E-content for

B.sc. Part-III Zoology Honours

Paper VII: Group A- Molecular Biology
Topic: Nucleic acid

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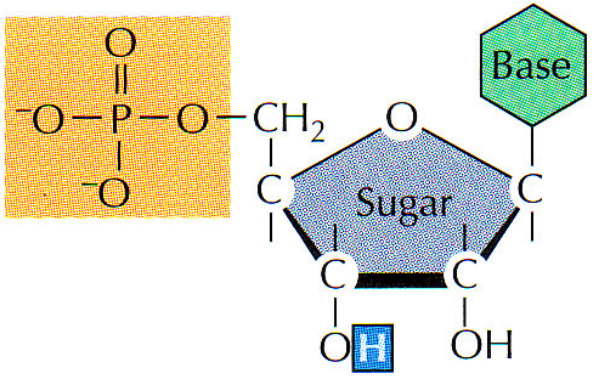
Nucleic acids

- **Principle information molecule in the cell.**
- **All the genetic codes are carried out on the nucleic acids.**
- **Nucleic acid is a linear polymer of nucleotides**

Nucleotides

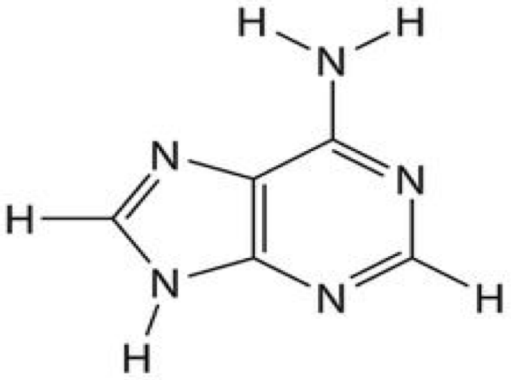
- **Nucleotides are the unit structure of nucleic acids.**
- **Nucleotides composed of 3 components:**
  - **Nitrogenous base (A, C, G, T or U)**
  - **Pentose sugar**
  - **Phosphate**



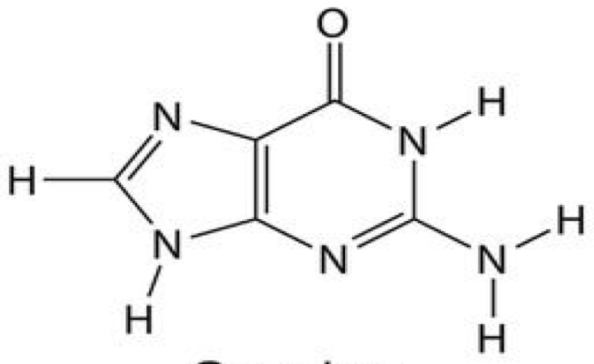
Nitrogenous bases

- There are 2 types:
  - Purines:
    - Two ring structure
    - Adenine (A) and Guanine (G)
  - Pyrimidines:
    - Single ring structure
    - Cytosine (C) and Thymine (T) or Uracil (U).

