves are formed because two sugars of a base pair protrude with respect to each other by an wide angle of 240^0 and narrow angle of 120^0 .

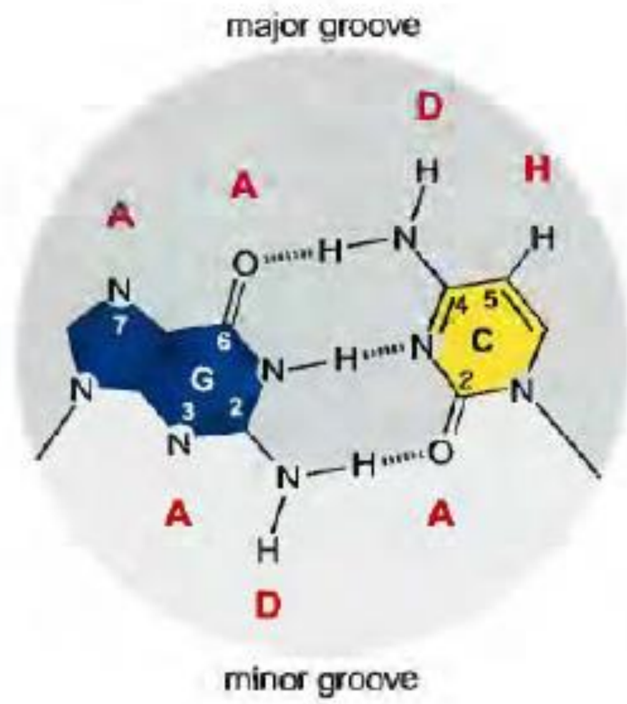
- Had two sugars protrude at an angle of 180^0 two grooves would have been equal.


180⁰
180⁰


major groove
minor groove
240°
120°


2.0 nm
5′
3′
One complete turn
3.4 nm
10.5 bp
Major groove
Minor groove
Sugar-phosphate backbone
5′
3′