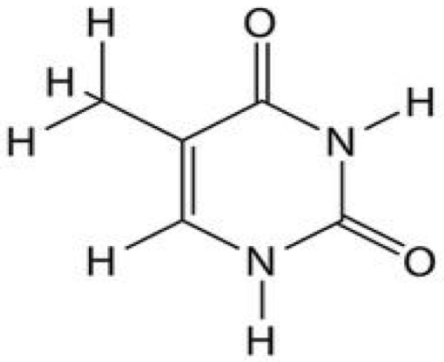
Nucleotide bases



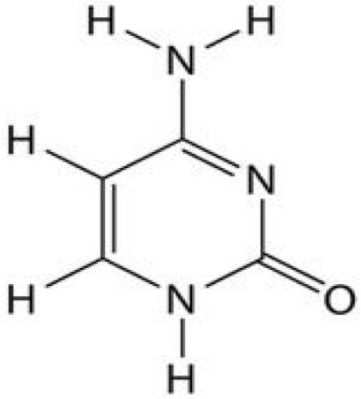
Adenine



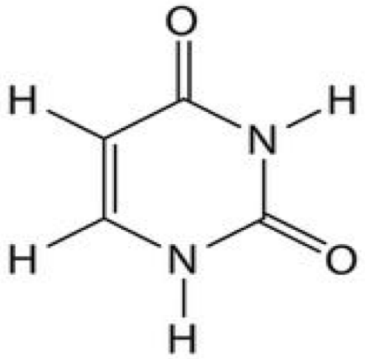
Guanine



Thymine



Cytosine

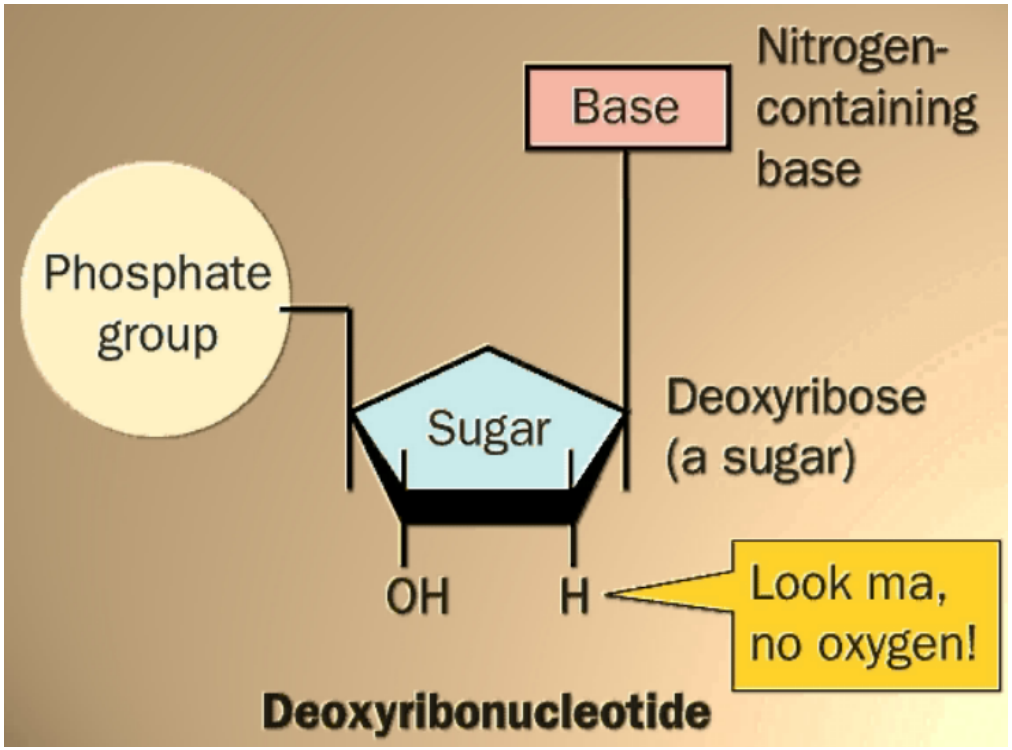


Uracil

Types of Nucleic acids

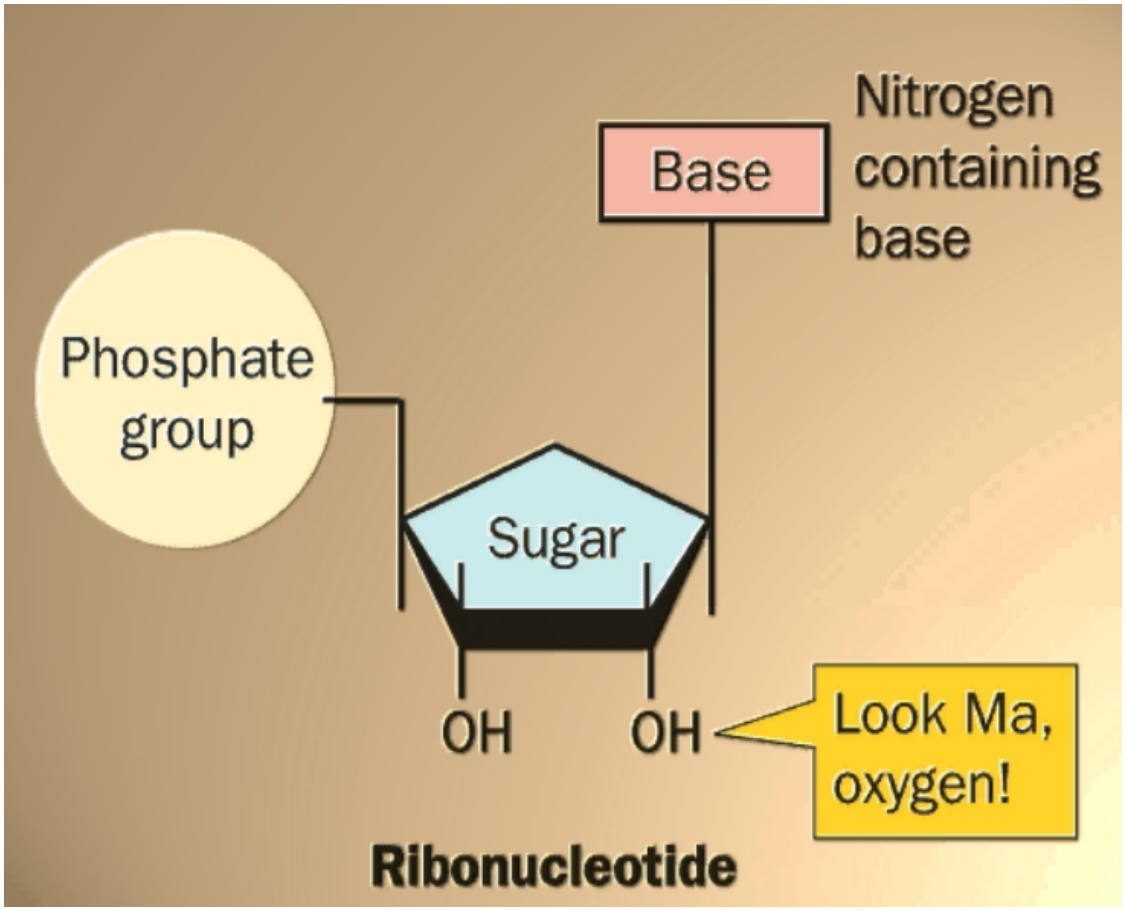
There are 2 types of nucleic acids:

1. <u>Deoxy-ribonucleic acid</u> (DNA)
   - Pentose Sugar is deoxyribose (no OH at 2' position)
   - Bases are Purines (A, G) and Pyrimidine (C, T).



2. Ribonucleic acid (RNA)

- **Pentose Sugar is Ribose.**
- **Bases are Purines (A, G) and Pyrimidines (C, U).**



Linear Polymerization of Nucleotides

- **Nucleic acids are formed of nucleotide polymers.**
- **Nucleotides polymerize together by *phospho-diester bonds* via condensation reaction.**
- **The phospho-diester bond is formed between:**
  - **Hydroxyl (OH) group of the sugar of one nucleotide.**
  - **Phosphate group of other nucleotide**

Polymerization of Nucleotides

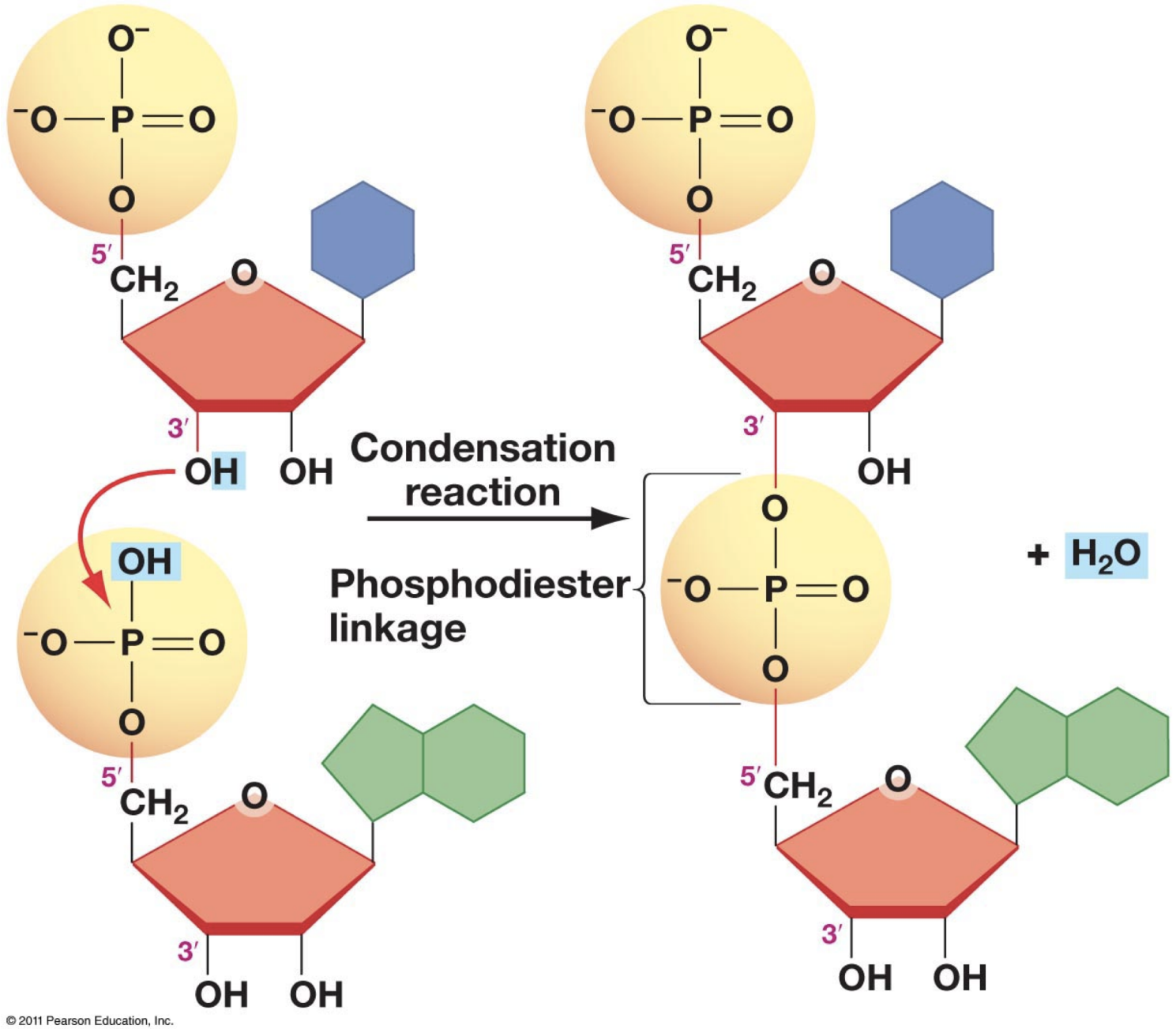
- The formed polynucleotide chain is formed of:
  - Negative (-ve) charged Sugar-Phosphate backbone.
    - Free 5’ phosphate on one end (5’ end)
    - Free 3’ hydroxyl on other end (3’ end)

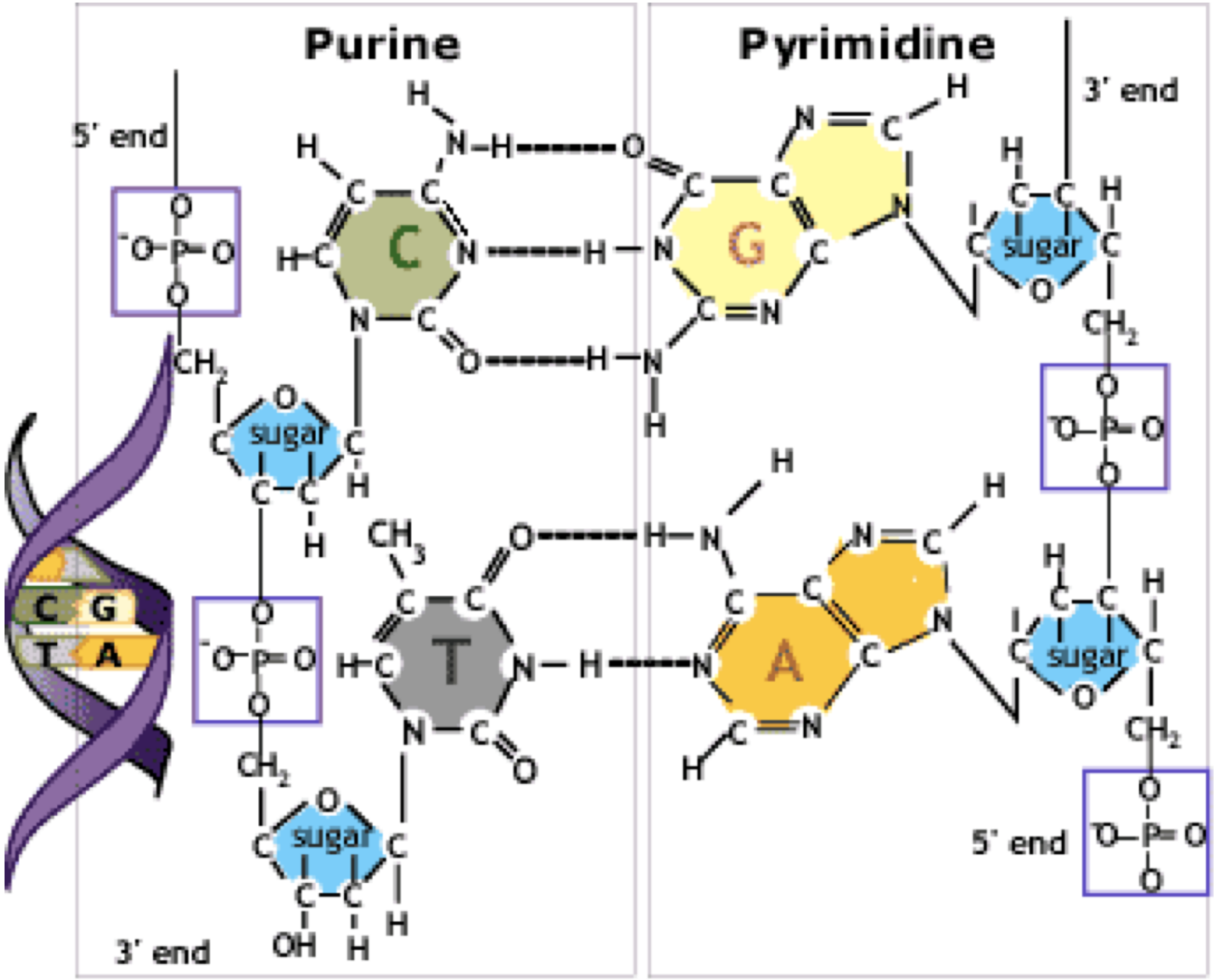
  - Nitrogenous bases are not in the backbone
    - Attached to the backbone
    - Free to pair with nitrogenous bases of other polynucleotide chain

Polymerization of Nucleotides

- **Nucleic acids are polymers of nucleotides.**
- **The nucleotides formed of purine or pyrimedine bases linked to *phosphorylated sugars* (nucleotide back bone).**
- **The bases are linked to the pentose sugar to form *Nucleoside*.**
- **The nucleotides contain one phosphate group linked to the 5' carbon of the nucleoside.**