Grooves Have Chemical Informations



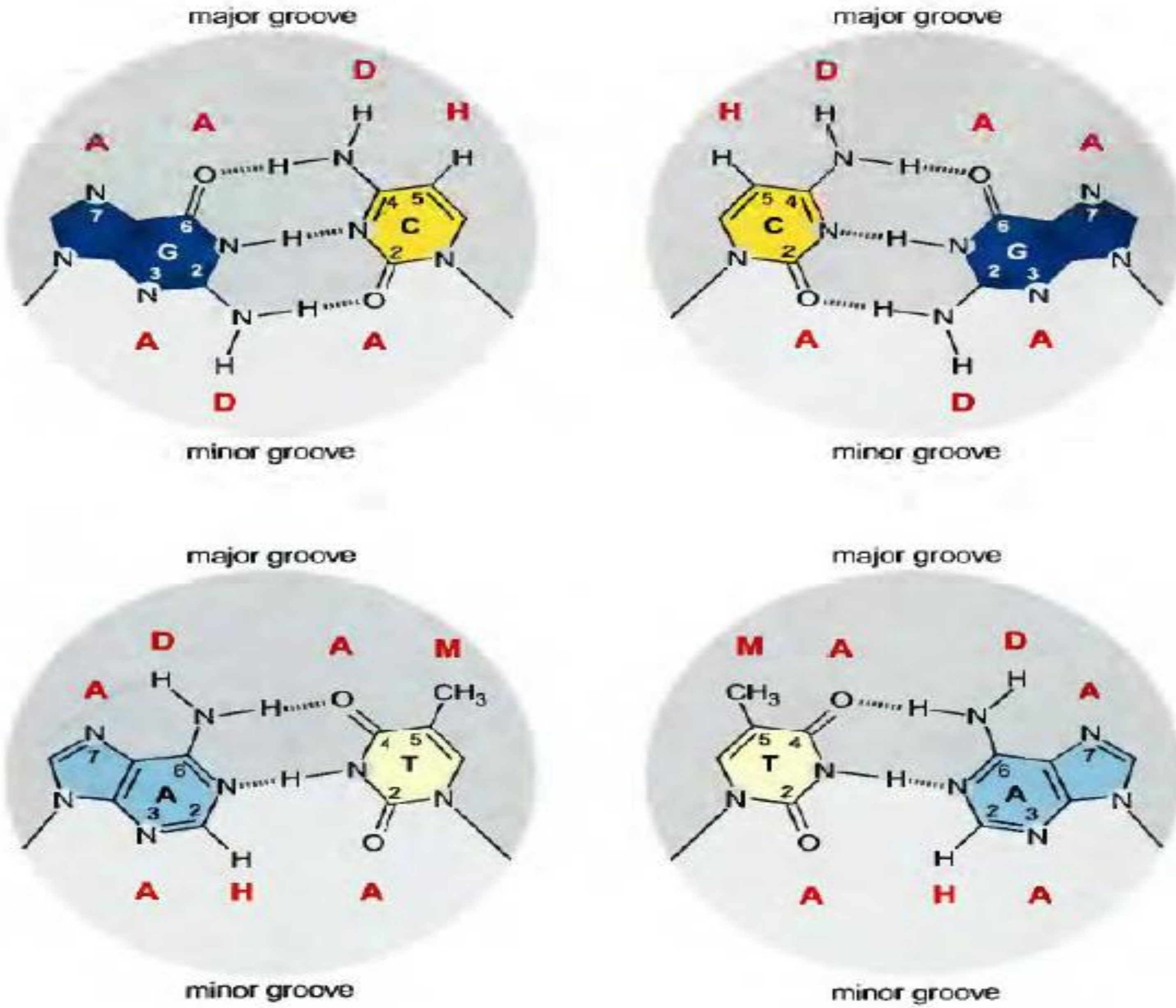
A = Hydrogen Bond Acceptor

D= Hydrogen Bond Donor

H= Nonpolar H atom

M= Mehyle group

Each base pair has a characteristic pattern of chemical information at major groove.



- Major Groove Has More Information

Molecular Biology of the Gene **Watson, J.D** (5ed)

- Major grove pattern A A D H Guanine: Cytosin (G:C)
- Major grove pattern H D A A   Cytosin :Guanine: (C:G)
- Major grove pattern A DA M Adinine: Thymine (A:T)
- Major grove pattern M A D A Thymine: Adinie (T:A)

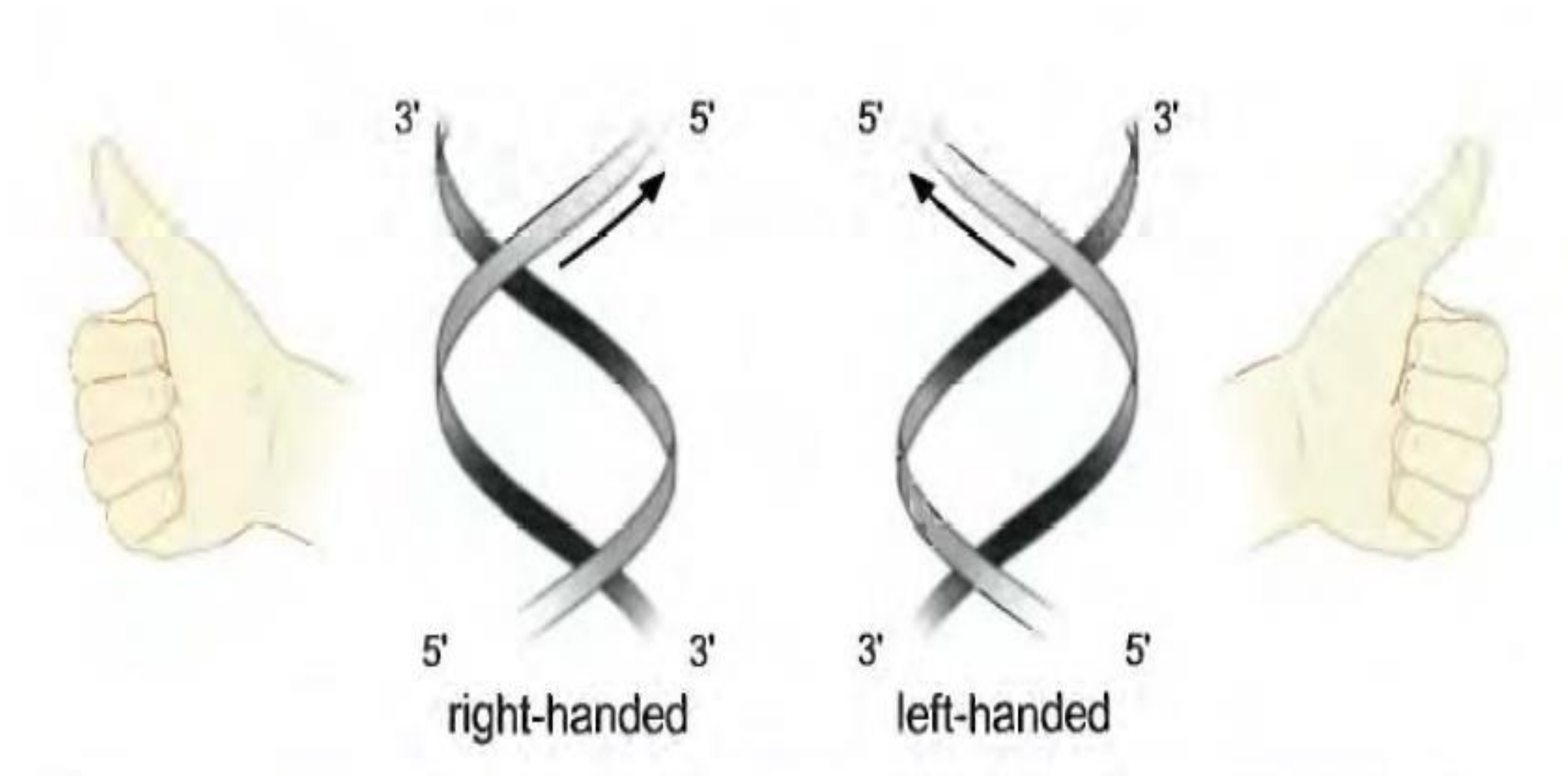
- A A D H
- Guanine: Cytosin (G:C)