Nucleotide = Nucleoside + Phosphate group




Purine
Pyrimidine
5' end
3' end
C
G
T
A
sugar
C G
T A
3' end
5' end

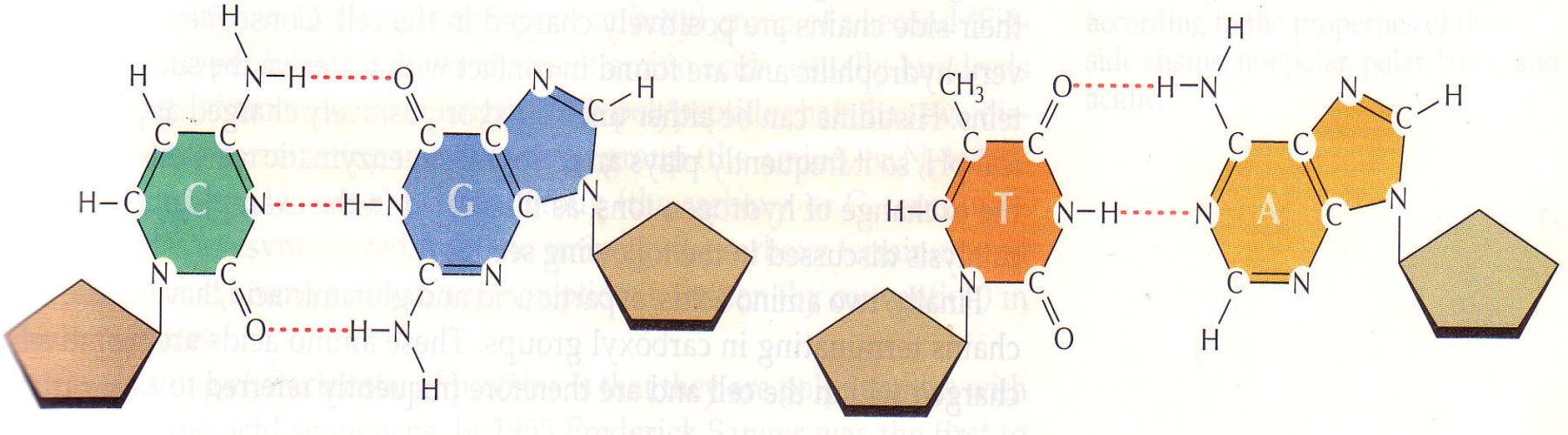
- **The polymerization of nucleotides to form nucleic acids occur by condensation reaction by making phospho-diester bond between 5' phosphate group of one nucleotide and 3' hydroxyl group of another nucleotide.**

- **Polynucleotide chains are always synthesized in the 5' to 3' direction, with a free nucleotide being added to the 3' OH group of a growing chain.**

Complementary base pairing

- It is the most important structural feature of nucleic acids
- It connects bases of one polynucleotide chain (nucleotide polymer) with complementary bases of other chain
- Complementary bases are bonded together via:
  - Double hydrogen bond between A and T (DNA), A and U (RNA) (A═T or A═U)
  - Triple H-bond between G and C in both DNA or RNA (G≡C)

Base pairing



Significance of complementary base pairing

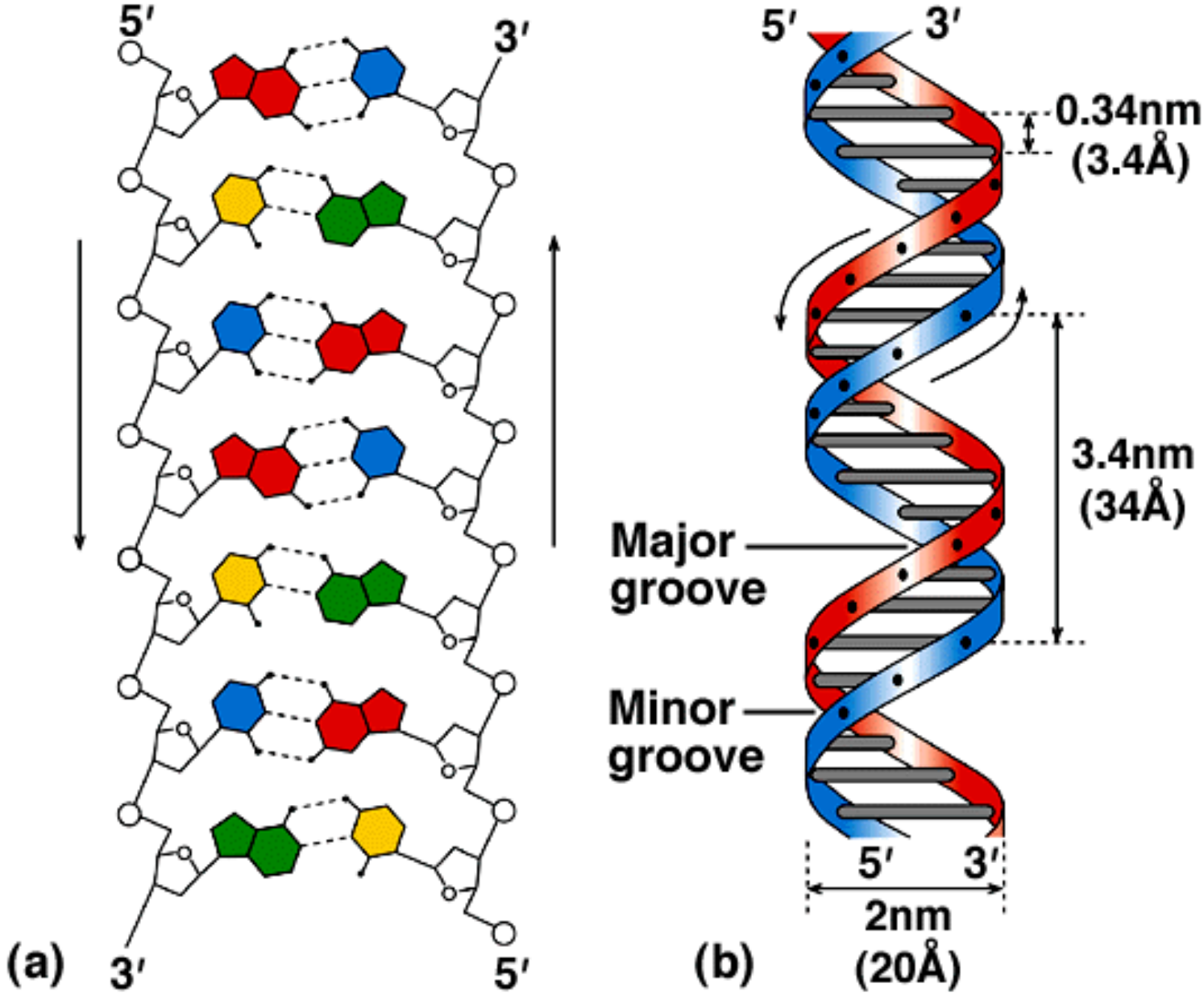
- **The importance of such complementary base pairing is that each strand of DNA can act as template to direct the synthesis of other strand similar to its complementary one.**

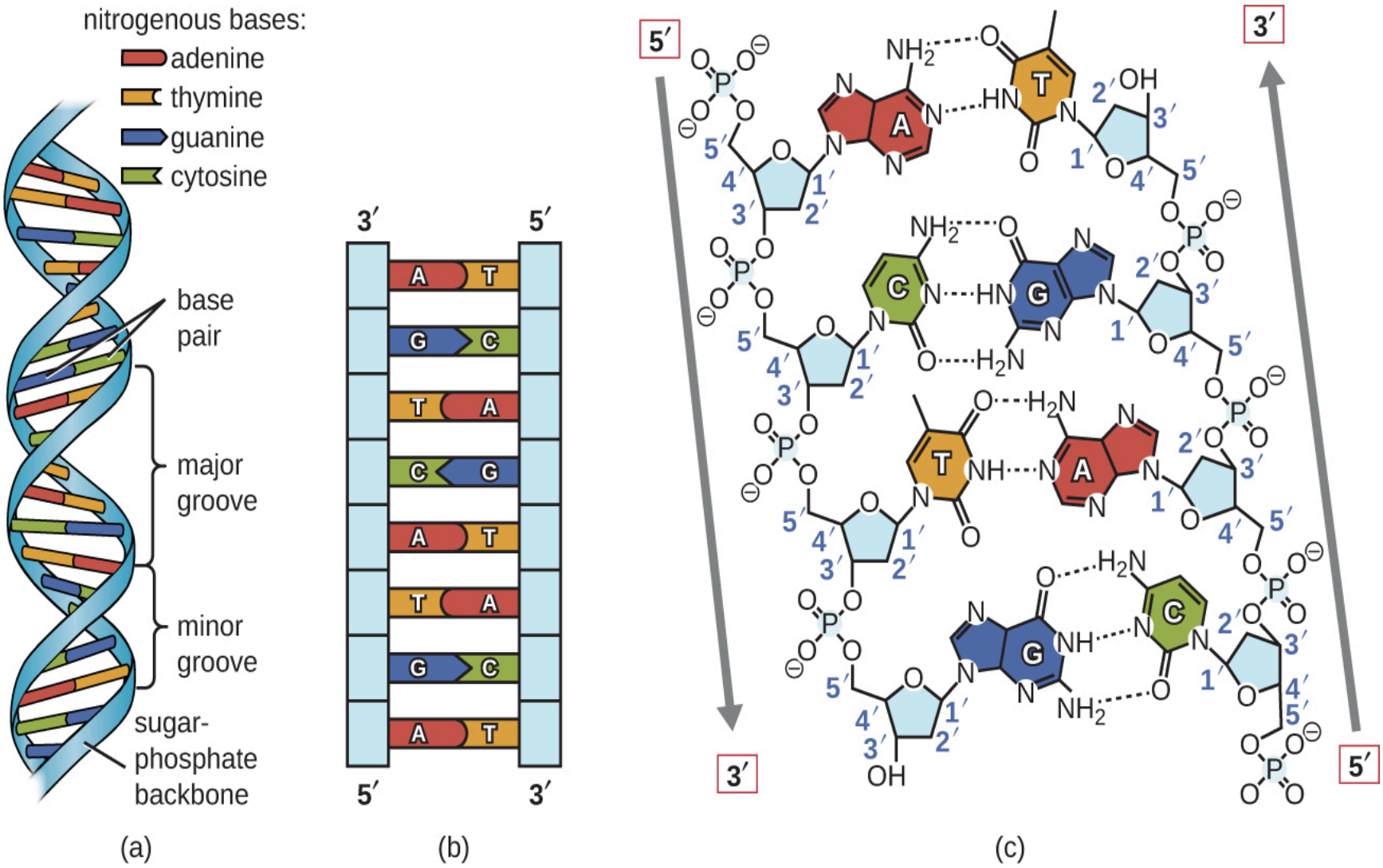
- **Thus *nucleic acids are uniquely capable of directing their own self replication*.**

- **The information carried by DNA and RNA direct the synthesis of specific proteins which control most cellular activities.**

DNA structure

- DNA is a double stranded molecule consists of 2 polynucleotide chains running in opposite directions.
- Both strands are complementary to each other.
- The bases are on the inside of the molecules and the 2 chains are joined together by double H-bond between A and T and triple H-bond between C and G.
- The base pairing is very specific which make the 2 strands complementary to each other.
- So each strand contain all the required information for synthesis (replication) of a new copy to its complementary.


5′
3′
3′
5′
(a)
5′
3′
0.34nm
(3.4Å)
3.4nm
(34Å)
Major
groove
Minor
groove
5′
3′
2nm
(20Å)
(b)


nitrogenous bases:
adenine
thymine
guanine
cytosine
base pair
major groove
minor groove
sugar-phosphate backbone
(a)
3′
5′
A T
G C
T A
C G
A T
T A
G C
A T
5′
3′
(b)
5′
3′
3′
5′
(c)

Forms of DNA

1- ***B-form helix***:

- It is the most common form of DNA in cells.
  - Right-handed helix
  - Turn every 3.4 nm.
  - Each turn contain 10 base pairs (the distance between each 2 successive bases is 0.34 nm)
  - Contain 2 grooves;
    - Major groove (wide): provide easy access to bases
    - Minor groove (narrow): provide poor access.

2- *A-form DNA*:

- Less common form of DNA , more common in RNA
  - Right handed helix
  - Each turn contain 11 b.p/turn
  - Contain 2 different grooves:
    - Major groove: very deep and narrow
    - Minor groove: very shallow and wide (binding site for RNA)

3- *Z-form DNA*:

- Radical change of B-form
  - Left handed helix, very extended
  - It is GC rich DNA regions.
  - The sugar base backbone form Zig-Zag shape
  - The B to Z transition of DNA molecule may play a role in gene regulation.

Denaturing and Annealing of DNA

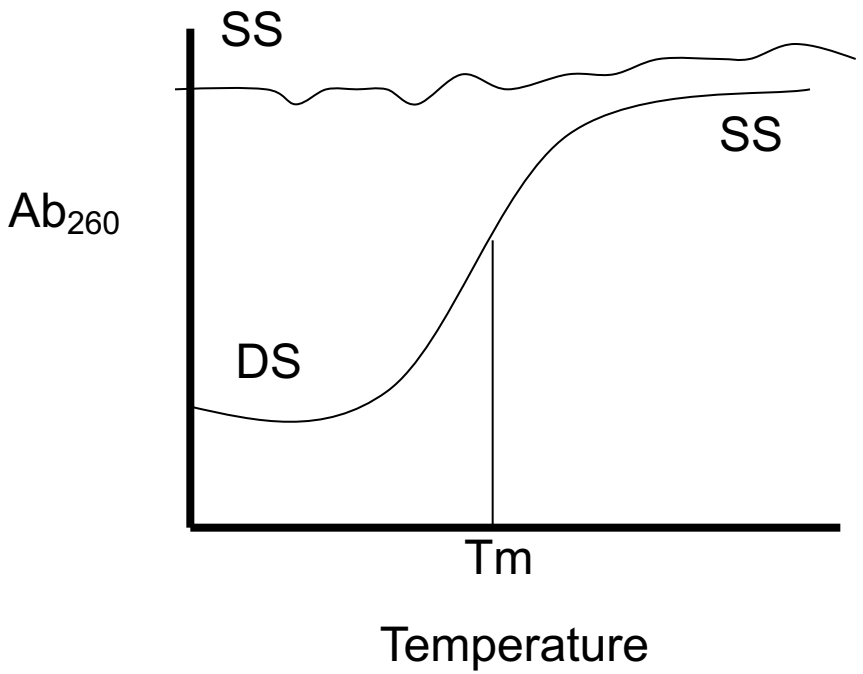
- **The DNA double strands can denatured if heated (95ºC) or treated with chemicals.**
  - **AT regions denature first (2 H bonds)**
  - **GC regions denature last (3 H bonds)**
- **DNA denaturation is a reversible process, as denatured strands can re-annealed again if cooled.**
- **This process can be monitored using the hyperchromicity (melting profile).**

Hyperchromicity (melting profile)

- It is used to monitor the DNA denaturation and annealing.
- It is based on the fact that single stranded (SS) DNA gives higher absorbtion reading than double stranded (DS) at wavelength 260º.
- Using melting profile we can differentiate between single stranded and double stranded DNA.