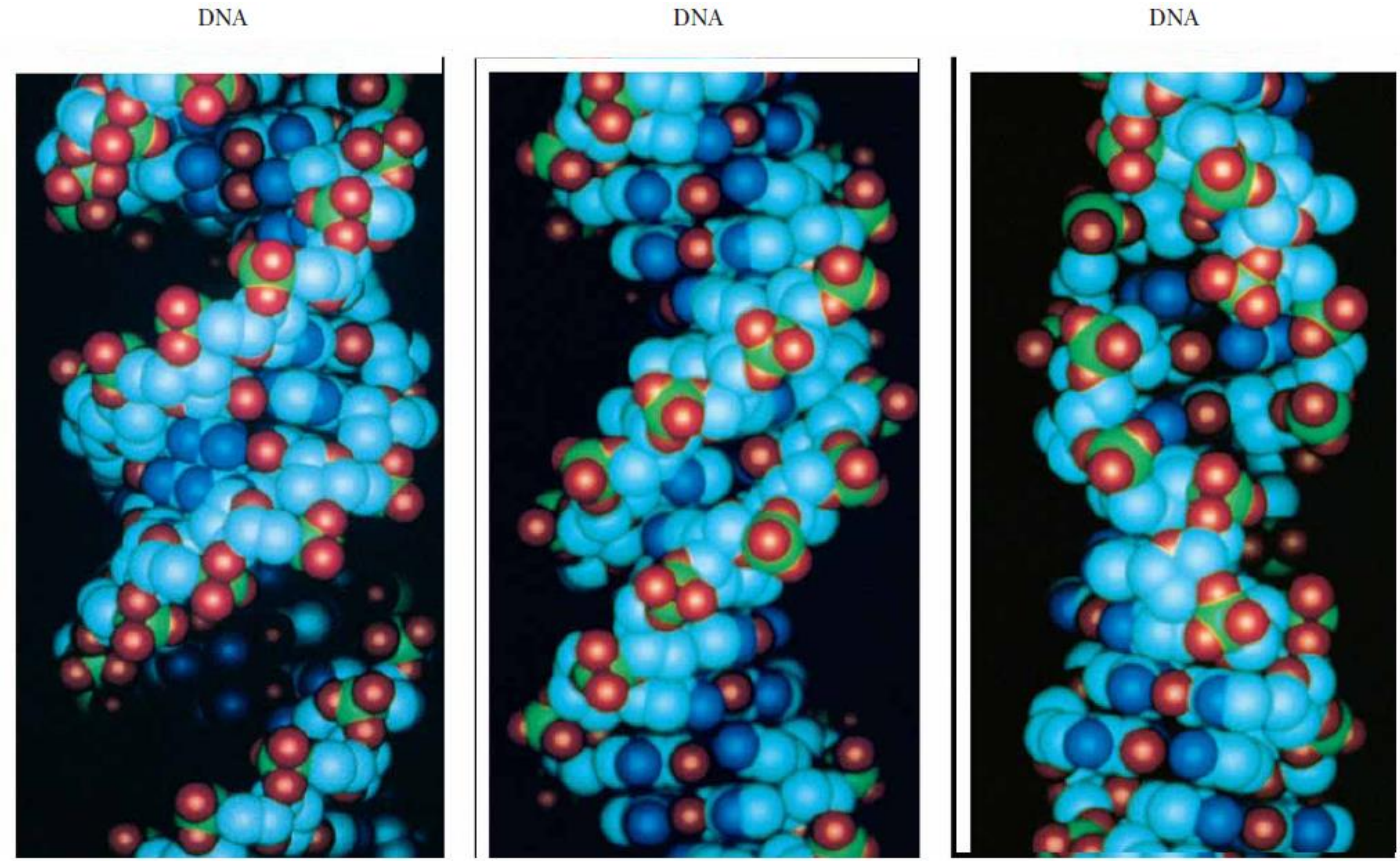
- H D A A
- Cytosin :Guanine: (C:G)

- A DA M
- Adinine: Thymine (A:T)

- M A D A
- Thymine: Adinie (T:A)