Hyperchromicity (melting profile)

Using melting profile we can differentiate between SS DNA and DS DNA



Tm (*melting temp.*): temp. at which 50% of DS DNA denatured to SS

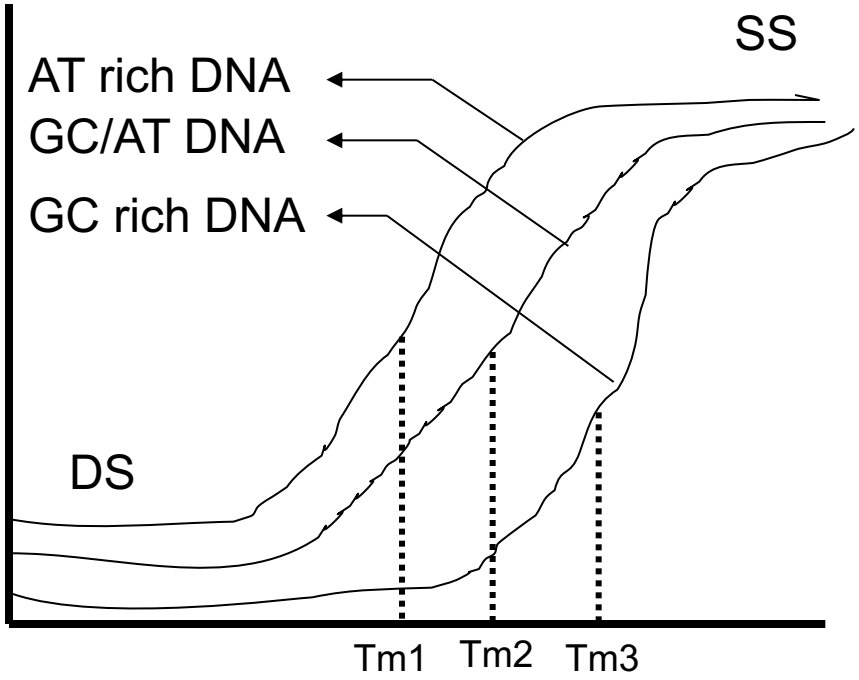
- Heating of SS DNA: little rise of Ab reading
- Heating of DS DNA: high rise of Ab reading

- **Melting profile can be also used to give an idea about the type of base pair rich areas using the fact that:**
  - **A═T rich regions: denatured first (low melting point)**
  - **G≡C rich regions: denatured last (higher melting point)**

Tm1: Small melting temp. of AT rich DNA

Tm2: higher melting temp. of AT/GC equal DNA

Tm3: Highest melting temp. of GC rich DNA



Importance of T_m

- Critical importance in any technique that relies on complementary base pairing
  - Designing PCR primers
  - Southern blots
  - Northern blots
  - Colony hybridization

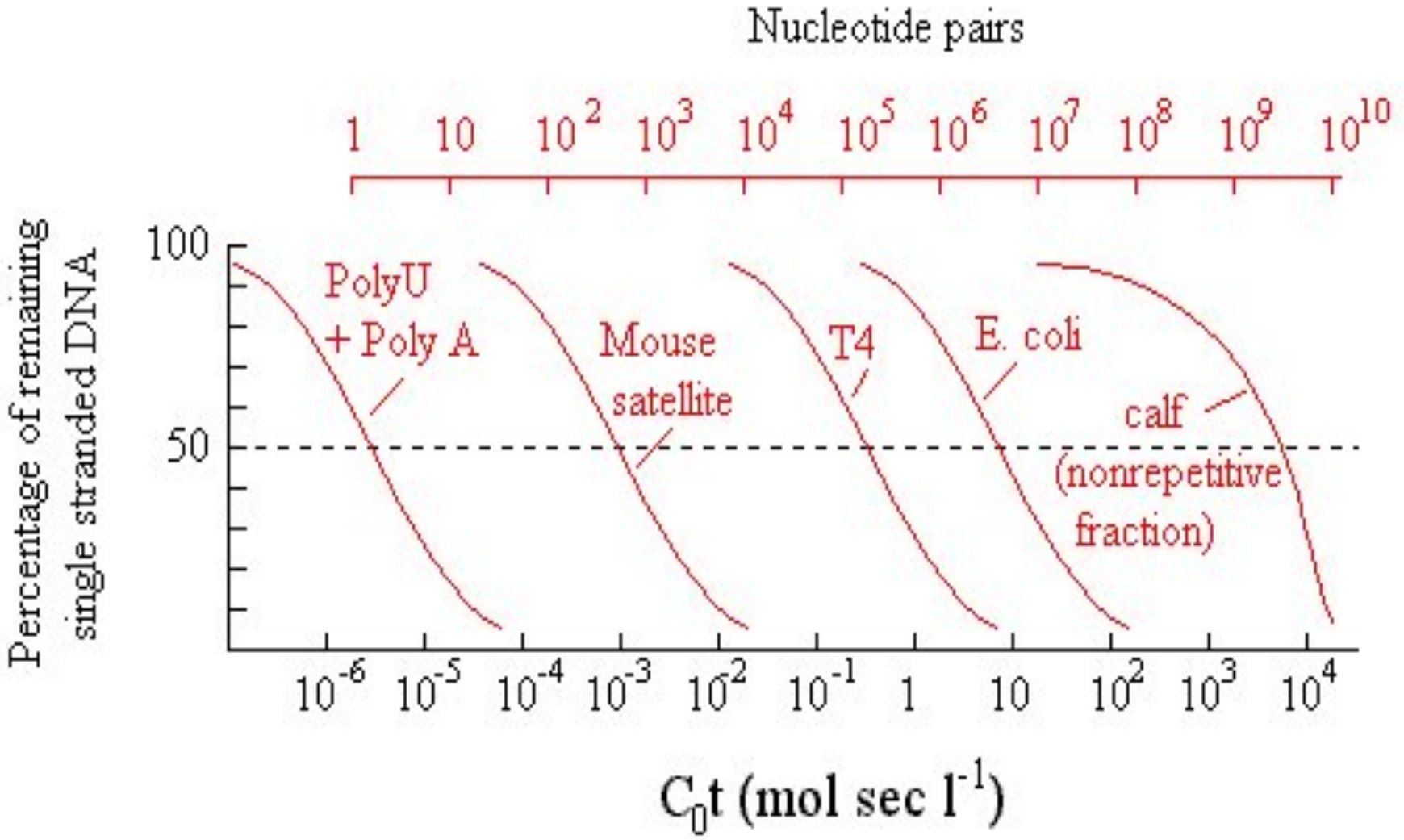
Renaturation

- Strands can be induced to renature (anneal) under proper conditions. Factors to consider:
  - Temperature
  - Salt concentration
  - DNA concentration
  - Time