B-DNA the Right Handed DNA



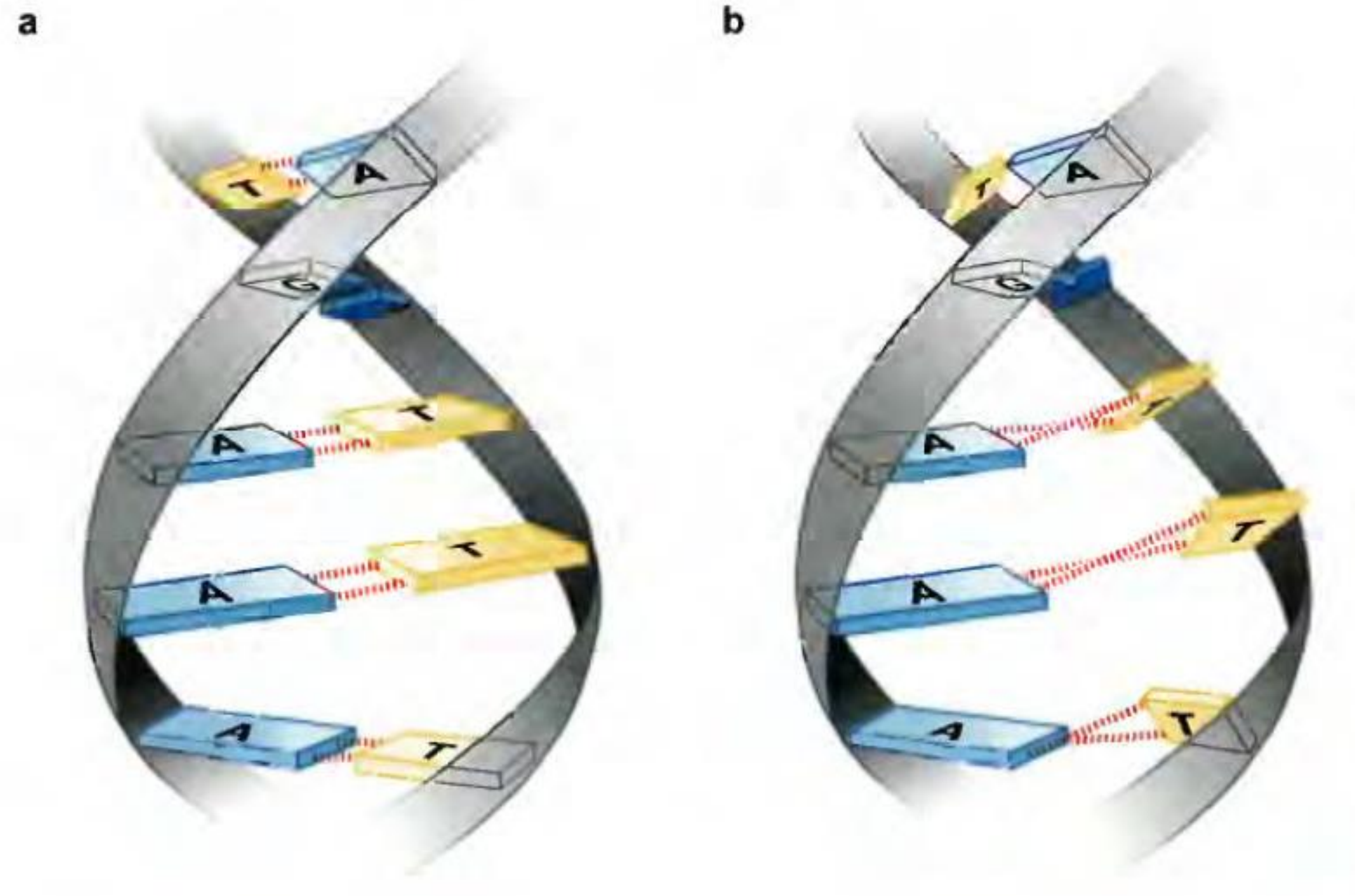


- Major and minor grooves in A, B and Z DNA

Alternative forms of DNA

	A-DNA	B-DNA	Z-DNA
Orientation	Right-handed	Right-handed	Left-handed
Major groove	Deep and narrow	Moderate depth, wide	Very shallow, virtually nonexistent, sometimes called a "single groove"
Minor groove	Shallow and broad (superficial)	Moderate depth, narrow	Very deep and narrow
Bases/turn	11	10.5	12
Conditions	Low humidity (75%), high salt	High humidity (95%), low salt	High $MgCl_2$ (> 3 M), NaCl, or ethanol In the presence of methylated cytosine: high humidity and low salt

* Other forms of DNA have been crystallized, including B′, C, C′, C″, D, E, and T. All of these are right-handed structures, and occur under unique conditions. For example, C-DNA forms in the presence of lithium salts and low humidity.



Propeller twist between purine and pyrimidene bases

Sourse: Agarwal etal. 1988 science 242: 899-907

Denaturation of DNA

- Two starnds of DNA can be sepatated by braking H bonds holding them.
- This can be achieved by gradually heating (up to about 100^{o} C) or by high pH conditions.
- This separation is called denaturation.
- It is reversible .