Factors Affecting T_m

- G-C content of sample
- Presence of intercalating agents (anything that disrupts H-bonds or base stacking)
- Salt concentration
- pH
- Length

$C_o t$ Curves


Nucleotide pairs
1
10
10^2
10^3
10^4
10^5
10^6
10^7
10^8
10^9
10^10
Percentage of remaining single stranded DNA
100
50
PolyU + Poly A
Mouse satellite
T4
E. coli
calf (nonrepetitive fraction)
10^-6
10^-5
10^-4
10^-3
10^-2
10^-1
1
10
10^2
10^3
10^4
C0t (mol sec l-1)

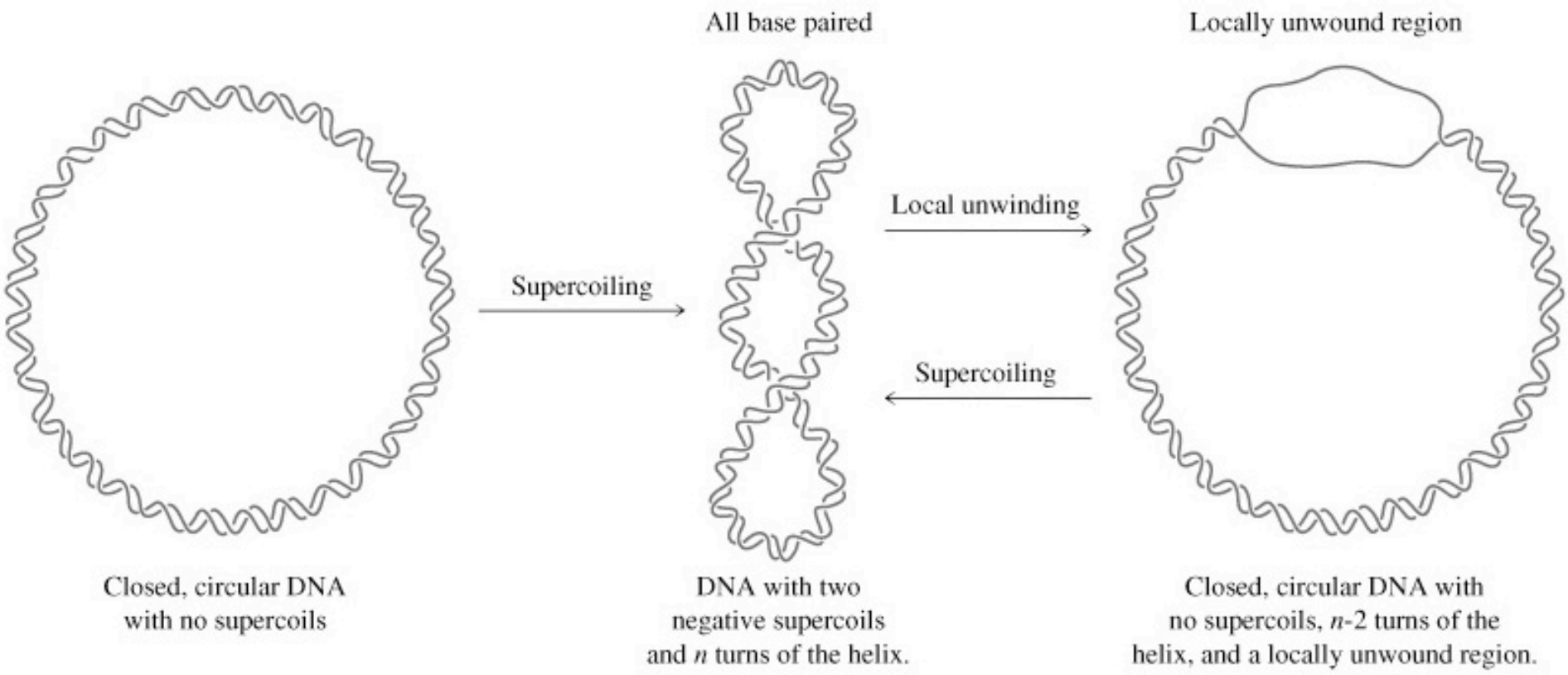
What Do $C_o t$ Curves Reveal?

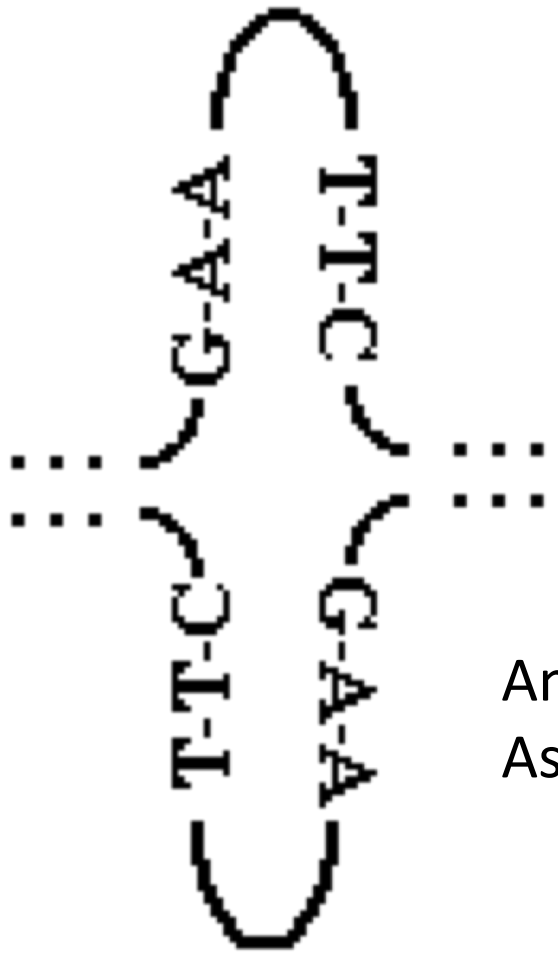
- Complexity of DNA sample
- Reveals important info about the physical structure of DNA
- Can be used to determine T_m for techniques that complementary base pairing.

DNA is Dynamic

- Like proteins, DNA has 3º structure
- Why so many deviations from normal conformation?
  - Effects on transcription (gene expression)
  - Enhances responsiveness
  - May also serve in packaging
- NOTE: most cellular DNA exists as protein containing supercoils

Supercoiling





Cruciform Structures

Another adaptation to supercoiling
Associated with palindromes

Negative and positive supercoils

- **Negative** supercoils twist the DNA about its axis in the opposite direction from the clockwise turns of the right-handed (R-H) double helix.
  - **Underwound** (favors unwinding of duplex).
  - Has right-handed supercoil turns.
- **Positive** supercoils twist the DNA in the same direction as the turns of the R-H double helix.
  - **Overwound** (helix is wound more tightly).
  - Has left-handed supercoil turns.

Components of DNA Topology : Twist

- The clockwise turns of R-H double helix generate a positive Twist (T).
- The counterclockwise turns of L-H helix (Z form) generate a negative T.
- T = Twisting Number

B form DNA: + (# bp/10 bp per twist)

A form NA: + (# bp/11 bp per twist)

Z DNA: - (# bp/12 bp per twist)

Components of DNA Topology : Writhe

- W = Writhing Number
- Refers to the turning of the axis of the DNA *duplex* in space
- Number of times the duplex DNA crosses over itself
  - Relaxed molecule W=0
  - Negative supercoils, W is negative
  - Positive supercoils, W is positive

Components of DNA Topology : Linking number

- L = Linking Number = total number of times one strand of the double helix (of a closed molecule) encircles (or links) the other.

- $L = W + T$

L cannot change unless one or both strands are broken and reformed

- A change in the linking number, ΔL, is partitioned between T and W, i.e.

- $$\Delta L = \Delta W + \Delta T$$

- if $\Delta L = 0$, then $\Delta W = -\Delta T$

DNA in most cells is negatively supercoiled

- The superhelical density is simply the number of superhelical (S.H.) turns per turn (or twist) of double helix.
- Superhelical density = σ = W/T = -0.05 for natural bacterial DNA
  - i.e., in bacterial DNA, there is 1 negative S.H. turn per 200 bp
    - (calculated from 1 negative S.H. turn per 20 twists = 1 negative S.H. turn per 200 bp)

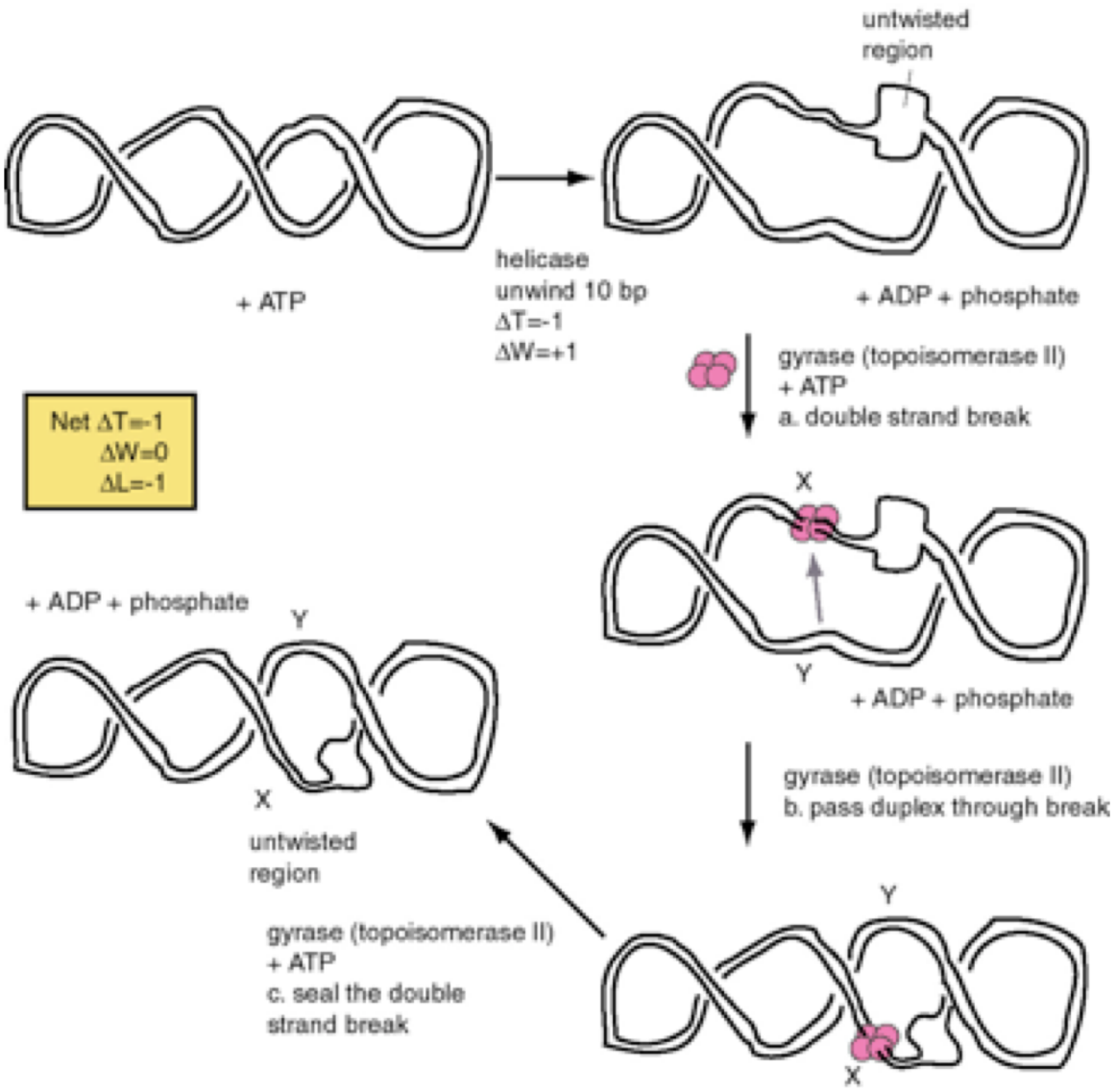
Topoisomerase I

- Topoisomerases: catalyze a change in the Linking Number of DNA
- Topo I = nicking-closing enzyme, can relax positive or negative supercoiled DNA
- Makes a **transient break in 1 strand**
- *E. coli* Topo I specifically relaxes negatively supercoiled DNA. Calf thymus Topo I works on both negatively and positively supercoiled DNA.

Topoisomerase II

- Topo II = gyrase
- Uses the energy of ATP hydrolysis to *introduce* negative supercoils
- Its mechanism of action is to make a transient **double strand** break, pass a duplex DNA through the break, and then re-seal the break.

TopoII: double strand break and passage



Complexity of DNA- Factors Repetitive Sequences

- Single Copy Genes
- Highly repetitive (hundreds to millions)
  - Randomly dispersed or in tandem repeats
  - Satellite DNA
    - Microsatellite repeats
    - Miniisatellite repeats
- Middle repetitive (10- hundreds)
  - Clustered
  - Dispersed
- Slightly repetitive (2-10 copies)

Renaturation curves of *E. coli* and calf DNA