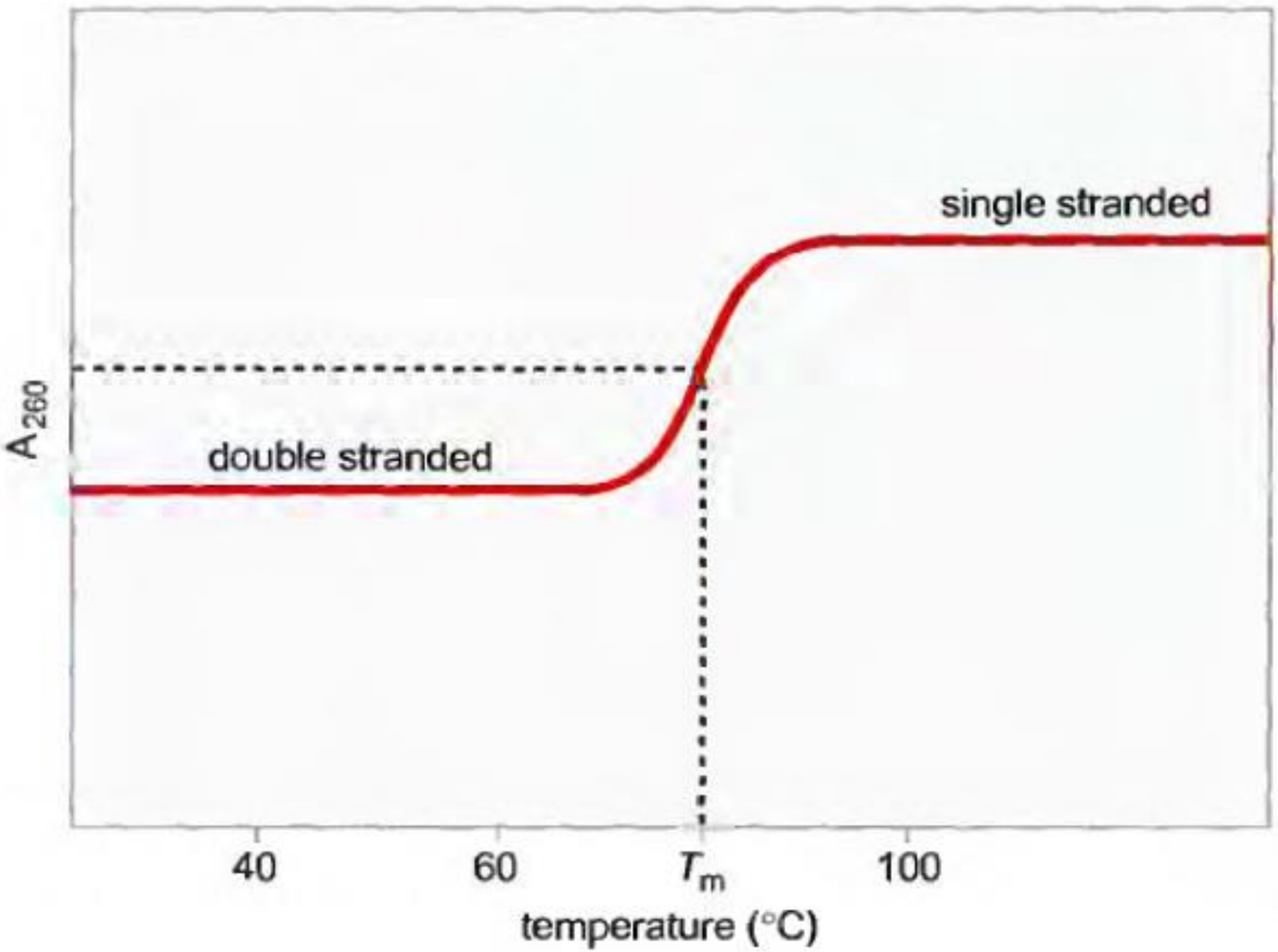
(if Denauration is achieved by increasing temperature the DNA is said to be Melt)

Hyperchromatic Shift

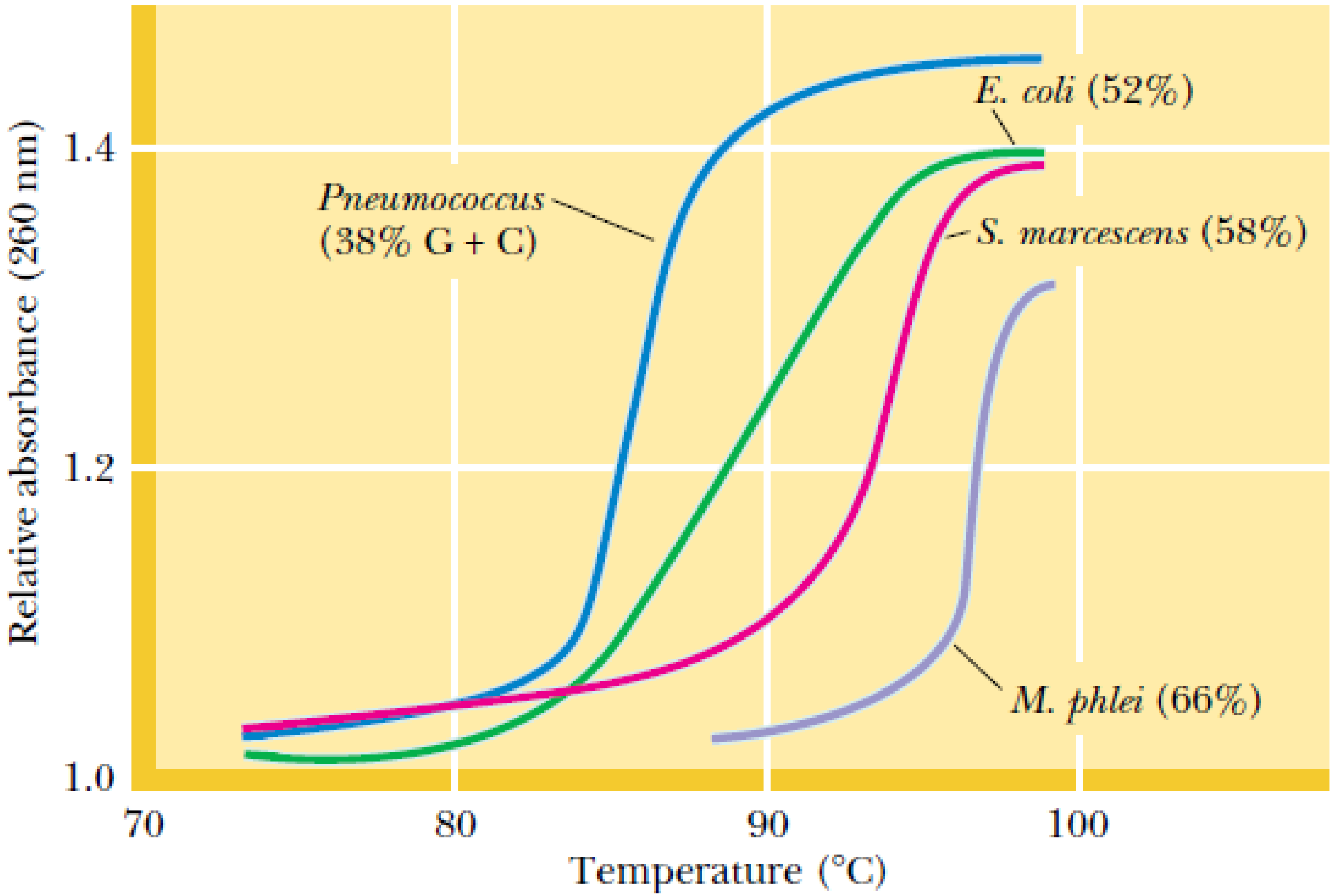
- The nitrogenous bases of DNA has absorbance for UV (at 260 nm).
- In double helix the absorbance is low (ie. DNA absorb less 260-nm radiation than expected for the nucleotide numbers) because π electron clouds is stacked together in the double helix.
- This absorbance is increased when DNA is denatured.
- The course of this dissociation can be followed spectrophotometrically.
- This increase in absorbance during denaturation is called **hyperchromic shift**

- The rise in absorbance coincides with strand separation.

- The midpoint of the absorbance increase is termed the **melting temperature, *Tm***



A typical DNA Denaturation Curve



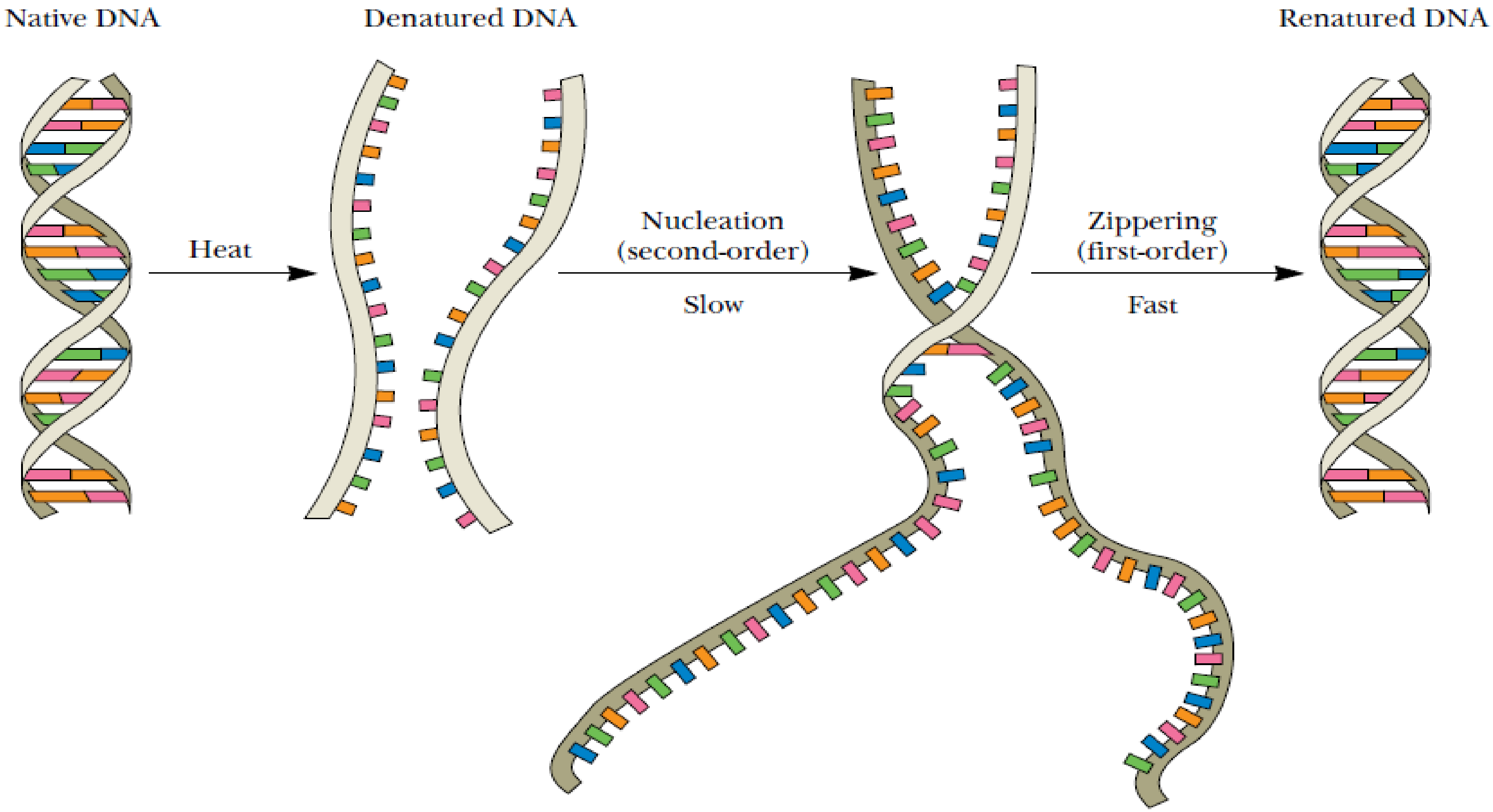
Melting Curves of DNA from Different Sources.

- DNAs differ in their *Tm values because they differ in relative* G+C content.
- The higher the G+C content of a DNA, the higher its melting temperature .
- *Tm also dependents on the ionic strength of the* solution.
- The lower the ionic strength, the lower the melting temperature. If dissolved in distilled water the DNA would melt even at the room temperature.

$$Tm = 69.3 + 0.41(\% G + C) \quad \text{at} \quad 0.2\ M\ Na^{+}$$

Renaturation of DNA

- Denatured DNA regains it’s duble helical structure if the conditions of denaturations are removed.
- This reassociation of the DNA strands is called reannealing.
- Many of the realignments are imperfect, and thus the strands must dissociate again to allow proper pairings .
- The process occurs more quickly if the temperature is warm enough to promote diffusion of the large DNA molecules but not so warm as to cause melting



- Steps in the thermal renaturation of DNA.

  1. Nucleation 2. Zippering

- The nucleation phase of the reaction is a second-order process depending upon sequence alignment of the two strands. This process takes place slowly.
- Once the sequences are aligned, the strands zipper up quickly.

Rates of Renaturation and DNA sequence complexity

- The rate of renaturation of DNA depends on its sequence complexity
- Strands with more complex sequences will take more time to reanneale.
- Thus for any given amount of DNA (in grams), sequences which are *more heterogeneous, (that is, more dissimilar) will take longer time to reanneale than sequences which are less heterogenous*

c_0t curves:
The Quantitative Analysis Of DNA Complexily

- Conceder, c , is the Concentration of single stranded DNA at time, t.
- The second order rate equation for the rate of decrease in c is given by

$$dc/dt = k_2c^2$$

where K_2 is the rate constant for second order reaction

- Starting with a concentration, c_0, of completely denatured DNA at t_0, *the amount of single-stranded DNA* remaining at some time *t is*

$$c/c_0 = 1/(1 + k_2c_0t)$$

- *The time* for half of the DNA to renature (when $c/c_0 = 0.5$) *is defined as* $t = t_{1/2}$.
- *Then,*

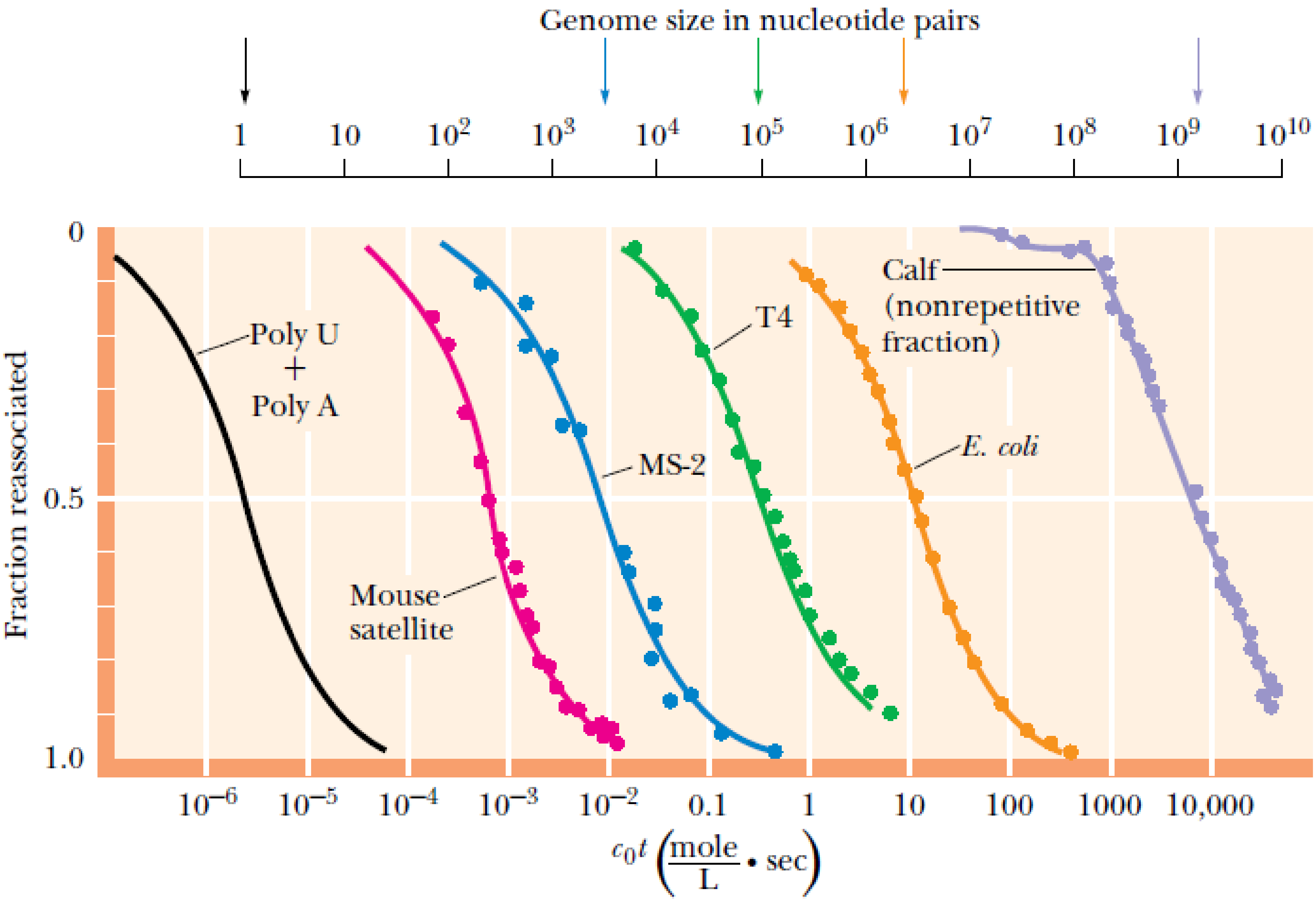
$$0.5 = 1/(1 + k_2c_0t_{1/2})$$

$$(1 + k_2c_0t_{1/2}) = 2$$

yielding

$$c_0t_{1/2} = 1/k_2$$

- A graph of the fraction of single-stranded DNA reannealed (c/c_0) *as a function* of c_0t *on a semi-logarithmic plot is referred to as a* **c_0t curve**



(From Britten, R. J., and Kohne, D. E., 1968. Science ***161:529–540.)***

NOTE

- relatively more complex DNAs take longer to renature.
- It is reflected by their greater $c_0t_{1/2}$ *values.*
- Thus the more is the compexity of DNA the greater is the value of $c_0t_{1/2}$

Nucleic Acid Hybridization

- If DNA from two different species are mixed, denatured, and allowed to cool slowly so that reannealing can occur, artificial **hybrid duplexes may form, provided** the DNA from one species is similar in nucleotide sequence to the DNA of the other.

- The degree of hybridization is a measure of the sequence similarity or *relatedness between the two species.*

Significance of Nucleic Acid Hybridization

- it can reveal evolutionary relationships
- it gives researchers the power to identify specific genes selectively against a vast background of irrelevant genetic material by using probe.
- **Probe is a**n labeled oligo- or polynucleotide, c**onstructed so that its sequence is** complementary to a target gene.

we shall continue to discuss further the DNA structure its Topology and Super-coiling some other Day

END